ASSOCIATION OF PARAOXONASE-2 GENETIC VARIATION WITH SERUM PARAOXONASE ACTIVITY AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Dedicated to

my mother Mrs. Susmita Dasgupta and my father Dr. Gautam Dasgupta

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SLE, a severe autoimmune disease is of major public health relevance since it predominantly affects women at child bearing age and even though immunosuppressives have increased the life span of SLE patients, lack of absolute cure is still troubling. Risk of premature coronary heart disease (CHD) is strikingly high in SLE women (35-44 years) than the general population. Low paraoxonase (PON) activity is associated with increased CHD as well as SLE risk. PON multigene (*PON1*, *PON2 PON3*) are anti-oxidants that cluster on chromosome 7q21-22 at 94.5-94.6 Mb, in close vicinity to a linkage peak for SLE on 7q21.1 at 77.5Mb. *PON1* (*PON1*/192, *PON1*/55) and *PON3* (*PON3*/10340,*PON3*/2115) single nucleotide polymorphisms (SNPs) are the known significant modulators of PON/paraoxon activity. The purpose of this study was to determine the impact of *PON2* tagSNPs with PON activity, SLE risk, lupus nephritis, parameters of LDL oxidation and subclinical carotid vascular disease measures. Nineteen *PON2* tagSNPs were screened from HapMap and SeattleSNP databases in 489 SLE and 569 healthy control women from two recruitment sites (Pittsburgh and Chicago), using Pyrosequencing, RFLP or TaqMan allelic discrimination methods. Pairwise linkage disequilibrium ($r^2 \geq 0.8$) identified 15

tagSNPs that captured all the 19 *PON2* variants in our sample. Although none of the *PON2* tagSNPs revealed any obvious association with SLE risk, low PON/paraoxon activity was independently associated with SLE. Two *PON2* variants [rs6954345(Ser311Cys) and rs987539] showed significant association with PON/paraoxon activity in Pittsburgh whites (cases+controls). Our data revealed few modest associations of *PON2* variants with lupus nephritis (rs17876205, rs17876183, rs10261470, rs987539, rs9641164) in white (Pittsburgh+Chicago) SLE cases, parameters of LDL oxidation [*PON2*/rs11545941(Ala148Gly), rs13306702, rs2286233, rs10261470, rs17876205, rs4729189] in white (Pittsburgh) SLE cases and consistent association of *PON2*/rs11981433 and rs12704795 SNPs with carotid intima media thickness and plaque in white (Pittsburgh+Chicago) SLE cases. In conclusion, our data suggest that *PON2* genetic variants have modest effect on serum PON activity, risk of lupus nephritis and subclinical carotid vascular disease measures in SLE patients.

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1.0 OVERVIEW OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

1.1 INTRODUCTION

Systemic lupus erythematosus is an autoimmune connective tissue disorder characterized by marked inflammation that can affect almost any organ system in our body. An individual is said to acquire an autoimmune state, when his or her immune system seizes to recognize its own elements as "self" and generates antibodies against it. An autoimmune response in SLE is attributed to antibodies generated primarily against the largest cellular organelle in our body, the nucleus. American College of Rheumatology (ACR) has assigned 11 classification criteria for SLE (Table 1, Table 2), of which the presence of any 4 would call a person with SLE or lupus (Tan et al. 1982, Hochberg et al. 1997).

The most obvious clinical observation in SLE is its predominance in young women at their reproductive years (Mills et al. 1994, Beeson et al. 1994). In the Allegany County, Pennsylvania, a five year study reported a five fold higher SLE incidence in Caucasian women and even greater in African American women compared to men who were diagnosed "definite" for SLE (McCarty et al. 1995). In general, incidence rates of SLE in the United States and Europe are estimated to be 2-8 cases per 100,000 while the prevalence varies between 20-60 cases per 100,000 (Cooper et al. 1998, Danchenko et al. 2006). Though use of exogenous corticosteroids and immunosuppressants have improved the life expectancy in 80-90% of SLE

patients over the last few decades, still the average incidence has been on the rise from 1.51 to 5.56 per 100,000 over the last 40 years (Uramoto et al. 1999). With no two SLE cases being exactly alike, the precise cause of the disease is still unknown and factors that increase the likelihood of developing SLE involve a complex interplay of multiple genes, hormones and environmental agents.

SLE is notable for its unpredictable exacerbations and remissions, and follows a bimodal pattern of mortality, first identified by Urowitz et al. (1976), where deaths in patients who survived longer were attributed to atherosclerotic vascular disease while deaths in those who survived less were endowed to active disease, severe infection and high dose of steroids. The term SLE generally refers to the systemic form of the disease, while two other forms are discoid and drug induced lupus.

Table 1. The 1982 Revised Criteria for Classification of Systemic Lupus Erythematosus *

1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a) Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusi
	b) Pericarditis--documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria greater than 0.5 grams per day or grater than 3+ if quantitation not performed b) Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a) Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance b) Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	a) Hemolytic anemia--with reticulocytosis,b) Leukopenia--less than 4,000/mm<>3<> total on 2 or more occasions c) Lyphopenia--less than 1,500/mm<>3<> on 2 or more occasions d)Thrombocytopenia--less than 100,000/mm<>3<> in the absence of offending drugs
10. Immunologic disorder	a) Positive LE cell preparation b) Anti-DNA: antibody to native DNA in abnormal titer c) Anti-Sm: presence of antibody to Sm nuclear antigen d) False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in tim and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

***adapted from Tan et al. 1982**

Table 2. The 1997 Update on immunologic disorder criteria of SLE*

Immunologic disorder	1982 SLE criteria (Tan et al. 1982)
(criteria #10 of 1982 SLE criteria)	a) Positive LE cell preparation b) Anti-DNA: antibody to native DNA in abnormal titer Anti-Sm: presence of antibody to Sm nuclear antigen.False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
	1997 update on SLE criteria (Hochberg et al. 1997)
	1. Delete item 10(a) ("Positive LE cell preparation"), and
	2. Change item 10(d) to "Positive finding of antiphospholipid antibodies based on 1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2) a positive test result for lupus anticoagulant using a standard method, or 3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test."

*** adapted from Feletar et al. 2003**

1.2 DIFFERENT FORMS OF SLE

Lupus is known as *discoid lupus* when it exclusively pertains to the cutaneous system of our body. This term was framed by Kaposi in (1875). People with discoid lupus develop rashes on the skin often observed on the face and scalp which is worsened upon sunlight exposure. With time, they may also develop the systemic form, though the percentage is small, varying between 5-10%.

Systemic lupus, a more severe form of lupus affects the entire body. Common complaints from systemic lupus patients are mostly low grade fever, prolonged fatigue, arthritis, ulcers of the mouth, nose, facial rash and sensitivity to sunlight.

A butterfly shaped rash over the ridge of the nose, clinically known as the malar rash, is the most noticeable feature in both discoid and systemic lupus. Hebra in year 1866 had first used the metaphor of a butterfly to describe this classic malar rash, though the history of lupus dates back to the 10th century (Mallavarapu et al. 2007).

Long term use of ceratin medications may also result in a new form lupus, known as drug induced lupus (DIL). Symptoms of DIL overlap with that of SLE which include rash, fever, pleuritic chest pain. However unlike SLE, drug induced lupus is generally reversible and the symptoms go away once the medications are discontinued.

1.3 HISTORY OF LUPUS

The term *lupus* was first quoted in the biography of St. Martin, in 963 AD (Mallavarapu et al. 2007) though most of the authorities associate this term with Rogerius Frugardi who had first described the erosive lesions observed in the face in 1230 AD. In 1530 AD, Giovanni Manardi further reported the lesions of the lower extremity (Blotzer et al. 1983). Laurent Theodore Biett of the Paris School of Dermatology, had initially named lupus as "*erythema centrifugum*" while his student Pierre Louis Alphee Cazenave, coined the more familiar term *lupus erythematosus* in 1952 (Smith et al. 1988).

Kaposi was the first person to differentiate between discoid lupus and the disseminated or systemic lupus. He was the first to illustrate the systemic symptoms that were mainly fever, weight loss, anemia, arthritis, while the more placeable ones like renal, cardiac and pulmonary

manifestations were recognized by Osler (1895-1903) (Hepburn 2001). Sir William Osler had also framed the present day term --"systemic lupus erythematosus."

The word lupus is originally a Latin word, meaning *wolf*, in view of the fact that the disease appearance resembled wounds caused by bite of wolf (Blotzer et al. 1983, Holubar et al. 1980). One of the cornerstone achievements in the history of lupus is the discovery of a lupus erythematosus (LE) cell (Hargraves et al. 1969). The name LE cell is derived from its exclusive presence in the bone marrow of patients who had confirmed or suspected SLE. The salient feature of this cell is that, its nuclei are phagocytosed by mature polymorphonuclear leucocytes. This landmark in the history of lupus was followed by innumerable studies which made major inroads to our present day knowledge about SLE pathogenesis.

The current consensus is that the disease burden bears a striking disparity with gender, age, region and race, which are discussed in the following sections.

1.4 RISK FACTORS FOR DEVELOPING SLE

1.4.1 Gender

One of the striking epidemiological observations of SLE is that, females are more inclined to the likelihood of developing SLE than males, as clinical evidence alone show that 80-90% of the patients are females (Siegel et al. 1973, Jacobson et al. 1997). Although females dominate, both sexes do not differ in terms of the disease severity. This female to male preponderance is reported as high as 9:1 at their child bearing years which implies that sex hormones play a vital role in SLE pathogenesis (D' Cruz et al. 2007).

1.4.2 Sex Hormone

Observational phenomenon on hormonal status indicate that SLE flares highly correspond to the periodicity of female menstrual cycle (Rose et al. 1944), where an elevated level of 17β-estradiol - the principal estrogen present in serum is correlated with an increased lupus risk (Lahita et al. 2000, McMurray et al. 2001). Apart from 17β-estradiol estradiol, other hormones with immunoregulatory properties like testosterone, progesterone, prolactin and dehydroepiandrosterone/dehydroepiandrosteronesulfate (DHEA/DHEAS) can also modulate SLE incidence risk (Whitacre et al. 1999, Olsen et al. 1996). In fact, hormone replacement therapy and use of oral contraceptives can also trigger SLE risk (Sanchez-Guerrero et al. 1997, Meier et al. 1998). However, although the sex hormonal status is found to act upon the tendency to SLE risk, the disease can occur at all ages varying from babies in their mother's womb to individuals at their 80s.

1.4.3 Age

Based on age of onset, SLE is categorized into 3 subtypes: neonatal lupus, pediatric lupus and late onset lupus.

Neonatal lupus is a rare congenital disorder caused by transfer of maternal autoantibodies from the affected mother to the infant through the placenta. Nearly 75% of babies with neonatal lupus develop skin rashes at birth.

Pediatric or childhood onset lupus is usually diagnosed between the ages of seven and puberty. Delayed diagnosis in most cases of pediatric lupus results in higher disease activity, resulting in damage of the kidneys, heart as well as the central nervous system.

The average age of onset for late onset lupus is 59 and interestingly in late onset lupus, the female to male ratio is less prominent (3:1) unlike its other forms (Lockshin et al. 2002). Symptoms mostly include achy joints, fatigue, and heart disease. The disease severity is also reported to be low at age of onset 50 or higher compared to SLE onset prior to age of 50 (Boddaert et al. 2004).

In females, the highest incidence and prevalence rates of SLE are observed at 15-44 and 45-64 years of age, respectively (Siegel et al. 1973, Manzi et al. 1997). In fact within females, and also in males, this disease risk highly differs based on the ethnic background of an individual.

1.4.4 Racial differences

A review article by Dancheko et al. (2006), which evaluates the world wide disease burden of SLE, reveals that people with non-white ancestry are at higher risk of SLE compared to the whites. African-American women are at 3-4 times higher risk of SLE than Caucasian females, which alone indicates that apart from hormonal influence, genetic elements play a major role in SLE etiology, which are discussed in the next section. Within racial groups, the highest prevalence rates of 21.9 per 100,000 per year have been reported in UK Afro-Caribbean residents (D'Cruz et al. 2007). However, in non-whites who have higher disease risk and thereby higher mortality rates from SLE compared to the Caucasians, a major confounding factor is their low socioeconomic status (SES) (Karlson et al. 1995, Simard et al. 2007).

1.4.5 Heredity

As complex as the name sounds, this trait has 100 or more genes involved in its pathogenesis (Tsao et al. 2004, Lindqvist et al. 1999). Genetic predisposition to SLE is substantiated by studies reporting a 10-fold higher risk in monozygotic twins than dizygotes with concordance rates 24%-58% and 2-5%, respectively, (Deapen et al. 1992), a high degree of familial aggregation (Sestak et al. 1999) and high heritability (>66%) (Lawrence et al. 1987). Apart from human studies, murine models have also shown that, the phenotypic expression of a lupus susceptible gene is highly dependant on the genetic background (Nadeau et al. 2001).

The two basic strategies that made enormous contribution to unravel the genetic elements that confer SLE risk are the genome wide linkage studies and hypothesis driven candidate gene association analysis. The potentially susceptible intervals for SLE risk that have been identified and replicated in indepedent studies are 1q22-24 (Moser et al. 1998), 1q41-42 (Tsao et al. 1997), 2q37 (Lindqvist et al. 2000), 4p16 (Gray-McGuire et al. 2000), 6p11-21, 16q12-13 (Gaffney et al. 1998, Gaffney et al. 2000) and 11p13 (Kelly et al. 2002), 12q24 (Nath et al. 2004). Apart from the strongest susceptibility loci, many other multiple loci with moderate linkage signals have been identified in several studies and it is their combined effect that helps in truly understanding the complex nature of the SLE genetics.

So far, the strongest evidence for linkage with SLE was found near human leukocyte antigen (HLA) DR2 and DR3 loci (Graham et al. 2007), other key candidate genes being interferon regulatory factor (IRF5) (Demirci et al. 2007, Graham et al. 2006), tyrosine kinase 2 (TK2) (Sigurdsson et al. 2005), C-reactive protein (CRP) (Russell et al. 2004), protein tyrosine phosphatase N22 (PTPN22) (Baca et al. 2006, Orozco et al. 2005), programmed cell death 1 (PDCD1) (Prokunina et al. 2002), signal transducer and activator of transcription (STAT4)

(Taylor et al. 2008). A recent study also confirms that a common haplotype in STAT4 gene along with two *IRF5* alleles correlate with anti-dsDNA production and SLE risk (Sigurdsson et al. 2008). Few studies have also found association of Toll-like receptor (*TLR-5, TLR-9*) variants with SLE pathology (Means et al. 2005), though this has not been confirmed by others (Demirci et al. 2007).

Recently, two genome wide association (GWA) scans on SLE identified many other susceptible loci, apart from the established variants on HLA regions. The first GWA study by the International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN) (Harley et al. 2008), scanned >300,000 SNPs using high–density Illumina HumanHap 300 BeadChip, in a European case-control cohort of 720 SLE women and 2337 controls and replicated in two independent cohorts. This study identified *ITGAM* on 16p11.2, *KIAA1542* on 11p15.5, *PXK* on 3p14.3 and rs10798269 on 1q25.1 and nine other regions that would confer SLE risk in addition to the previous SLE susceptible regions on *HLA, IRF5, FCGR2A, PTPN22* and *STAT4*. The second GWA study (Hom et al. 2008) screened 13311 and 1783 case-control individuals with more number of SNPs (>500,000) than the first GWA study, using Illumina HumanHap550 bead chip and replicated in a Swedish case-control cohort, identified more SLE susceptible loci which were *BLK*, *C8orf13* on 8p23.1 in addition to the established ones on *HLA, ITGAM-ITGAX* regions.

In a genomewide microsatellite marker screen performed in 82 sib pair families with SLE by Gaffney et al. (2000), reported an interval at 7q21 with a LOD score of 2.40 which satisfies the criteria for suggestive linkage of LOD score > 2.2 by Lander et al. (1995). This region harbors the paraoxonase (PON) gene cluster with paraoxonase-2 (*PON2*) gene as one of its members, which maps to chromosome 7q21.3 at 94.6 Mb, near to the linkage signal for SLE

found at 77.5 Mb on 7q21.3. This qualifies *PON2* as positional candidate for SLE. Our group has already reported associations of certain haplotypes of *PON1* with SLE risk (Tripi et al. 2006) but not *PON3* variants (Sanghera et al. 2008). To our knowledge, SLE risk with respect to *PON2* variants has not been analyzed yet.

So this study investigated the role of *PON2* SNPs with SLE susceptibility and its accompanying phenotypes.

1.4.6 Environmental triggers

Environmental agents that are found to increase SLE risk mainly include photosensitivity, occupational exposure to silica, and water contaminated with trichloroethate (TCE). Photosensitivity or exposure to sunlight acts a major stimulus in triggering certain variants of discoid lupus erythematosus.

Next to sunlight, exposure to silica can also increase SLE risk. Silica particles enter our body through the respiratory pathway where it gets ingested by alveolar macrophages. These particles are known to play the role of an adjuvant, which stimulates migration of macrophages to their site of deposition resulting in an inflammatory response (Parks et al. 2002, Finckh et al. 2006, Lippmann et al. 1973).

Exposure to silica is mainly occupational, and men are more exposed than women since most of these are traditionally male jobs. Brown et al. (1997) reported that out of 1130 men with silicosis who showed a relative SLE risk of 23.8 (95% CI;11.9-86.3) majority had to be treated for SLE as well. A very high prevalence of SLE, as high as 93 per 100,000 cases was found in a cohort of 1500 miners exposed to silica by Conrad et al. (1996). In case of women who are

occupationally exposed to silica, Nagata et al. (1992) reported an odds ratio of 3.9 (95% CI;1.6-9.7) for SLE risk.

1.4.7 Smoking, Alcohol consumption and Diet

Cigarette smoking, alcohol consumption, as well as diet of a person can also add up to SLE risk (Hardy et al. 1998, Cooper et al. 1998). Noxious chemicals effused during smoking increase systemic inflammation, impair T-cell function and reduce the count of natural killer cells (Bermudez et al. 2002, Hughes et al. 1985). A meta-analysis study shows that current smokers are at 1.5 times higher risk of SLE than non-smokers (Costenbader et al. 2004), while seropositivity for anti-dsDNA antibodies was found to be four times higher than non-smokers (Freemer et al. 2006). However unlike a consistent association of SLE with cigarette smoking, studies on alcohol consumption show conflicting results (Ghaussy et al. 2001). Interesting enough, food habit of a person can also contribute to the development of inflammatory and autoimmune disease (Parke et al. 1996). For SLE patients, a diet comprising anti-inflammatory agents (omega 3 fatty acids) and anti-oxidants (vitamin A, C, E) are highly recommended. This fact has been verified in lupus-prone mice models treated with fish oil rich in omega 3 fatty acids or anti-oxidants which showed delayed onset of lupus (Cooper et al. 1998).

1.4.8 Infectious agents

In SLE patients, the onset of the disease is often followed by an infection. A possible infectious agent that has been identified to trigger SLE is the Epstein Barr virus (EBV). In humans, association of EBV with SLE risk is modified by age, gender and race (Parks et al. 2005).

Introduction of the whole viral nuclear antigen 1 into lupus prone mouse models is found to increase IgG antibody, specific for double stranded and Sm DNA, supporting the role of EBV in SLE pathogenesis (Sundar et al. 2004).

Memory cells or B cells are the main residing places for the EBV virus, which triggers proliferation of more memory cells after infection, culminating into an inflammatory response (Gross et al. 2005). Inflammatory reaction in SLE is unique as it involves the production of auto-antibodies against its own components. This autoimmune reaction lies central to SLE pathogenesis, which we discuss in the following section.

1.4.9 Inflammation

The hallmark feature in SLE inflammation is that, antibodies are mainly produced against the chromatin components which encompass dsDNA, histones and nucleosomes. It is interesting to note that the body does produce antibodies to nuclear antigens during any protective immune response, though they are not sufficient to elicit an autoimmune reaction (Ramanujam et al. 2008). In SLE, antibodies are generated against a multitude of nuclear antigens.

Antibodies like antinuclear, anti-Ro, anti-La, and antiphospholipid (aPL) precede the onset of SLE by many years, whereas anti-Sm and anti-nuclear ribonucleoprotein antibodies are found only months before clinical onset of SLE, the time when the disease manifestations start to show. Antibodies to double-stranded DNA (anti-dsDNA), which mainly correlates with disease activity (Schur et al. 1968), appear intermediate to that of other antibodies (Arbuckle et al. 2003). Higher the disease activity, higher is the inflammatory effecter response, which ends up in wide spread organ damage. Organ damage over time is evaluated by the Damage Index assigned by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology

(SLICC/ACR) (Gladman et al. 1997). Serum CRP level is considered to be a sensitive marker of inflammation (Ridker et al. 2000). Some of the severe clinical manifestations which result in the irreversible organ damage in SLE are lupus nephritis, accelerated atherosclerosis and an elevated level of aPL**.**

1.5 CLINICAL MANIFESTATIONS IN SLE

1.5.1 Lupus Nephritis

One of the serious and common clinical complications of SLE is lupus nephritis or glomerulonephritis. Lupus nephritis or kidney inflammation is caused by an over active immune system, as a result of which antigen antibody complexes are deposited in the kidney (Foster et al. 2007). The immune complex is mainly comprised of DNA and antibodies to it, so majority of the patients show serpositivity for anti-DNA antibody. Along with a rising anti-DNA antibody titer, a low titer of compliment factors, especially C3, is also seen in most of the lupus nephritis patients and these two are considered to be a strong predictor of active lupus renal flares (Mortensen et al. 2008, Rovin et al. 2007).

The clinical spectrum of lupus nephritis ranges from asymptomatic low grade proteineuria to a rapidly progressive course with hypertension leading to renal insufficiency within days. Eight cohort studies comprising of 2149 SLE patient have reported that, the prevalence rate of renal disease varies widely between 31 to 65% (Wallace et al. 1996). The general consensus is that 50% of SLE patients at some point of time during their course of illness will certainly develop clinically relevant nephritis, though in most of the cases, nephritis is

diagnosed in the early stages of the disease. Like the disease risk, lupus nephritis has also gender and race specific prevalence while renal involvement is more pronounced in males than in females and Africans have more severe kidney inflammation than the Caucasians. Genetic elements, like HLA antigens which predispose to SLE risk, are also found to predispose to lupus nephritis (Korbet et al. 2007). Maggi et al. (1994) have shown that the renal disease is associated with enhanced oxidation of low density lipoprotein (LDL), which is a prerequisite for cardiovascular complications. An additional phenomenon that is observed in SLE patients is glomerular thrombosis that results from the hypercoagulability, accompanying antibodies directed against negatively charged phospholipid-protein complexes. This is observed in a group of SLE patients who have higher titers of antiphospholipid antibodies (aPL). Raised anticardiolipin antibody (aCL) levels are observed in patients with lupus nephritis (Loizou et al. 2000). Importance of antiphospholipid antibodies in SLE are therefore discussed in the following section.

1.5.2 Antiphospholipid antibodies (aPL)

In general, large percentages of SLE patients (20-60%) show persistent positivity for higher levels of antiphospholipid antibodies (aPL) that are associated with a spectrum of clinical manifestations such as recurrent venous and arterial thrombosis, fetal loss, thrombocytopenia, and neurological symptoms (Harris et al. 1983). aPL binds to plasma proteins with an affinity for phospholipid and includes anticardiolipin antibodies (aCL), lupus anticoagulant (LAC) and anti *β2*-glycoprotein I or anti-apolipoprotein H, (antiβ2-GPI or anti-apoH).

Raised levels of aCL and LAC are associated with myocardial infarction (Hamsten et al. 1986, Petri et al. 2004). Cross reactivity reported between aPL and antibodies to oxLDL

(Vaarala et al. 1993), in SLE patients implies a possible link between atherosclerotic and inflammatory events in SLE. Among the clinical challenges in SLE, one of the most compelling is the high incidence of accelerated atherosclerosis in women at their child bearing age.

1.5.3 Premature Atherosclerosis

Given the well known fact that coronary heart disease (CHD) is the leading cause of mortality in females, majority of deaths reported in SLE women is also attributed to premature atherosclerosis (Manzi et al. 1997, Ward et al. 1999, Petri et al. 1992, Bruce et al. 2005). Although coronary heart disease is the prime cause of death in women, incidence of coronary events, in particular myocardial infarction is observed 10-20 yrs later compared to the males (Kannel et al. 1995). Young women with lupus show 50 times higher morality rates from myocardial infarction than the general population (Mosca et al. 1997, Rich-Edwards et al. 1995). Traditional risk factors for CHD though increase the risk of atherosclerosis, they do not fully rationalize for the observed accelerated atherosclerosis in SLE (Esdaile et al. 2001). Factors which also contribute to premature atherosclerosis in SLE are inflammation, adverse effects of corticosteroids, renal involvement, and higher levels of antiphospholipid antibodies. Premature atherosclerosis in SLE was first reported in a necroscopy study by Bulkley et al. (1975) while 6 to 12% of SLE patients show signs of clinical atherosclerotic events, almost half of SLE subjects (40%) show subclinical plaque formation. Carotid plaque and carotid intima media thickness (IMT) are considered to be the two very sensitive markers of atherosclerosis (Manzi et al. 1999, Salonen et al. 1993). Non-invasive techniques like arterial ultrasound of the carotid arteries are used to measure the thickness of the specific layers of artery wall (IMT), and carotid plaque formation which offers a partial way to track the disease progression.

Atherosclerotic plaque formation:

Atherosclerosis derived from the Greek word "*athero*" meaning paste and "*sclerosis*" meaning hard, literally stands for hardening of the arteries. This progressive disease is built on a "response to injury' hypothesis, where an endothelial cell gets injured triggering an inflammatory response, which may further be aggravated by chronic inflammation and immune system dysregulation that are observed in SLE (Ross 1993). Hardening of the artery wall mainly occurs due to accumulation of cellular debris, cholesterol, calcium, and fat deposits in the inner lining of the vessel wall. This build up is termed as plaque which is a progressive event, where the earliest event is the development of a fatty acid streak. A major pre-requisite for this plaque formation is the entrapment of oxidized low density lipoprotein (oxLDL)-loaded monocytes into subendothelial space of the arteries. For this, both transfer and retention of LDL and other lipoproteins to the artery wall are the essential phenomenon (Steinberg et al. 1989, Young et al. 1994, Schwenke et al. 1989).

After its transport, LDL gets oxidized by oxidative wastes secreted into the membranes that can literally seed LDL with reactive oxygen species. LDL is first oxidized to a mild form (MM-LDL), which is later modified to its higher oxidized form (Palinski et al. 1990, Witztum et al. 1991, Witztum etal. 1994, Parthasarathy et al. 1994). This high oxidized LDL then induces adhesion of monocytes to the endothelial lining and also acts a potent inducer of other inflammatory molecules like MCP-1, M-CSF, GRO and P-selectin which eventually leads to the formation of plaque (Vlaicu et al. 1985, Navab et al. 1996). Figure 1 illustrates the role of oxidized LDL (oxLDL) in atherosclerosis development.

Navab et al. (1996) suggested that although these oxidizing forces help in the progression of fatty acid streak, at the same time opposing forces try to attenuate the formation of

atherosclerosis. These opposing forces comprise of the high density lipoprotein (HDL) associated enzyme systems --- paraoxonase-1 (PON1) and PAF acetylhydrolase (Stafforini et al. 1993, Watson et al.1995, Mackness et al. 1993). These enzyme systems act by preventing oxidation of LDL which is illustrated both *in vivo* (Klimov et al. 1993) and *in vitro* (Mackness et al. 1993) and thereby accounts for the inverse relationship of HDL-level with atherosclerosis risk (Parthasarathy et al. 1990).

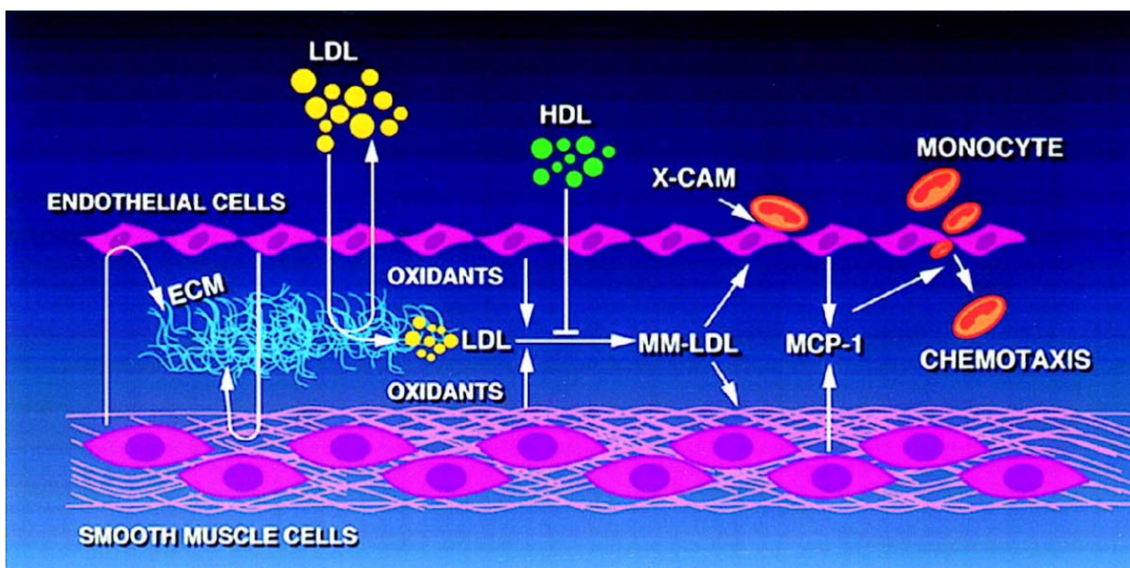


Figure 1. Role of oxidized LDL in atherosclerosis development.
adapted from Navab et al. (1996)

In particular, PON1 enzyme is suggested to destroy the biologically active lipid peroxides in MM- LDL, which in turn attenuates the inflammatory response at the artery wall (Mackness et al. 1987, Mackness et al.1991). Like PON1 enzyme, paraoxonase -2 (*PON2*) enzyme also has anti-atherogenic properties (Ng et al. 2006, Rosenblat et al. 2003), though unlike PON1 enzyme, *PON2* enzyme is not associated with HDL.

The paraoxonase enzyme activity or PON activity is often associated with cardiovascular disease risk and many studies suggest that PON activity is better measure for CHD risk than the variants in the gene itself.

1.6 SERUM PARAOXONASE ACTIVITY, CARDIOVASCULAR DISEASE, AND SLE

Serum paraoxonase (PON) is a calcium dependant esterase. Paraoxonase specificity towards endogenous serum is not yet well characterized, therefore synthetic substrates are used to monitor the enzyme's activity. PON activity is substrate dependent (Furlong et al. 1988).
PON activity polymorphism has historical importance as it was the first genetic marker found to be linked to cystic fibrosis gene and thereby maps to the long arm of chromosome 7. Several studies in non-SLE cohorts (McElveen et al. 1986, Durrington et al. 2001, Mackness et al. 2004 , Ayub et al. 1999, Jarvik et al. 2000, Mackness et al. 2001, Jarvik et al. 2003) have implied that low PON activity may affect the risk for CHD. Serum PON activity was reported to be low in diabetic cases who also show vast cardiovascular complications (Letellier et al. 2002). Our previous study has shown that low PON activity is an independent risk factor for SLE (Tripi et al. 2006).

2.0 OVERVIEW OF THE PARAOXONASE (PON) GENE FAMILY

2.1 BACKGROUND

The name paraoxonase (PON) is derived from its catalytic role of hydrolyzing paraoxon. PON is an active metabolite of parathion. Parathion belongs to a class of insectides that assumed importance when organophosphates came into widespread use over 50 years ago. Liver and other tissues catalyze this oxidative desulphuration of parathion to paraoxon. PON enzyme not only hydrolyses paraoxon but also hydrolyses a variety of other substrates such as diazinon, chlorpyrifos, nerve reagents such as sarin, soman, aromatic esters, and lactones. For *PON1*, phenylacetate is the most commonly used substrate for measuring serum PON activity (Draganov et al. 2005), while for *PON2*, dihydrocoumarin (Rosenblat et al. 2003) is the only substrate reported so far. *PON1*, *PON2* and *PON3* are the 3 members of the paraoxonase gene family that cluster on the long arm of chromosome 7. Similarities at the exon and intronic junctions in all three PON multigene members suggest that they arose by gene duplication and therefore they share considerable structural and functional similarity. These genes are 70% identical at the nucleotide level and 60% identical at the amino acid level (Primo-Parmo et al. 1996). This high degree of conservation suggests that the entire gene family plays an important physiological role, though so far, not completely understood.*PON2* and *PON3* differ from *PON1* in their cDNA sequences, where *PON1* has three nucleotides in its codon 106, which is missing

in both *PON2* and *PON3* (Primo-Parmo et al. 1996, Draganov et al. 2004). Although *PON1, PON2, PON3* exhibit anti-oxidant properties, they differ in their expression silhouettes. *PON1* and *PON3* are primarily synthesized in liver, a portion of which when secreted in the plasma gets associated with HDL, while *PON2* is ubiquitously expressed in cells including liver, macrophage, artery wall, brain and kidney but is not present in plasma (Ng et al. 2005).

Of all 3 members of the paraoxonase family, our gene of interest in this study is *PON2*. The following sections therefore address the molecular aspects of *PON2*, *PON2* polymorphisms and its associations reported so far.

2.2 MOLECULAR ASPECTS OF *PON2*

PON2 is the oldest member of the paraoxonase gene family as suggested by phylogenetic reports, followed by *PON3* and then *PON1* (Draganov et al. 2004). Location of the *PON2* in the *PON* multigene family is shown in Figure 2. Similar to their evolutionary time points, *PON2* lies near to the telomere, while *PON1* near the centromere and *PON3* in between them, on chromosome 7 (Ng et al. 2005, Draganov et al. 2004).

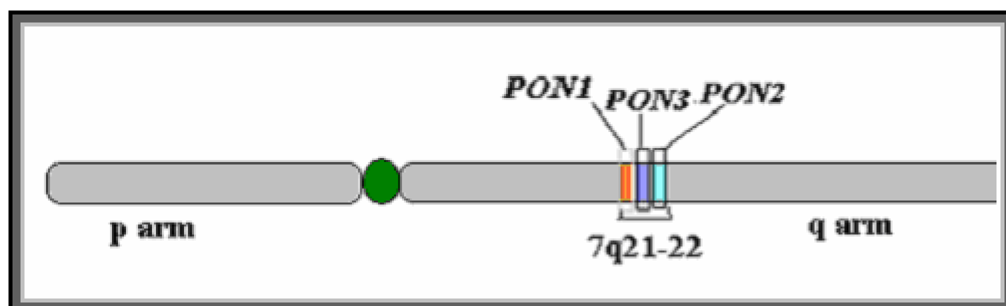


Figure 2. Location of PON2 gene in PON multigene family

PON2 protein has a molecular mass of 44 KDa (355 amino acids), although its exact physiologic or pathophysiologic role is still unknown. *PON2* spans 30 kb on chromosome 7. *PON2* along with *PON1* and *PON3,* lie on the long arm of chromosome 7 (7q21-22) at 94.5-94.6 Mb which lie in close proximity to the linkage peak on 7q21.1 at 77.5 Mb for SLE (Gaffney et al. 2000). Therefore *PON2* is a positional candidate gene for SLE.

***PON2* expression:**

Anti-oxidant property of *PON2* has been evaluated through *in vtiro* studies, where over expression of this gene in HeLa cells was found to inhibit LDL lipid peroxide formation, invert oxidation of mildly oxidized LDL (MM-LDL), and thereby inhibit chemotaxis of monocytes by MM-LDL (Ng et al. 2001).

In vivo studies in mice by Ng et al. (2006) have also shown that an elevated level of *PON2* expression is capable of preventing LDL oxidation, and *PON2* deficient mice with lower levels VLDL/LDL cholesterol develop larger atherosclerotic lesions compared to their wild type counterparts (Ng et al. 2006). Though some studies claim that *PON2* expression levels are enhanced during an oxidative stress, unlike *PON1*, some others report that this is not the case always. *PON2* expression in mouse macrophages was elevated by oxidative stress (Rosenblat et al. 2003, Shiner et al. 2004), while in monocyte-derived macrophages in hypercholesterolemic individuals showed lower *PON2* expression compared to subjects with normal cholesterol levels (Rosenblat et al. 2004). A recent study also showed that in human carotids both mRNA and protein levels of *PON2* decline sharply with the progression of plaque (Fortunato et al. 2008), and lithium exposure mimicking high oxidative stress was also found to down regulates *PON2* expression (Allagui et al. 2007). This differential response of *PON2* may be attributed to its cell specific regulatory functions, which remains to be explored.

2.3 *PON2* POLYMORPHISMS AND THEIR EFFECTS

PON2 has 9 exons, and approximately 216 polymorphisms have been identified, some of which are associated with pathophysiologic conditions (http:// www.ncbi.nlm.nih.gov/sites/entrez). The two well-known functional polymorphisms, in *PON2* are rs11545941(Ala148Gly), and rs6954345(Ser311Cys).

The Cys311 is hypothesized to be a catalytic center for hydrolysis of oxidized lipids (Augustinsson, 1968). Majority of the studies reported so far have examined *PON2* variants together with *PON1* variants, *PON2*/Ser311Cys polymorphism was found to be associated with CHD risk in conjunction with *PON1*/codon192 polymorphism in Asian Indian and US ancestry (Sanghera et al. 1998, Chen et al. 2003). A recent report by Saeed et al. (2007) also supports interaction between *PON1* variants (Gln192Arg and C-108 T) and *PON2* variants (Ala148Gly and Ser311Cys) and their association with myocardial infarction in a Pakistani cohort. *PON*2/Ser311Cys is also associated with cardiovascular disease (CVD) in a familial hypercholesterolemic cohort (Leus et al. 2001), higher HDL-C (Pan et al. 2002), ischemic stroke in patients with diabetes mellitus type 2 (Wang et al. 2003), reduced bone mass in post menopausal women (Yamada et al. 2003), microvascular complications in diabetes mellitus (Mackness et al. 2005), CAD (Martinelli et al. 2004, Wang et al. 2003), pathogenesis of Alzheimer's disease (AD), either involving apolipoprotein E4 or independent of its status (Shi et al. 2004 and Janka et al. 2002), albumin excretion rate (Thameem et al. 2008).

Some studies found association of *PON2*/Ala148Gly polymorphism with plasma lipoprotein levels (Boright et al. 1998) but other studies did not (Sanghera et al. 1997). However, a meta-analysis of 43 studies (Wheeler et al. 2004) does not suggest any associations with either

PON1/codon55 or *PON1*/codon192 and *PON2*/Ser311Cys with CHD. In addition to being a positional candidate gene, *PON2* is also a biologic candidate gene, for the CHD risk in SLE. PON activity is in part genetically determined, however, the correlation between functional and non functional PON polymorphisms and PON activity is not a direct one because in many cases PON polymorphisms do not predict any risk to cardiovascular disease. However, at the same time serum PON activity, using paraoxon as substrate is found to be associated with CHD risk (Durrington et al. 2001, Mackness et al. 2004, Ayub et al. 1999, Jarvik et al. 2000, Mackness et al. 2001, Jarvik et al. 2003, Rozek et al. 2005, Bhattacharyya et al. 2008).

In mouse models, Aviram et al. (1998) showed an inverse relation of serum PON activity with atherosclerotic plaque progression. A recent tag single nucleotide polymorphism (tagSNP) report on PON gene cluster by Carlson et al. (2006) also provide the evidence that low PON activity is a predictor of CHD. For a given individual the serum PON activity remains stable over time and patients with familial hypercholesterolemia and diabetes (Mackness et al. 1991, Abbott et al. 1995) and children chronic renal failure (Ece et al. 2006) have decreased PON activity. PON activity has also been found to be low in SLE cohorts (Tripi et al. 2006, Alves et al. 2002). Association study of PON activity with antiphospholipid antibodies, however, gave inconsistent results (Lambert et al. 2000, Tripi et al. 2006).

PON activity analyzed with paraoxon as the substrate is credited mostly to the *PON1*/Q192R SNP. Our studies investigating the relationship between *PON1* and *PON3* SNPs and PON activity have confirmed that PON1/Q192R is the major contributor to PON activity, others being *PON1*/L55M, *PON3*/2115 and *PON3*/10340 (Tripi et al. 2006, Sanghera et al. 2008).

PON2, the oldest member of the PON multigene family not only qualifies as a positional candidate for SLE risk but also as a biologic candidate for CHD risk in SLE. To our knowledge the role of *PON2* genetic variation in relation to SLE risk, oxidised LDL, CHD in SLE, nephritis and PON activity has not been examined. Therefore, we examined *PON2* genetic variation in relation to SLE susceptibility, accompanying phenotypes and PON activity.

3.0 RESEARCH OBJECTIVES

The objective of this research was to investigate the role of common *PON2* variants with SLE risk, SLE-related phenotypes such as lupus nephritis, carotid vascular disease (IMT and carotid plaque), parameters of LDL oxidation and variation in serum PON activity.

Following are the specific aims:

a) To determine the relationship of *PON2* tagSNPs and haplotypes with SLE risk in Caucasian and African-American subjects who are a apart of SLE case-control cohorts collected from Pittsburgh (Pittsburgh Lupus Registry, Central Blood Bank of Pittsburgh) and Chicago (SOLVABLE study).

b) To determine the association of *PON2* tagSNPs with lupus nephritis in Caucasian lupus patients.

c) To determine the association of the *PON2* genetic variation with parameters of LDL oxidation.

d) To determine the association of *PON2* tagSNPs with carotid vascular disease measures in Caucasian SLE cases.

e) To characterize the extent of contribution of *PON2* tagSNPs on serum paraoxonase activity.

4.0 EXPERIMENTAL DESIGN

4.1 SELECTION OF CASE-CONTROL COHORT

In order to test the role of common *PON2* variants with SLE risk and its phenotypes we performed a case control study. The case cohort comprised of 489 SLE women (396 from Pittsburgh and 93 from Chicago) who fulfill the ACR classification criteria for SLE (Tan et al. 1982, Hochberg et al. 1997). They are obtained from two sources, the Pittsburgh Lupus Registry and the Chicago SOLVABLE study (Study of Lupus Vascular and Bone Long-term Endpoints). The control cohort comprised of 569 women (496 from Pittsburgh and 73 from Chicago) with no apparent history of SLE, who belong to either the Central Blood Bank of Pittsburgh or the SOLVABLE study for the Chicago part. The controls were geographically matched to cases to minimize the potential effect of differential sampling. The mean ± SD age of cases were 43.48 ±11.29 years and controls were 45.99 ± 13.01 years.

Blood samples from all participants were collected at the baseline visit, who gave informed consent for this study. This study was also approved both by the University of Pittsburgh Institutional Review Board and Northwestern University Institutional Review Boards, Table 3 summarizes the composition of the case-control cohorts for our *PON2* study. A further detailed description of cases and controls from Pittsburgh Lupus Registry and the Chicago SOLVABLE study are summarized below.

Table 3. Composition of the case-control cohort

Population	White		Black	
	Cases	Controls	Cases	Controls
Pittsburgh site	348	454	48	42
Chicago site	68	57	25	16
Total	416	511	73	58

4.1.1 Case-Controls from Pittsburgh site

A total of 396 SLE women (348 whites and 48 blacks) from Pittsburgh Lupus Registry were enrolled in our study. These women were 17 years of age or older (mean ±SD =43.20±11.48 years) and were diagnosed and further followed up either at University of Pittsburgh since 1980 or by private rheumatologists residing in the metropolitan area since 1991. The Pittsburgh Lupus registry has 1784 patients (which includes both living and the deceased), majority of which reside within a radius of 200 miles which represent an admix of rural and urban community. This mix portrays homogeneity with respect to sampling of cases in contrast to sampling of SLE patients seen strictly at a referral centre and also in terms of the extensive disease heterogeneity.

Patients who are diagnosed with lupus nephritis fulfill any of the three criteria a) renal biopsy showing lupus nephritis b) at least 2 readings of proteineuria >0.5gm/24 hrs or 3+ protein by dipstick c) red blood cell casts. Information on traditional risk factors like age, race, smoking

habits, BMI, total cholesterol, LDL, HDL, triglyceride levels, vascular risk factors like carotid IMT and carotid plaque index measurement, and SLE related disease factors like renal disease are described elsewhere (Selzer et al. 2001).

A total of 496 controls (454 whites and 42 blacks) were recruited from the Central Blood Bank of Pittsburgh with a mean ± SD age of 45.62± 13.47 years.

4.1.2 Case –Controls from Chicago site

The SLE cases that are recruited from SOLVABLE study comprised of 68 whites and 25 blacks with a mean ±SD age of 43.67 ±10.47 years, while controls (57 whites and 16 blacks) had a mean ±SD age of 48.25±9.49 years.

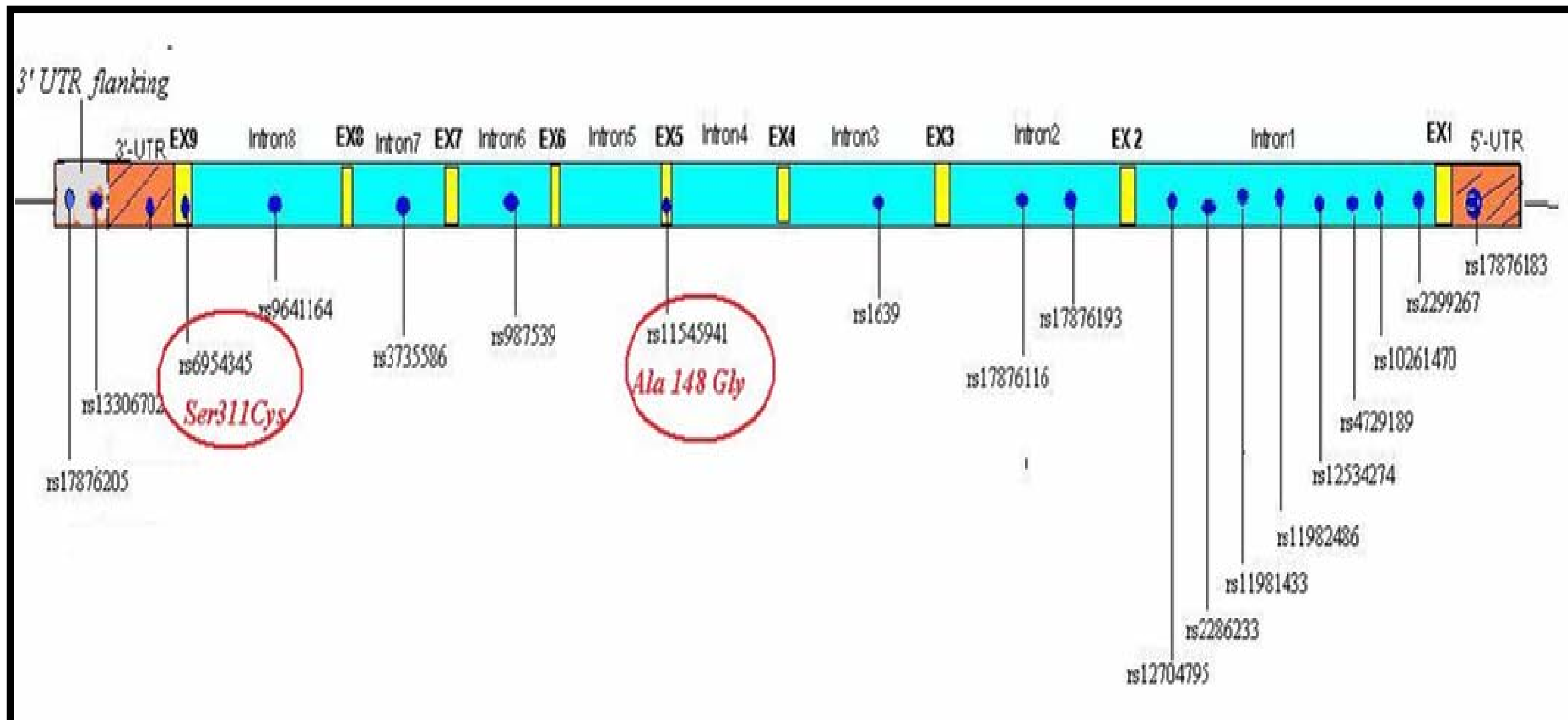
4.2 SELECTION OF *PON2* TAGSNPS (HAPMAP AND SEATTLE)

We selected a total of nineteen *PON2* tagSNPs based on the linkage disequilibrium (LD) select program provided by international HapMap project (www.hapmap.org) and SeattleSNPs database (http://pga.gs.washington.edu/) for this study.

While SeattleSNPs or Seattle programs for genomic applications (PGA) submitted a total of 23 highly informative tagSNP bins for the entire *PON2* in its European registry, HapMap database tags this information to 11 tagSNPs. This lower number of tagSNP information in HapMap compared to the Seattle database is attributed mostly to the following reasons: a) Seattle database employed sequencing approach to identify the polymorphic sites whereas HapMap used genotyping of selective SNPs to identify the tagSNPs, b) HapMap database has more number of people (90) compared to Seattle (23) in their respective Caucasian cohorts.

The threshold for the minor allele frequency (MAF) for all the tagSNPs except two, included in our study is reported to be MAF$\geq$ 5%, in either of the databases. TagSNPs *PON2*/rs17876183 (MAF=3%) and *PON2*/rs17876205 (MAF=2%) are submitted by SeattleSNPs database only. We have included these two SNPs due to their positional relevance .[rs17876183 at 5'-UTR and rs17876205 after 3'-flanking region].

The intron and the exon locations of the selected *PON2* tagSNPs is illustrated in Figure 3. and a summary of the Seattle numbers of each SNP with their corresponding reference ID numbers in NCBI Entrez SNP database, their allelic status, their location and their MAFs are shown in Table 4.



` **Figure 3. Intron-Exon locations of the selected 19 *PON2* tagSNPs in PON2 gene**

Table 4. Minor allele frequencies (MAF) of the selected 19 SNPs in HapMap (CEU*) and SeattleSNP(ED) database**

SNPs	dbSNP	Seattle ID	Location	Alleles	MAF(CEU*) in HapMap	MAF(ED**) in Seattle
1	rs17876183	680	5'-UTR	G>A	not reported	0.05
2	rs2299267	3035	intron 1	A>G	0.2	0.28
3	rs10261470	3628	intron 1	G>A	0.108	0.17
4	rs4729189	6998	intron 1	A>T	0.186	0.28
5	rs12534274	7447	intron 1	G>A	0.268	0.196
6	rs11982486	9359	intron 1	T>C	0.333	0.21
7	rs11981433	10621	intron 1	T>C	0.408	0.39
8	rs2286233	10704	intron 1	A>T	0.083	0.07
9	rs12704795	10954	intron 1	T>G	not reported	0.39
10	rs17876193	13311	intron 2	C>G	not reported	0.2
11	rs17876116	17484	intron 2	G>T	not reported	0.12
12	rs1639	21122	intron 3	T>G	0.23	0.3
13	rs11545941(codon 148)	23956	exon5	C>G	0.24	0.2
14	rs987539	27982	intron 6	T>C	0.492	0.46
15	rs3735586	29392	intron 7	T>A	0.233	0.2
16	rs9641164	30153	intron 8	A>T	0.237	0.33
17	rs6954345 (codon 311)	30199	exon 9	C>G	0.24	0.17
18	rs13306702	30931	after 3'-UTR	G>C	0.017	0.05
19	rs17876205	31873	after 3'-UTR	G>C	not reported	0.043

*CEU- also known as CEPH (Centre d'Etude du Polymorphisme Humain) refers to the European Ancestry (90 individuals) in HapMap database

**ED --refers to the European Descent (23 individuals) in Seattle database

4.3 QUANTIFICATION OF PARAMETERS OF LDL–OXIDATION

Initially LDL was isolated from plasma by ultracentrifugation followed by copper induced oxidation or by malondialdehyde (MDA) as described elsewhere (Palinski et al. 1990). With the help of chemiluminescence immunoassay, the amount of antibody (IgG and IgM) bound to both copper-modified oxLDL [anti-cu-modified oxLDL (IgG) and anti-cu-modified ox-LDL (IgM)] and MDA-modified oxLDL [anti-MDA-modified LDL (IgG), anti-MDA-modified LDL (IgM)], were measured, details of which were described in Frostegard et al. (2005). Another LDL oxidation parameter the EO6 epitope concentration on Apo B-100 particle (oxLDL-EO6 epitope), which measures oxPL/ApoB was also determined by chemiluminescence assay, where an anti-Apo B-100 antibody, MB47 and a biotin labeled anti-oxLDL antibody (EO6) were used to quantify the amount of EO6 epitope on LDL. Parameters like LDL immune complexes [LDL immune complexes (IgG), LDL immune complexes (IgM)], were also measured by chemiluminescence immunoassays for antibody binding, with IgG and IgM antibodies. Lp(a) parameters for LDL oxidation were determined using macro L(p)a enzyme-linked immunoabsorbent assay (ELISA) (Ali S. et al. 1998).

Data from all parameters quantifying LDL oxidation were expressed in relative light units (RLUs), measured over 100 msec.

4.4 QUANTIFICATION OF PON ACTIVITY

Serum Paraoxonase (PON) activity was measured in both cases and controls using paraoxon as the substrate as described elsewhere (Furlong et al. 1989). All the samples were measured in triplicates.

Briefly, 20 µl of serum was diluted in 180 µl of dilution buffer (9 mM Tris pH 8.0, 1 mM $CaCl_2$), then the assay buffer (2.0 M NaCl, 0.1M Tris HCl pH 8.5, 2.0 mM $CaCl_2$) containing paraoxon substrate (1.2 m*M* paraoxon) was added to the samples which are in a 96 well plate. Next the plate was transferred to the plate reader (Spectramax ™ M2), where it was mixed for 5 seconds at 37°C, and read at 405 nm (1 measurement every 15 seconds for 3 minutes). The output was noted in optical density per minute (OD/minute) and the PON1 activity (in units/liter) was calculated using the equation mOD/minute × 11,120. Each sample was run in triplicates for the PON activity measurement. The above mentioned procedure for serum PON activity measurement was carried out for Pittsburgh case-control cohort while for Chicago cohort, keeping everything same, the raw pathlength values were also measured for each sample. This time the optical density (OD)/ minute was divided by the pathlength for each sample and PON activity (in units/liter) was calculated by [(mOD/minute)/(pathlength)] X 6.1.

4.5 DETERMINATION OF *PON2* GENOTYPES

4.5.1 DNA isolation

Genomic DNA was isolated from buffy coat using QI Amp kit (Qiagen Chatsworth CA). For each SNP, the DNA regions of interest were then amplified by polymerase chain reaction (PCR).

4.5.2 DNA amplification by Polymerase Chain Reaction (PCR)

PCR was used to amplify the target DNA fragments containing the SNP prior to their discrimination by allelic discrimination assay. PCR primers were designed by Biotage software. Either the forward or the reverse primer was biotinylated. In short, 1 μg–4 μg of genomic or whole genome amplified DNA was amplified in a 25 μL or 50 μL PCR reaction mixture which comprised of 5 μL of 10 X PCR buffer, 1-4 mM $MgCL_2$, 1.25 mM each dNTP (Pharmacia), 0.2-0.4 uM of each primer, 0-3 mM dimethylsulfoxide (DMSO), 0.3 uL of *Taq* DNA polymerase (Invitrogen or Amplitaq). Each PCR reaction starts with an initial denaturation event at 95°C followed by denaturation and annealing at lower temperatures for multiple cycles, which is terminated by a final extension at 72°C. The annealing and cycling conditions were different for each fragment. Thermal cyclers mostly Hybaid and 9700 PCR amplification system were used to amplify the fragments whose correct size was checked by running 5 μL of the amplified PCR

product in a 2%-3% agarose gel. PCR conditions, sequences of PCR primers and length of the fragment amplified for each *PON2* variant are summarized in Table 5.

Table 5. PCR conditions, PCR primer sequences and amplicon lengths of *PON2* variants

SNP	Primer Orientation	PCR primer sequence	PCR conditions		Amplicon length (bp)
rs11982486	**Forward** **Reverse**	TCCTTTGACCACCCACAATTATC CCAAACCTCAGCATCAGACAATAT	95ºC--5 min 95ºC--30 sec 60ºC--30 sec 72ºC--30sec 72ºC---5min	*45 cycles*	185
rs2286233	**Forward** **Reverse**	TGAGGCTTACAGTCATTTTTCACG GTTGTGGGAAAAGAGTTCCAGAT	95ºC--5 min 95ºC--30 sec 60ºC--30 sec 72ºC--30sec 72ºC---5min	*45 cycles*	86
rs17876116	**Forward** **Reverse**	TTGACTGCTCCTGACATAATCACA GCCACTACTGCAGGAAGGTTTTA	95ºC--5 min 95ºC--30 sec 62ºC--10 sec 72ºC--30sec 72ºC--5min	*45 cycles*	69
rs11545941	**Forward** **Reverse**	AACCACCCAGAATTCAAGAATACA TGACTGTTTTCAGATGCAACAGAG	95ºC--5 min 95ºC--30 sec 59ºC--30 sec 72ºC--30sec 72ºC---5min	*45 cycles*	82
rs17876193	**Forward** **Reverse**	CCAACAGAAATAACCCCAAAGA TGTTTGCAAATGCACTGAAACTA	95ºC--5 min 95ºC--30 sec 58ºC--30 sec 72ºC--30sec 72ºC---5min	*45 cycles*	72
rs3735586	**Forward** **Reverse**	GGCAGGAAGGTTACCTCTAAATT CACCAGTGTATCCAGCTCAAGTA	95ºC--5 min 95ºC--30 sec 61ºC--30 sec 72ºC--30sec 72ºC---5min	*45 cycles*	100
rs9641164	**Forward** **Reverse**	ATGCATGTACGGTGGTCTTATATT AATGTTCTGGATGCGGAGA	95ºC--5 min 95ºC--30 sec 59ºC--15 sec 72ºC--30sec 72ºC---5min	*45 cycles*	68
rs13306702	**Forward** **Reverse**	GGCCATATTAATTTCTCTTGTGGA TGGGAATTTGAGTTGCAATATTT	95ºC--5 min 95ºC--30 sec 58ºC--30 sec 72ºC--30sec 72ºC---5min	*35 cycles*	100
rs10261470	**Forward** **Reverse**	GATATGTGGAGCCCCAAATG CACCACCTACCCCAACATTCT	95ºC--5 min 95ºC--30 sec 58ºC--10 sec 72ºC--30sec 72ºC---5min	*45 cycles*	51
rs6954345	**Forward** **Reverse**	AACAGGGCTTATTGATGATTGAGT ACAGACCCATTGTTGGCATAA	95ºC--5 min 95ºC--30 sec 59ºC--20 sec 72ºC--45sec 72ºC---5min	*45 cycles*	145

Next, the allelic status of the amplified DNA product was determined by various genotyping methods which are discussed in the following sections.

4.5.3 Genotyping methods

The allelic discrimination assays that were employed to genotype the 19 tagSNPs were restriction fragment length polymorphism (RFLP), Pyrosequencing ™ and TaqMan ®.

4.5.3.1 Restriction fragment length polymorphism (RFLP)

RFLP is one of the commonly used gel electrophoresis based genotyping methods employed for screening known polymorphisms. A restriction enzyme is highly specific in nature which cuts DNA within its precise recognition sequence known as the restriction site. Presence of a genetic variant can either abolish or create a restriction site. TagSNP *PON2*/rs9954345(Ser311Cys) was genotyped by RFLP using endonuclease *Dde*I, (Table 6) where the genotypes were evaluated by counting the number of different sized DNA bands visualized on a 3% NuSieve agarose gel.

The initial PCR amplified product was 145 bp. Digestion by *Dde*I gave the following restriction patterns: an intact 145 bp fragment corresponds to the minor allele in its homozygous form or the GG genotype, coexistence of fragments 100 bp and 45 bp correspond to the major CC genotype while the heterozygous CG genotype shows all three fragments sizes (145bp, 100bp and 45bp) for a particular individual. Figure 4 depicts the RFLP pattern of the fragment (145bp) containing the *PON2*/rs6954345 variant by *DdeI.*

Table 6. RFLP conditions for tagSNP *PON2*/rs6954345(Ser311Cys)

Restriction enzyme	Recognition sequenc	Digestion conditions
D*deI(NEB cutter)*	5'...CTNAG...3' 3'...GANTC...5'	37ºC for 16 hours

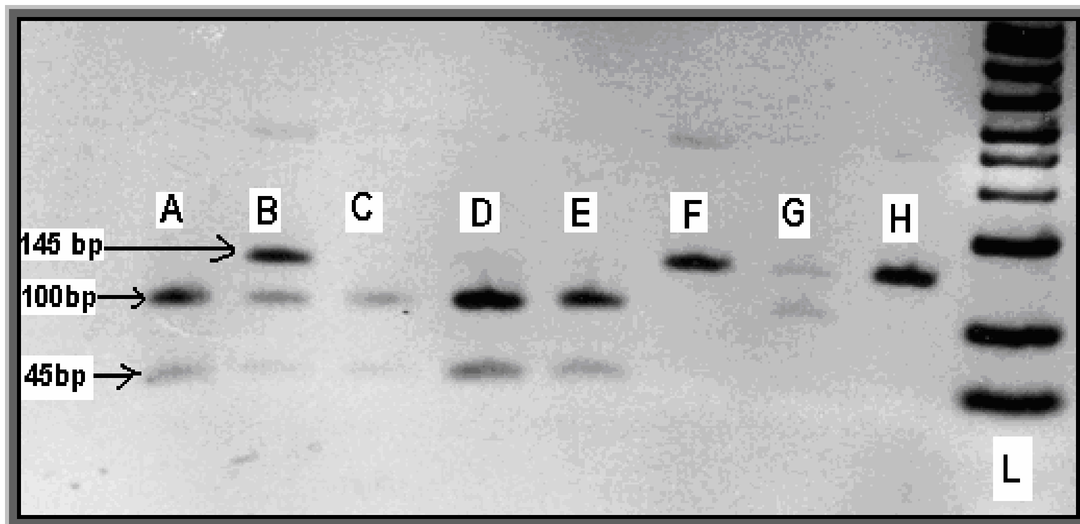


Figure 4. Restriction pattern of *PON2*/rs6954345(Ser311Cys) SNP by *DdeI*
Restriction patterns of the PCR-amplified 145-bp fragment after digestion with *Dde*I. Lanes A, C, D E—represent CC genotype. Lanes B, G represent CG genotype. Lane F, H --represent GG genotype. Lane L represents the ladder.

4.5.3.2 Pyrosequencing ™

Pyrosequencing ™ (PSQ) developed by Ronaghi et al. (1998) is based on real time pyrophosphate (PPi) detection, released during sequencing-by synthesis-reaction. PSQ technique is not only time and cost effective but also can determine bi- tri-, tetra allelic polymorphisms, multiple SNPs, deletions/insertions and mutations at the same time.

In short, 40µL of binding buffer (10mM Tris-HCl pH7.6, 2M NaCl, 1mM EDTA, 0.1% Tween20), 2 µL Streptavidin Sepharose beads (Amersham biosciences, Piscataway NJ) and 18 µL of high purity water were added to 10-15µL of the amplified biotinylated PCR product. This mix was then vortexed for 10 min, which allowed binding of the biotinylated PCR DNA to the streptavidin beads. DNA molecules once immobilized on the streptavidin beads were next captured onto filter probes using a vacuum prep tool, followed by a wash procedure in 70% ethanol for 10 sec, in a denaturation solution (0.2M NaOH) for 10 sec, and at last in a washing buffer (10mM Tris Acetate pH 7.6) for 20 sec. The theory behind this denaturation step is to separate the dsDNA into its single stranded template (ssDNA). In the next step, the vacuum was turned off in order to release the ssDNA from the filter probes of the vacuum prep tool to a PSQ 96 plate containing 9.5 µL of annealing buffer (20mM Tris-Acetate, 2mM $MgAc_2$ pH7.6) and 0.5µM of sequencing primer in each well. This 96 well plate is next placed on a heating block with lid, at 90° C for 2min, followed by another 2 min on the bench top with the heated lid and at last without the heated lid for 4 min. This entire heating process promotes annealing the ssDNA with the sequencing primer. Table 7 encompasses the sequences of the sequencing primers and the target sequence to analyze for each *PON2* variants genotyped by Pyrosequencing ™.

Table 7. Pyrosequencing primer sequences and the sequences analyzed for *PON2* variants screened by Pyrosequencing

SNP		Sequence
rs11982486	Sequence to Analyze S1*	TAC/TA/GTA AACTTTTTTT TTCTCTCTCT CTT AGATTACTACCACATTAGGC
rs2286233	Sequence to Analyze S1	GA/TAGCCCT GTCTTCCTTC TGAAGAGTC AGTTCCAGATGTAGAACCA
rs17876116	Sequence to Analyze S1	CCG/TAAGAG GGGATACACG ATGATAAAAC GCTCCTGACATAATCACA
rs11545941	Sequence to Analyze S1	AAGC/GAGAA AATTCTCTGT TGCATCTGAA A GGAAATTTTTAAATTTGAAG
rs17876193	Sequence to Analyze S 1	GCTC/TCC/G TTTTTTATCT TTCCTGATAG TTT AAATAACCCCAAAGATAAA
rs3735586	Sequence to Analyze S1	ATT/ATTGAC AATTTCT AAGTTTCTCATGTCATTTAG
rs9641164	Sequence to Analyze S1	A/TTGAATAT AAGACCACCG TACATGCA TTCAGGGGATACAAAGT
rs13306702	Sequence to Analyze S1	GG/CACTCTT AATT/GTGAA ACAACAACAA C GGAAAGCTGAAAGTGAAT
rs10261470	Sequence to Analyze S1	AC/TGTGAGA ATGTTGGGGT AGGTGGTGG GGAGCCCCAAATGGGCTG
rs6954345	Sequence to Analyze S1	GA/TAGCCCT GTCTTCCTTC TGAAGAGTC CGCATCCAGAACATTCTA

*S1--Sequencing primer

Pyrosequencing reactions were next performed following the manufacturers instructions using PSQ96 SNP Reagent kit, which contains the enzyme, substrate and the nucleotides. The enzyme, substrate contains DNA polymerase, ATP sulfurylase, luciferase, apyrase, adenosine 5´ phosphosulfate (APS) and luciferin. Analysis starts with the dispensation of enzyme, substrate into the plate. Essentially, DNA polymerase catalyses synthesis of a DNA strand complimentary to the original sequence, which in turn coupled to a chemiluminescent reaction, generating light proportional to the number of nucleotides incorporated and detected by a charge coupled device (CCD) camera built inside the PSQ system. PSQ software automatically analyzes the quantitative data, which is easily evaluated by the user. Figure 5, 6, and 7 show an outline view of Pyrosequencing ™ assay, the general principle behind different Pyrosequencing reaction systems and a diagrammatic representation of the Pyrosequencing cascade,respectively.

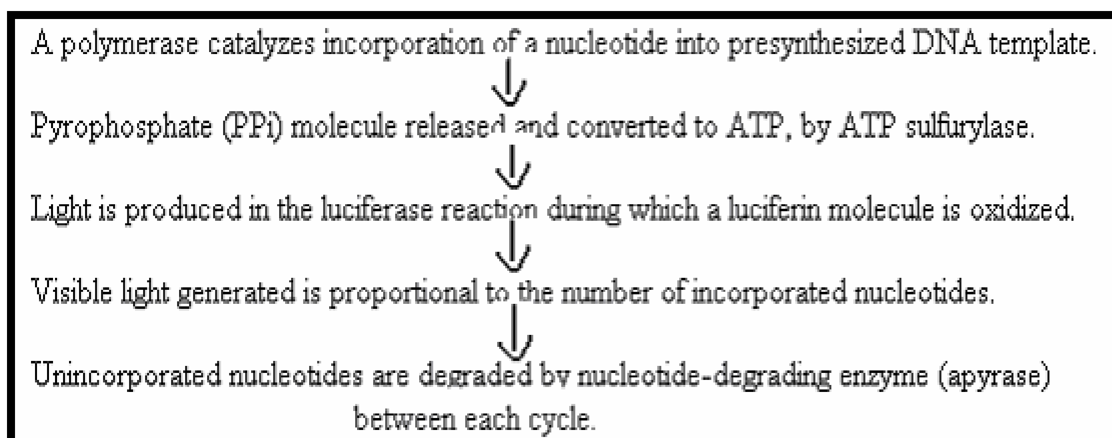


Figure 5. An outline view of Pyrosequencing assay

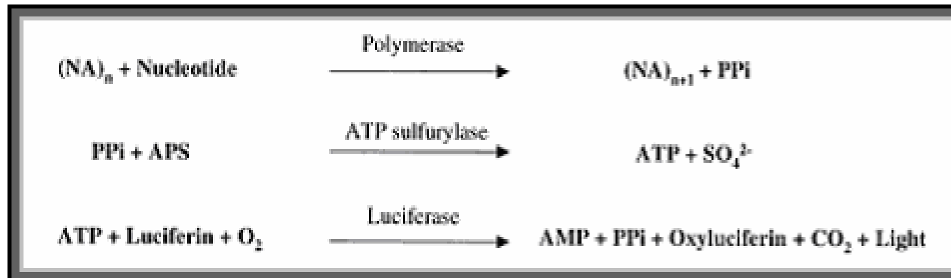


Figure 6. The general principal behind Pyrosequencing assay, adapted from Ronaghi et al. (2001).

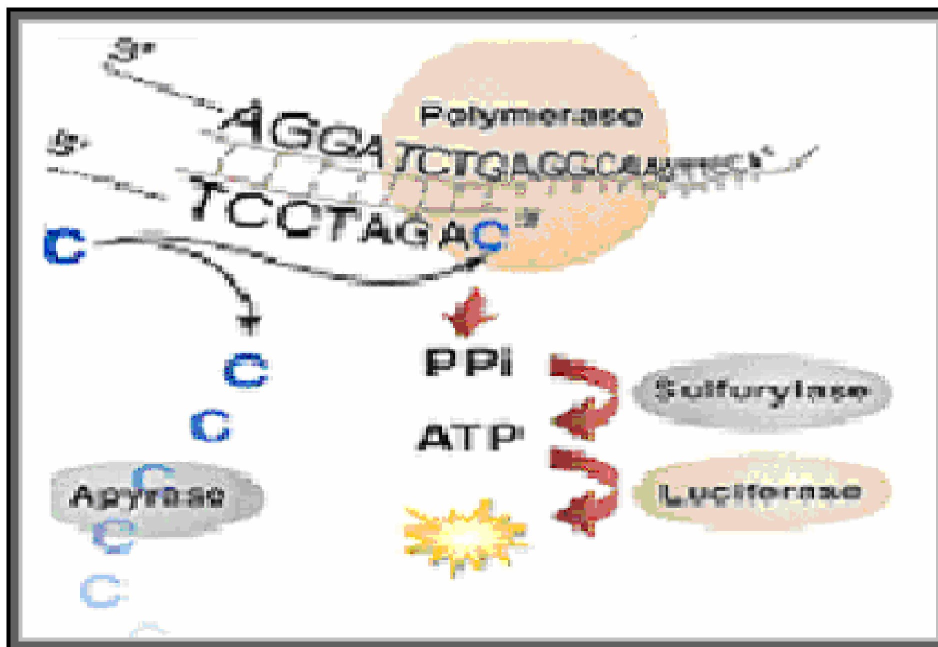


Figure 7. A diagrammatic representation of Pyrosequencing assay adapted from Medical Research Council, Available: at http://www.har.mrc.ac.uk/services/GEMS/mapping.html, accessed, December 2008.

In our study SNP pairs [rs3735586, rs1154594199Ala148Gly)] and (rs10261470, rs17876116) were genotyped simultaneously under the duplex Pyrosequencing assay. Examples

of pyrograms generated by the PSQ software while analyzing a simplex assay and a duplex assay are shown in Figure 8 and Figure 9, respectively.

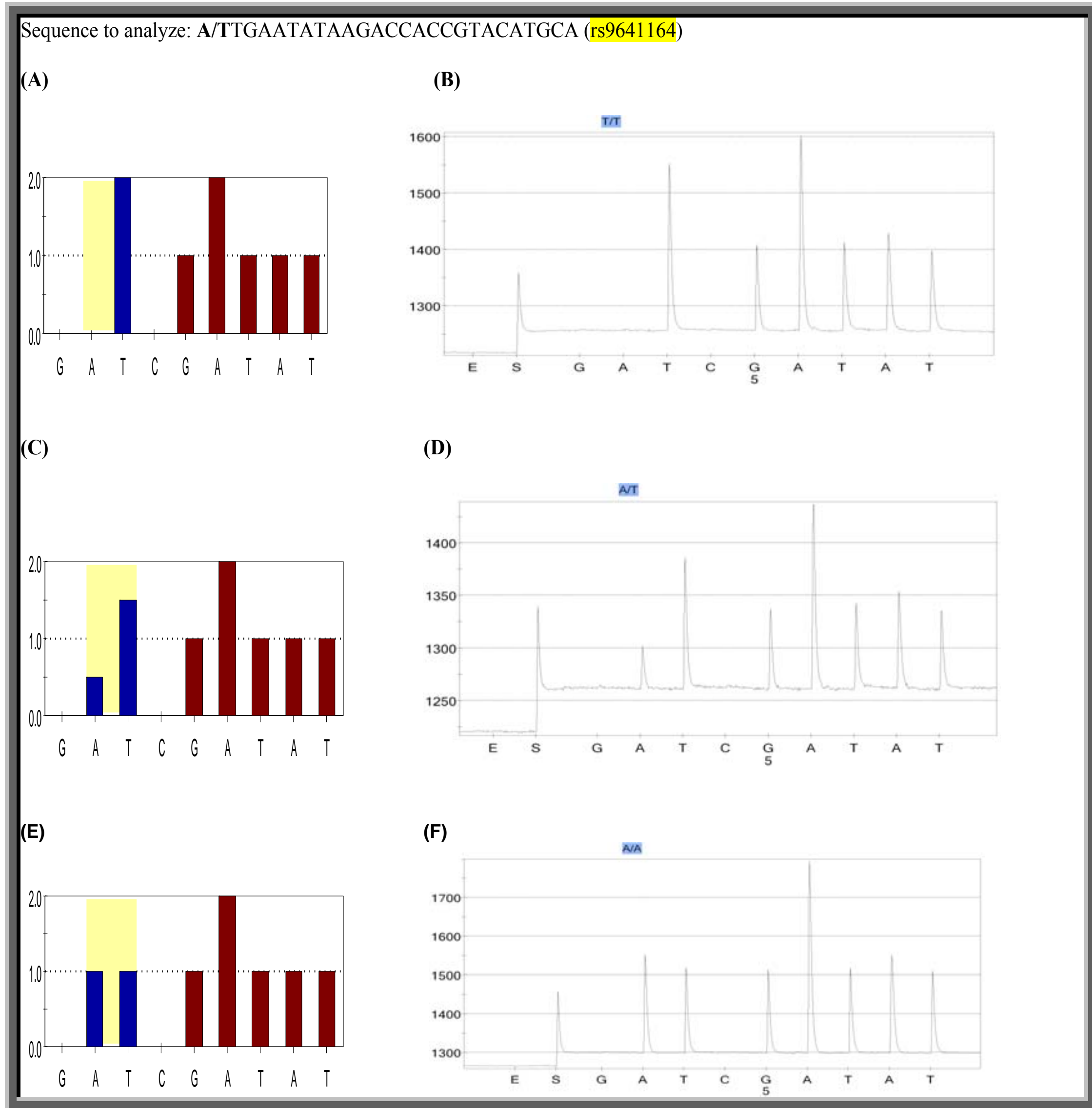


Figure 8. Pyrograms of simplex assay *PON2*/rs9641164
Part (A) -theoretical outcome of (T/T), Part (B) - experimental outcome of (T/T)
Part (C) -theoretical outcome of (A/T), Part (D) - experimental outcome of (A/T)
Part (E) -the theoretical outcome of (A/A), Part (F) - the experimental outcome of (A/A).

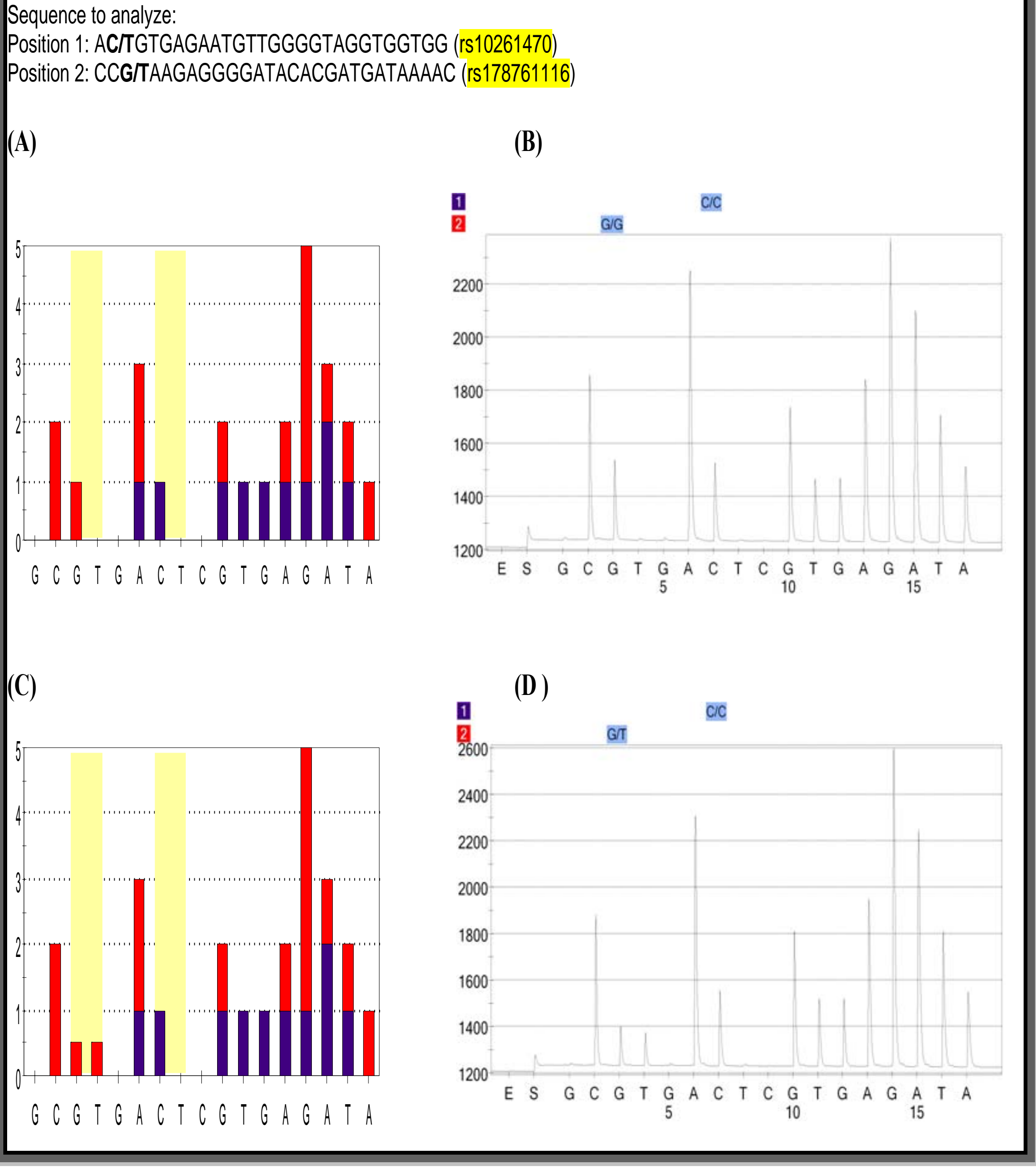


Figure 9. Pyrograms of duplex assay *PON2*/(rs10261470 and rs17876116)

Part (A) -theoretical outcome of (G/G //C/C), Part (B) - experimental outcome of (G/G// C/C).
Part (C) -theoretical outcome of (G/T //C/C), Part (D) - experimental outcome of (G/T //C/C).

4.5.3.3 TaqMan ®

TaqMan ® builds on a homogenous solution hybridization technology. This high throughput allelic discrimination assay is based on quantitative real time PCR reaction which utilizes the 5' nuclease activity of the *Taq* polymerase to detect a fluorescent reporter signal generated during the reaction (Figure 10). An outline view of TaqMan ® allelic discrimination assay is shown in Figure 11. Though the set up of the reaction is very similar to conventional PCR, in TaqMan PCR, the probe is also added at the beginning of PCR reaction, unlike other methods where the probe is added after the PCR reaction.

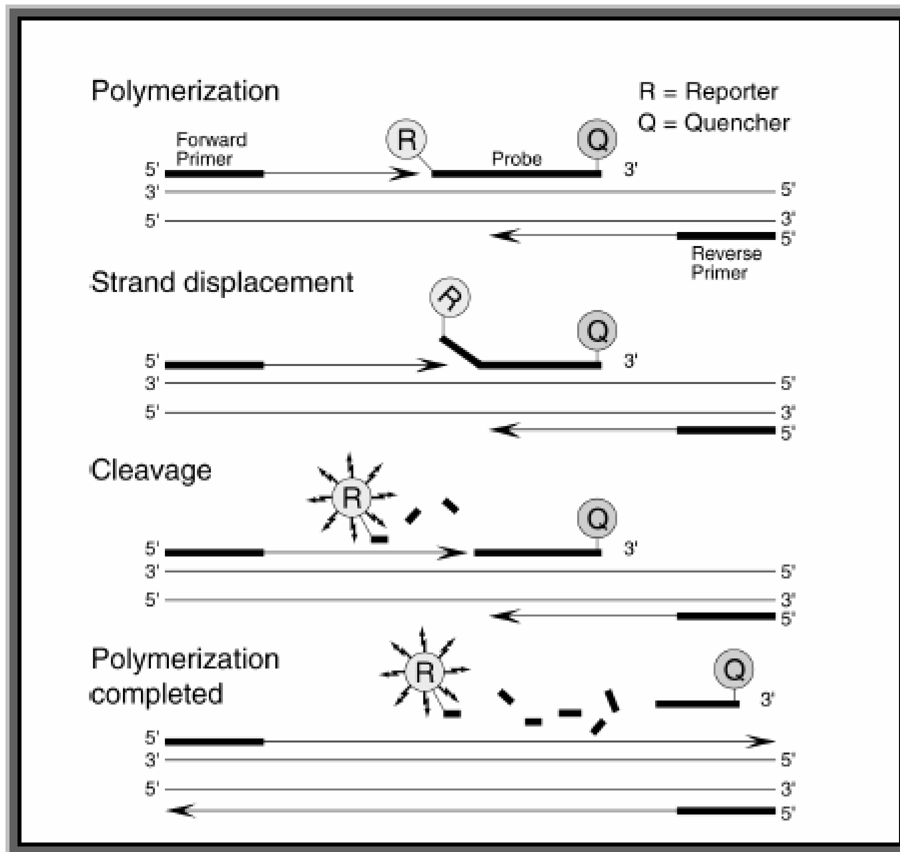


Figure 10. Diagrammatic representation of TaqMan assay

Graphic adapted from Applied Biosystems, available at

http://www.icmb.utexas.edu/core/DNA/Information_Sheets/Real-time%20PCR/7900taqAllelicDiscrim.pdf, accessed December 2008

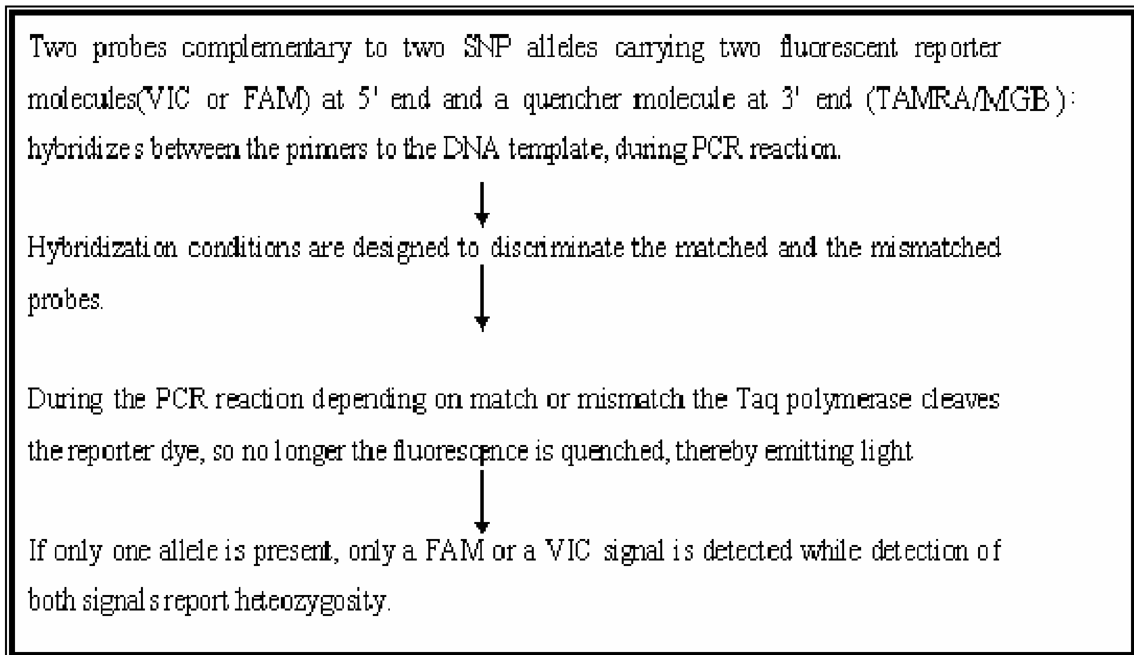


Figure 11. An outline view of TaqMan allelic discrimination assay

Eight *PON2* SNPs: rs2299267, rs12534274, rs987539, rs4729189, rs11981433, rs12704795, rs1639 and rs17876205 were screened by TaqMan allelic discrimination assay, using SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). The SNP genotyping assays used for each these SNPs were (C___2630173_1_ for rs2299267),(C__31373224_10 for rs12534274),(C___8952813_10 for rs987539),(C__27922117_10 for rs4729189), (C___2630169_10 for rs11981433), (C__26570646_10 for rs12704795), (C__11708890_10 for rs1639), and (C__59001801_10 for rs17876205).

Briefly, regular DNA or whole genome amplified DNA was aliquoted in 384 well plates. DNA was subjected to an initial heating process for 10 min under the following temperatures 94° C for 5min, 50° C for 5 min. Next, TaqMan reaction mix was prepared. For a plate which comprised of 384 samples, the TaqMan reaction mix had 998 µL of distilled water, 1026 µL of

TaqMan master mix and 26 μL of the respective TaqMan assay (40X concentration). 5 μL of this reaction mixture was added to each well in the plate, which was next subjected to PCR conditions: 95° C for 10 min, 95° C for 15 sec (30cycles),60° C for 1 min (50cycles). The PCR condition was the same for all SNPs. Next the plate was read with the help of a TaqMan machine. During the assay plate read, the data analysis software (SDS) employs an advanced algorithm to calculate the allele/marker signal contributions from each sample well. The genotype calls were determined by the SNP auto caller feature, built in within the analysis software that generates a cluster plot for each genotype thereby allowing the user to visualize data across samples, an example of which is shown in Figure 12.

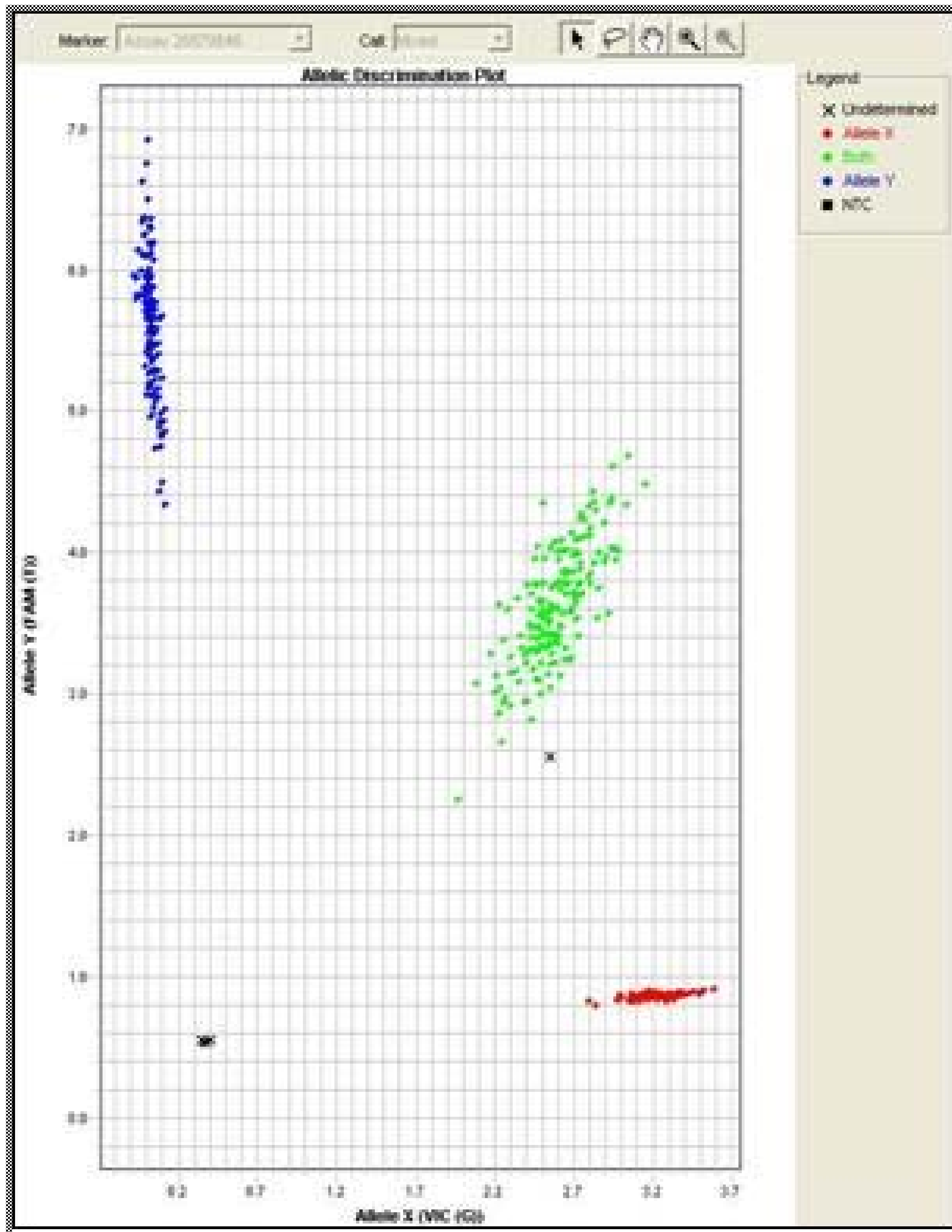


Figure 12. A display of the TaqMan cluster plot for SNP *PON2*/rs12704795
The blue cluster signifies the major homozygote (TT), the red cluster for minor homozygote (GG) and the green intermediate cluster signifies the heterozygote TG.

4.6 STATISTICAL ANALYSIS

Allele frequency for each tagSNP was calculated by allele counting. X^2 goodness of fit test was applied to determine any deviance of the observed genotype frequencies from the frequencies expected under Hardy-Weinberg Equilibrium (HWE) measures. For each tagSNP, allele and genotype frequencies were calculated by ethnicity (blacks and whites) and further within each ethnic group by SLE status, risk of lupus nephritis (within white SLE patients) and risk of carotid plaque (within white SLE patients). Z test for binomial proportions was used to evaluate the differences between allele frequencies, while Fisher's exact test between genotype frequencies. Covariates, like recruitment site (Pittsburgh and Chicago) and age were included in models when testing for association of each SNP with SLE risk and lupus nephritis. Covariates like body mass index (BMI), smoking, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, age and recruitment site were included in association models for carotid plaque and carotid IMT. Odds ratio adjusted for the appropriate covariates was calculated for different genetic models (codominant and recessive).

PON activity, carotid IMT and oxidized LDL-cholesterol (oxLDL) are continuous traits, therefore, they were presented by mean and standard deviation (SD) values. Raw measurements of PON activity, carotid IMT and parameters of LDL oxidation [anti-cu-modified oxLDL (IgG), anti-cu-modified oxLDL (IgM), oxLDL -EO6 (oxPL/Apo B), LDL immune complexes (IgG), LDL immune complexes (IgM), serum Lp(a), Lp(a)Mg, anti-MDA-modified LDL (IgG), anti-MDA-modified LDL (IgM)] were not normally distributed, so all statistical analyses were

performed on transformed data which pertains to their normal distribution. Carotid IMT values were transformed by a factor of 1- (1/ raw IMT data)], PON activity by square root transformation and oxidized LDL parameters by square root and log transformations. Mean ± SD measurements and association values of PON activity were adjusted for age, BMI and smoking. Similarly, for carotid IMT, age, BMI, smoking, cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and recruitment site were used as covariates. For oxidized LDL parameters, which were analyzed only in Pittsburgh recruitment cohort, the covariates used were age, BMI, smoking, cholesterol, triglycerides, HDL- cholesterol and high blood pressure.

Haploview (www.hapmap.org) program was used to compute pairwise LD pattern between *PON2* tagSNPs and also between the *PON2* SNPs and the reported major contributors of PON activity (*PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115) variants, values presented in LD parameters of D' and r^2. Taking into account the LD statistics, *PON2* haplotypes were evaluated for SLE risk. To determine the independent contribution of *PON2* variants towards PON activity, these variants were further analyzed along with *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 SNPs, adjusting for disease status, age, BMI and smoking in multiple regression models.

5.0 RESULTS

5.1 AGE DISTRIBUTION

Within each ethnic group (blacks or whites), the cases were slightly younger than controls as summarized in Table 8 (whites) and Table 9 (blacks).

Table 8. Mean ±SD age years in whites

	cases			[n*]	controls			[n]	
All whites	43.56	±	11.351	[416]	45.676	±	12.859	[471]	0.01
Pittsburgh white	43.416	±	11.395	[348]	45.28	±	13.144	[414]	0.039
Chicago white	44.253	±	11.258	[68]	48.548	±	10.211	[57]	0.029

n*--- number of people with age information

Table 9. Mean ±SD age years in blacks

	cases			[n*]	controls			[n]	
All blacks	41.828	±	10.824	[73]	48.753	±	14.133	[54]	0.002
Pittsburgh black	41.688	±	12.113	[48]	49.395	±	16.365	[38]	0.014
Chicago black	42.098	±	8.001	[25]	47.231	±	6.454	[16]	0.037

n*--- number of people with age information

5.2 HARDY WEINBERG EQUILIBRIUM (HWE)

The statistical results for Hardy-Weinberg equilibrium are shown in Table 10 and Table 11 in whites and blacks, respectively. The only polymorphism that was out of HWE was *PON2*/rs12534274 in Pittsburgh white SLE cases (*P*=0.02). All other 18 *PON2* polymorphisms maintained HWE in all groups.

Table 10. *P* value from deviation of the Hardy-Weinberg proportions in whites

PON2 tag SNP	Whites			
	Pittsburgh SLE	Chicago SLE	Pittsburgh controls	Chicago controls
rs17876183	0.779	0.984	0.953	0.996
rs2299267	0.850	0.972	0.632	0.906
rs10261470	0.920	0.854	0.717	0.538
rs4729189	0.171	0.765	0.658	0.780
rs12534274	**0.020**	0.925	0.090	0.135
rs11982486	0.157	0.304	0.065	0.521
rs11981433	0.117	0.688	0.465	0.151
rs2286233	0.423	0.430	0.398	0.795
rs12704795	0.078	0.248	0.477	0.198
rs17876193	0.886	0.733	0.051	0.944
rs17876116	0.855	0.854	0.954	0.971
rs1639	0.223	0.452	0.821	0.914
rs11545941	0.054	0.750	0.091	0.323
rs987539	0.045	0.989	0.484	0.159
rs3735586	0.046	0.331	0.119	0.115
rs9641164	0.200	0.248	0.783	0.807
rs6954345	0.046	0.939	0.338	0.599
rs13306702	0.834	0.965	0.648	0.941
rs17876205	0.975	0.997	0.974	0.990

Table 11. *P* value from deviation of the Hardy-Weinberg proportions in blacks*

PON2 tagSNP	Blacks			
	Pittsburgh SLE	Chicago SLE	Pittsburgh controls	Chicago controls
rs17876183	NA*	NA	0.995	0.987
rs2299267	0.734	0.463	0.998	0.658
rs10261470	0.932	0.863	0.687	0.658
rs4729189	0.702	0.730	0.633	0.695
rs12534274	0.793	1.000	0.890	0.614
rs11982486	0.648	0.701	0.793	0.894
rs11981433	0.744	0.701	0.938	0.729
rs2286233	0.845	0.730	0.988	0.815
rs12704795	0.744	0.701	0.938	0.894
rs17876193	0.949	0.992	0.986	NA
rs17876116	0.996	NA	NA	NA
rs1639	0.912	0.721	0.683	0.986
rs11545941	0.972	1.000	0.905	0.370
rs987539	0.374	0.096	0.599	0.611
rs3735586	0.453	0.860	0.971	0.778
rs9641164	0.943	0.491	0.974	NA
rs6954345	0.924	0.276	0.971	0.527
rs13306702	0.988	0.929	0.941	0.962
rs17876205	NA	NA	0.995	NA

*NA—*P* value could not be determined because the SNP was monomorphic

5.3 LINKAGE DISEQUILIBRIUM (LD) STRUCTURE

Figures 13, 14 and 15 show the LD pattern analyzed for Pittsburgh whites (cases+controls), Chicago whites (cases+controls) and the combined (Pittsburgh+Chicago) whites (cases+controls), respectively. The LD pattern between *PON2* variants in Pittsburgh whites (cases+controls) was similar to that of Chicago whites (cases+controls). Therefore, in combined (Pittsburgh+Chicago) whites pairwise tagging of *PON2* variants ($r^2 \geq 0.8$) identified four SNP pairs in high LD [rs3735586 & rs6954345(Ser311Cys), r^2= 0.922], [rs11545941(Ala148Gly) & rs6954345(Ser311Cys), r^2=0.819), [rs1639 & rs9641164, r^2=0.871] and [rs11981433 & rs12704795, r^2=0.989] (Table 12) .

Since the number of African American sample was considerably small, we analyzed LD only for the combined (Pittsburgh+Chicago) blacks (cases+controls) (Figure 16). *PON2*/SNP rs17876205 was monomorphic, therefore pairwise LD was computed for 18 of 19 *PON2* polymorphisms that were included in our study. Although in blacks, tagger analyses ($r^2 \geq 0.8$) identified only one highly correlated SNP pair (*PON2*/rs11981433 and rs12704795, r^2 =0.98), there were some similarities in the overall LD pattern to that of whites.

Table 12. Tagger analyses ($r^2 \geq 0.8$) of the selected 19 *PON2* variants in whites

Pittsburgh (Whites)(n*=798)		Chicago (Whites)(n=124)		Overall (Whites)(n=922)	
Test	Alleles Captured	Test	Alleles Captured	Test	Alleles Captured
rs6954345 (Ser311Cys)	rs3735586,rs11545941(Ala148Gly)	rs3735586 rs11545941(Ala148Gly)	rs6954345 (Ser311Cys)	rs695434(Ser311Cys)	rs3735586, rs11545941(Ala148Gly)
rs12704795	rs11981433	rs11981433	rs12704795	rs11981433	rs12704795
rs9641164	rs1639	rs9641164 rs1639		rs9641164	rs1639
rs2286233		rs2286233		rs2286233	
rs12534274		rs12534274		rs12534274	
rs987539		rs987539		rs987539	
rs17876183		rs17876183		rs17876183	
rs2299267		rs2299267		rs2299267	
rs17876193		rs17876193		rs17876193	
rs11982486		rs11982486		rs11982486	
rs17876116		rs17876116		rs17876116	
rs10261470		rs10261470		rs10261470	
rs17876205		rs17876205		rs17876205	
rs13306702		rs13306702		rs13306702	
rs4729189		rs4729189		rs4729189	

*n---number of individuals included in tagger analyses

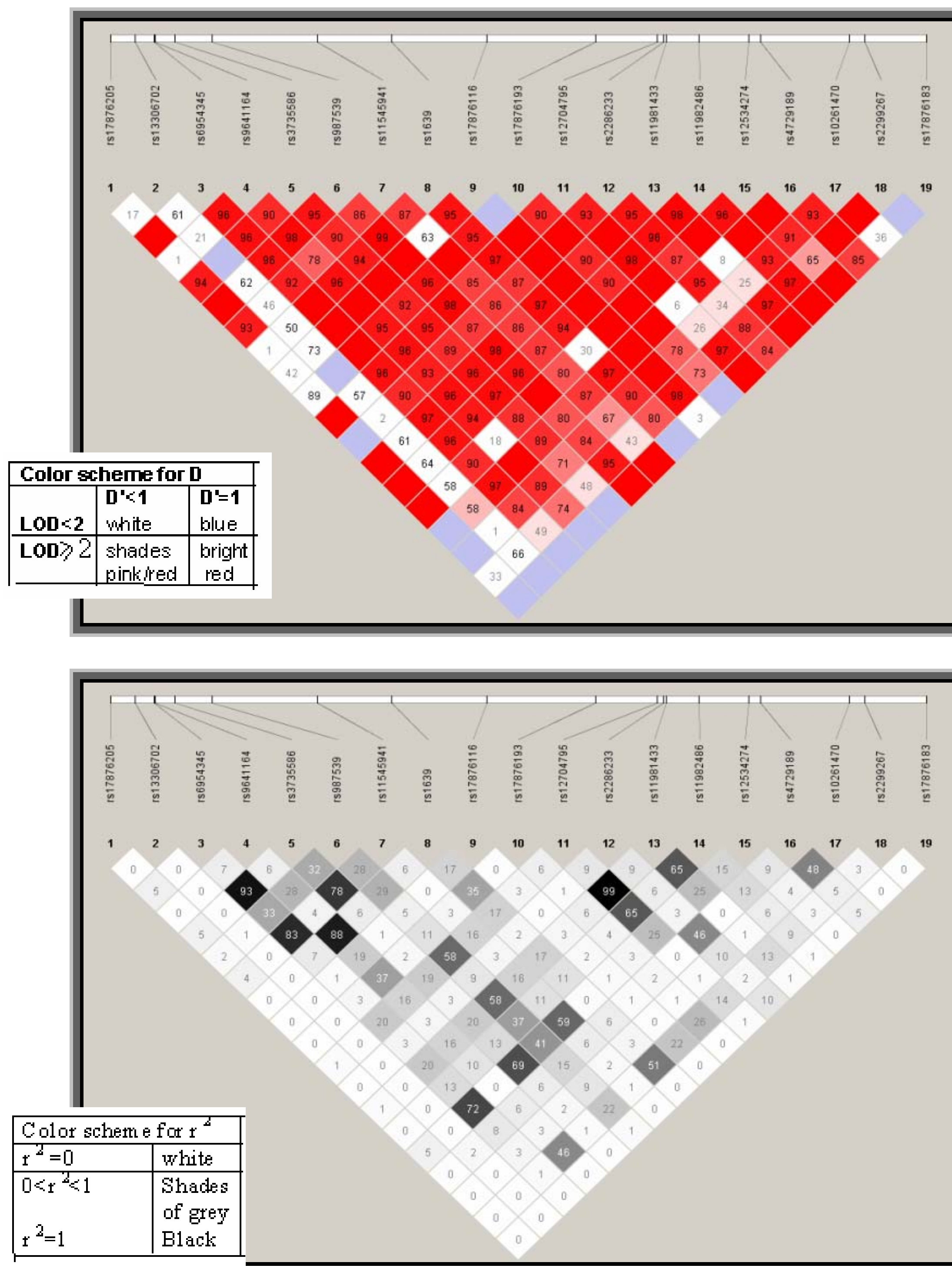


Figure 13. LD plot of 19 *PON2* SNPs in Pittsburgh whites

Total: 798 individuals (after 4 individuals missing >50% genotype are excluded)

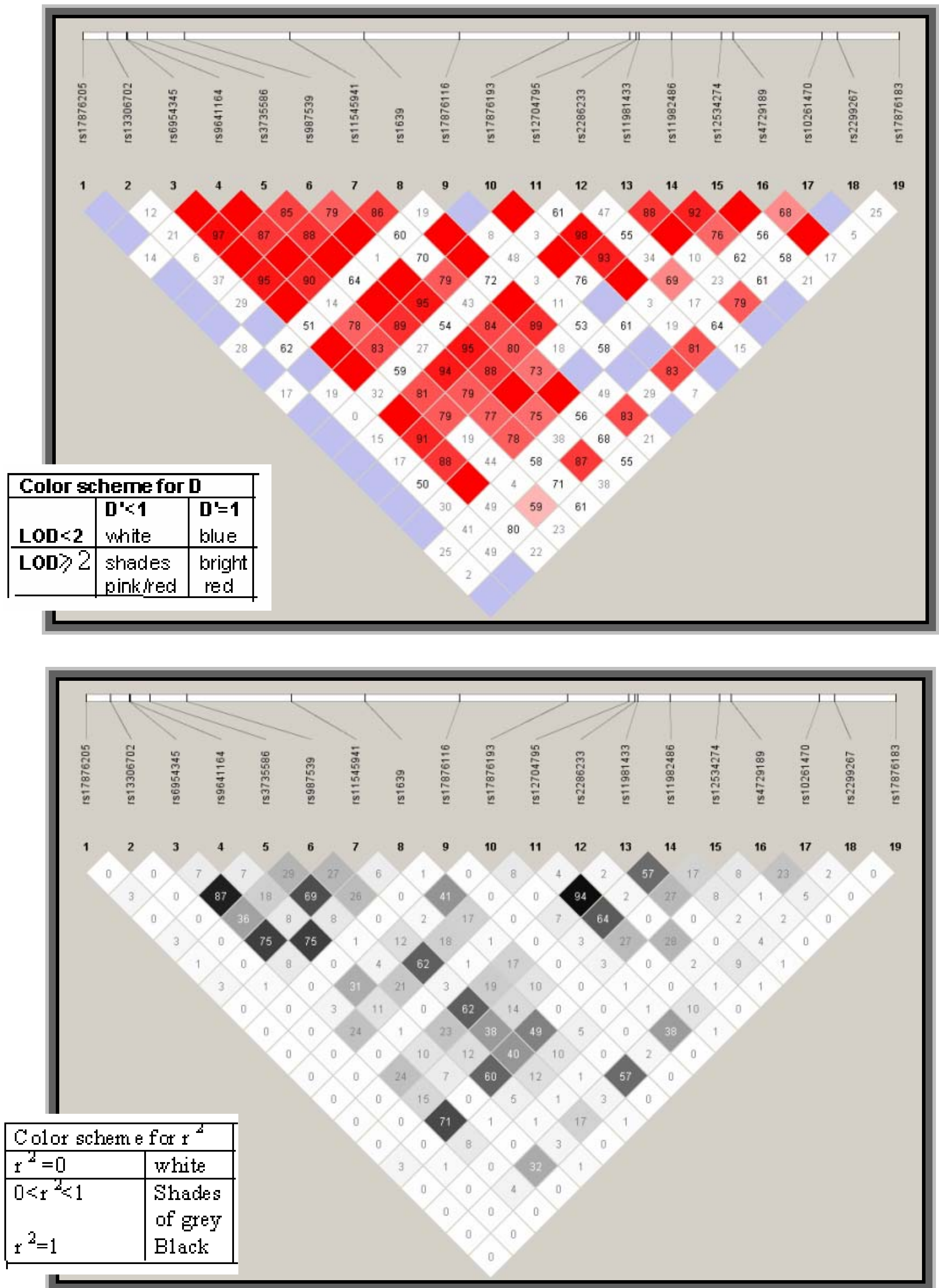


Figure 14. LD plot of 19 *PON2* SNPs in Chicago whites.

Total: 124 individuals (after 1 individual missing >50% genotype are excluded)

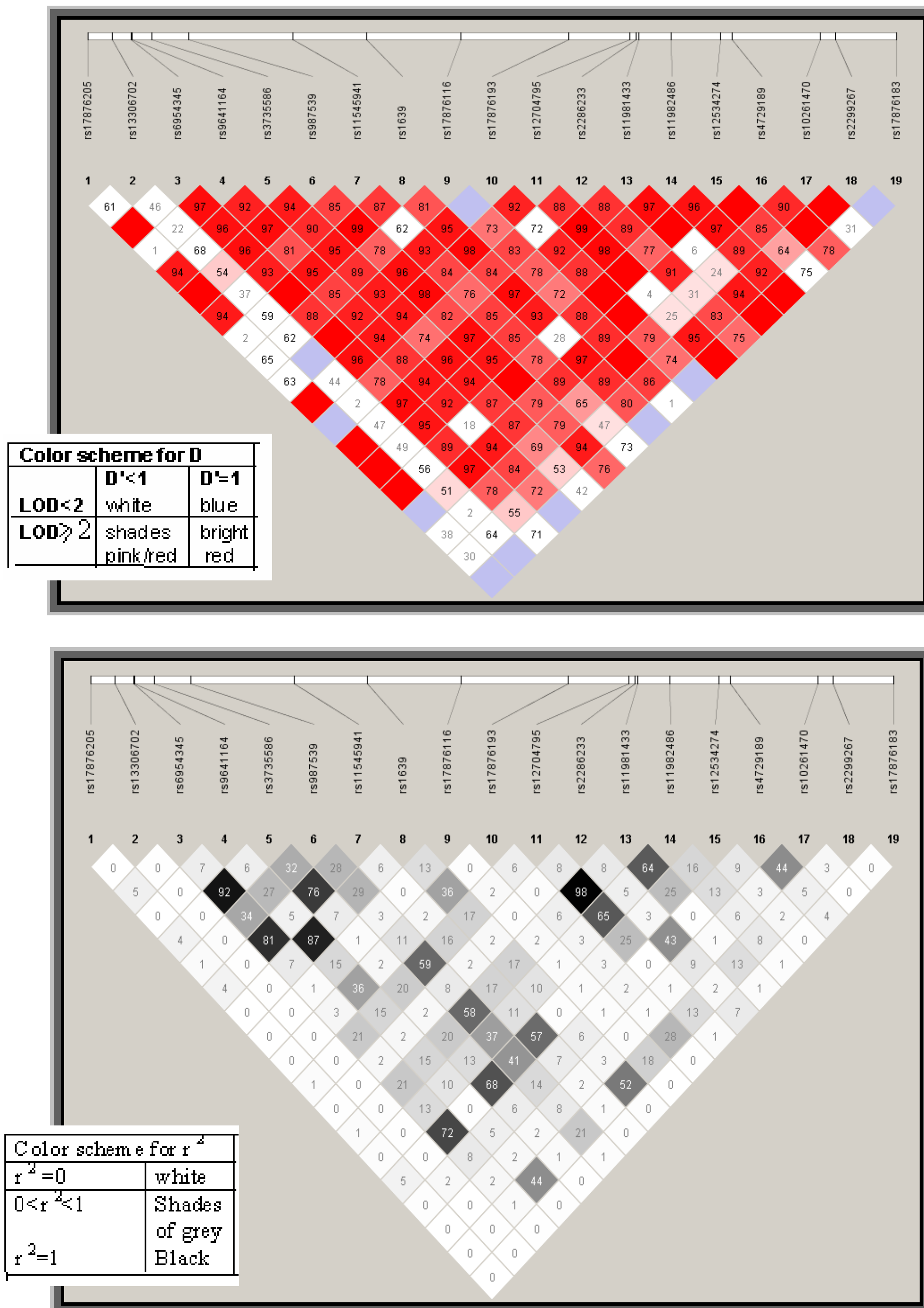


Figure 15. LD plot of 19 *PON2* SNPs in combined (Pittsburgh+Chicago) whites

Total: 922 individuals (after 5 individuals missing >50% genotype are excluded)

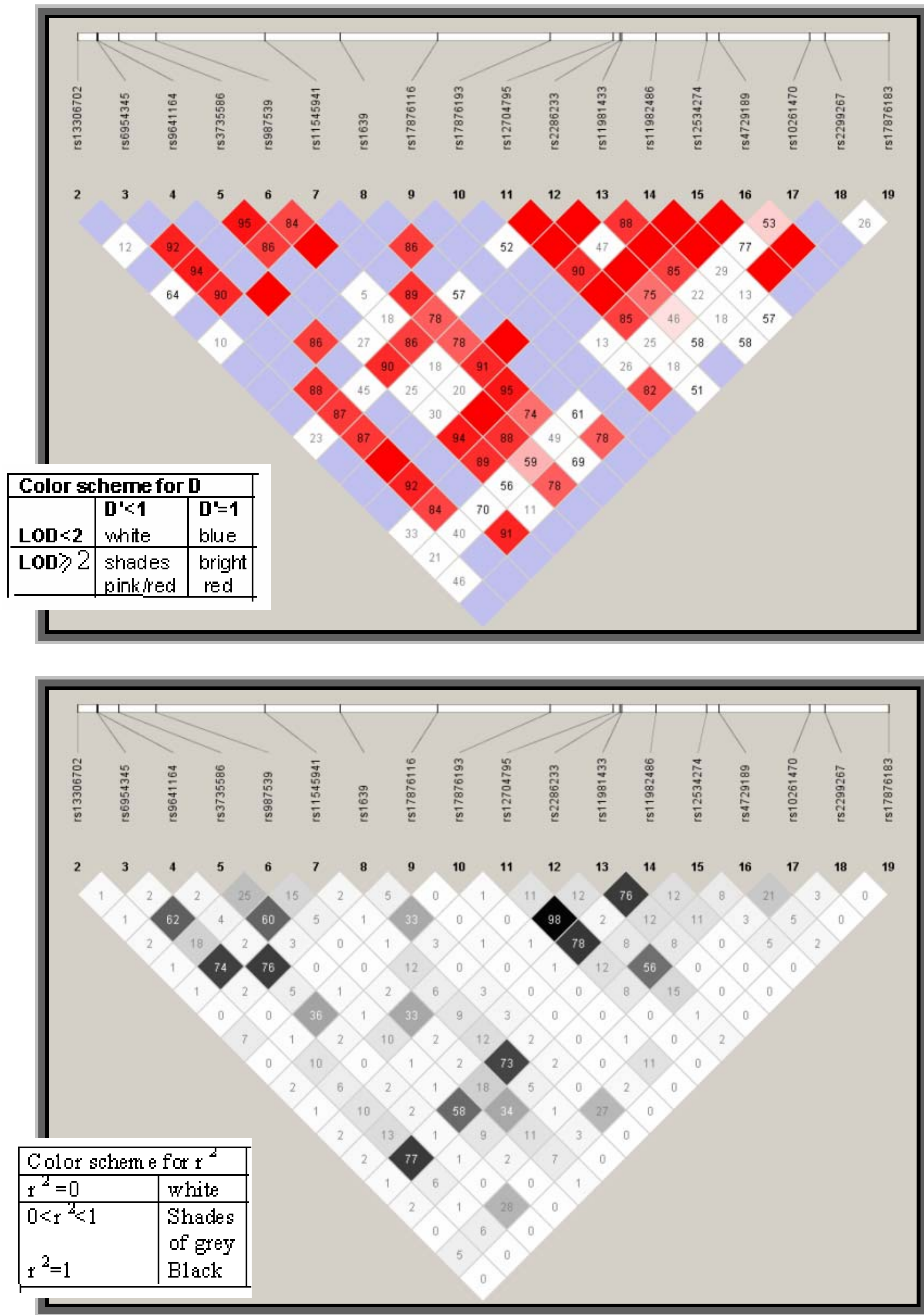


Figure 16. LD plot of 19 *PON2* in combined (Pittsburgh+Chicago) blacks

Total: 129 individuals (after 2 individual missing >50% genotype are excluded)

5.4 *PON2* SNP DISTRIBUTION BETWEEN WHITES AND BLACKS

Table 13 shows the comparison of SNP distribution between whites and blacks. Nine *PON2* polymorphic sites (rs11981433, rs2286233, rs12704795, rs17876193, rs17876116, rs1639, rs987539, rs9641164, and rs17876205) showed significant difference ($P \leq 0.01$) for both allele frequency difference and genotype distribution (adjusted for recruitment site) between these two ethnic groups. For *PON2*/rs3735586 variant only the allele frequency difference was significant with a p value of 0.0181.

Table 13. Comparison of Genotype and Allele frequencies of *PON2* tagSNPs between blacks and whites

			Genotype (%)						Alleles	
rs17876183		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(5'UTR)	**Whites**	**919**	883	(96.08)	35	(3.81)	1	(0.11)	0.980	0.020
	Blacks	129	127	(98.45)	2	(1.55)	0	(0.00)	0.992	0.008
					*P** 0.3400				*P*	0.0520
rs2299267		**Total**	**AA**		**AG**		**GG**		**A**	**G**
(intron 1)	**Whites**	**919**	636	(69.21)	254	(27.64)	29	(3.16)	0.830	0.16975
	Blacks	**130**	93	(71.54)	32	(24.62)	5	(3.85)	0.83846	0.162
					*P** 0.8300				*P*	0.7370
rs10261470		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**Whites**	**917**	694	(75.68)	203	(22.14)	20	(2.18)	0.868	0.132
	Blacks	**129**	89	(68.99)	37	(28.68)	3	(2.33)	0.833	0.167
					*P** 0.1300				*P*	0.1634
rs4729189		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**Whites**	**914**	566	(61.93)	299	(32.71)	49	(5.36)	0.78282	0.217177
	Blacks	**127**	77	(60.63)	46	(36.22)	4	(3.15)	0.787	0.212598
					*P** 0.5400				*P*	0.8674
rs12534274		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**Whites**	**909**	518	(56.99)	314	(34.54)	77	(8.47)	0.743	0.257
	Blacks	**129**	77	(59.69)	46	(35.66)	6	(4.65)	0.775	0.225
					*P** 0.3123				*P*	0.2430
rs11982486		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**Whites**	**919**	426	(46.35)	379	(41.24)	114	(12.40)	0.670	0.330
	Blacks	**130**	65	(50.00)	52	(40.00)	13	(10.00)	0.700	0.300
					*P** 0.5700				*P*	0.3207
rs11981433		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**Whites**	**914**	323	(35.34)	419	(45.84)	172	(18.82)	0.583	0.417
	Blacks	**127**	60	(47.24)	58	(45.67)	9	(7.09)	0.701	0.299
					*P** 0.0007				*P*	0.0001
rs2286233		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**Whites**	**921**	699	(75.90)	203	(22.04)	19	(2.06)	0.869	0.131
	Blacks	**128**	77	(60.16)	45	(35.16)	6	(4.69)	0.777	0.223
					*P** 0.0008				*P*	0.0007
rs12704795		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 1)	**Whites**	**912**	318	(34.87)	421	(46.16)	173	(18.97)	0.5795	0.421
	Blacks	**128**	62	(48.44)	57	(44.53)	9	(7.03)	0.707	0.293
					*P**<0.0001				*P* <	0.0001

P value for Allele frequency difference, unadjusted

**P* value for genotype distribution, adjusted for recruitment site

Table 13 (cont'd).

			Genotype (%)						Alleles	
rs17876193		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(intron 2)	**Whites**	**919**	761	(82.81)	147	(16.00)	11	(1.20)	0.908	0.969
	Blacks	**128**	120	(93.75)	8	(6.25)	0	(0.00)	0.969	0.031
					*P** 0.0013				*P*	< 0.0001
rs17876116		**Total**	**GG**		**GT**		**TT**		**G**	**T**
(intron 2)	**Whites**	**921**	835	(90.66)	84	(9.12)	2	(0.22)	0.952	0.048
	Blacks	**129**	128	(99.22)	1	(0.78)	0	(0.00)	0.996	0.004
					*P**<0.0001				*P*	< 0.0001
rs1639		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 3)	**Whites**	**903**	583	(64.56)	277	(30.68)	43	(4.76)	0.799	0.201
	Blacks	**120**	104	(86.67)	14	(11.67)	2	(1.67)	0.925	0.075
					*P**<0.0001				*P*	< 0.0001
rs11545941		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon5)	**Whites**	**919**	538	(58.54)	310	(33.73)	71	(7.73)	0.754	0.246
Ala148Gly	**Blacks**	**130**	72	(55.38)	47	(36.15)	11	(8.46)	0.735	0.265
					*P** 0.8700				*P*	0.5045
rs987539		**Total**	**CC**		**CT**		**TT**		**C**	**T**
(intron 6)	**Whites**	**906**	282	(31.13)	418	(46.14)	206	(22.74)	0.542	0.458
	Blacks	**128**	22	(17.19)	54	(42.19)	52	(40.63)	0.383	0.617
					*P**<0.0001				*P*	< 0.0001
rs3735586		**Total**	**TT**		**TA**		**AA**		**T**	**A**
(intron 7)	**Whites**	**919**	553	(60.17)	298	(32.43)	68	(7.40)	0.764	0.236
	Blacks	**130**	65	(50.00)	50	(38.46)	15	(11.54)	0.692	0.308
					*P** 0.1100				*P*	0.0181
rs9641164		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 8)	**SLE**	**921**	604	(65.58)	276	(29.97)	41	(4.45)	0.806	0.194
	Controls	**130**	116	(89.23)	12	(9.23)	2	(1.54)	0.938	0.062
					*P**<0.0001				*P*	< 0.0001
rs6954345		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon 9)	**Whites**	**916**	545	(59.50)	308	(33.62)	63	(6.88)	0.763	0.237
Ser311Cys	**Blacks**	**129**	75	(58.14)	45	(34.88)	9	(6.98)	0.756	0.244
					*P** 0.9500				*P*	0.7984
rs13306702		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**Whites**	**917**	876	(95.53)	38	(4.14)	3	(0.33)	0.976	0.024
	Blacks	**129**	116	(89.92)	13	(10.08)	0	(0.00)	0.950	0.050
					*P** 0.0900				*P*	0.0608
rs17876205		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**Whites**	**916**	886	(96.72)	30	(3.28)	0	(0.00)	0.984	0.016
	Blacks	**128**	128	(100.00)	0	(0.00)	0	(0.00)	1.000	0.000
					*P** 0.0100				*P*	< 0.0001

P value for Allele frequency difference, unadjusted

**P* value for genotype distribution, adjusted for recruitment site

5.5 RELATIONSHIP OF *PON2* SNPS WITH SLE RISK

5.5.1 Univariate analysis of *PON2* SNPs with SLE risk

A summary of the allele frequencies and the genotype distribution stratified by case-control status is shown in Table 14 for whites (416 cases and 515 controls) and Table 15 for blacks (73 cases and 58 controls). In univariate analysis, none of the *PON2* variants showed any obvious association with SLE disease risk, neither in whites nor in blacks. Whites analyzed by combining the Pittsburgh with the Chicago samples identified significant allele frequency difference (*P*= 0.0350) for *PON2*/rs17876116G>T variant (the 17876116T allele frequency was 6%/3.8% in white cases/white controls). Similarly, in blacks the difference in allele frequency of rs9641164A>T variant was significant (*P*= 0.0217) (the rs9641164T allele frequency was 9%/2.6% in black cases/black controls,). However, none of the above variants showed association with disease risk in their genotype distribution.

Table 14. Univariate analysis of *PON2* tagSNPs with SLE risk in whites

			Genotype (%)						Alleles	
rs17876183		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(5'UTR)	**SLE**	**410**	397	(96.83)	12	(2.93)	1	(0.24)	0.983	0.017
	Controls	**509**	486	(95.48)	23	(4.52)	0	(0.00)	0.977	0.023
					*P** 0.3553				*P*	0.3950
rs2299267		**Total**	**AA**		**AG**		**GG**		**A**	**G**
(intron 1)	**SLE**	**410**	275	(67.07)	121	(29.51)	14	(3.41)	0.818	0.182
	Controls	**509**	361	(70.92)	133	(26.13)	15	(2.95)	0.84	0.160
					*P** 0.4970				*P*	0.2230
rs10261470		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**SLE**	**409**	316	(77.26)	86	(21.03)	7	(1.71)	0.878	0.122
	Controls	**508**	378	(74.41)	117	(23.03)	13	(2.56)	0.859	0.141
					*P** 0.4370				*P*	0.2420
rs4729189		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**SLE**	**407**	261	(64.13)	126	(30.96)	20	(4.91)	0.796	0.204
	Controls	**507**	305	(60.16)	173	(34.12)	29	(5.72)	0.772	0.228
					*P** 0.7580				*P*	0.2160
rs12534274		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**SLE**	**406**	231	(56.90)	140	(34.48)	35	(8.62)	0.741	0.259
	Controls	**503**	287	(57.06)	174	(34.59)	42	(8.35)	0.744	0.256
					*P** 0.9300				*P*	0.9166
rs11982486		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**SLE**	**410**	189	(46.10)	173	(42.20)	48	(11.71)	0.672	0.328
	Controls	**510**	237	(46.47)	207	(40.59)	66	(12.94)	0.662	0.332
					*P** 0.8410				*P*	0.8570
rs11981433		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**SLE**	**407**	149	(36.61)	184	(45.21)	74	(18.18)	0.592	0.408
	Controls	**507**	174	(34.32)	235	(46.35)	98	(19.33)	0.575	0.425
					*P** 0.7630				*P*	0.4590
rs2286233		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**SLE**	**412**	319	(77.43)	82	(19.90)	11	(2.67)	0.874	0.126
	Controls	**509**	380	(74.66)	121	(23.77)	8	(1.57)	0.865	0.135
					*P** 0.3570				*P*	0.5955
rs12704795		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 1)	**SLE**	**405**	146	(36.05)	185	(45.68)	74	(18.27)	0.589	0.411
	Controls	**507**	172	(33.93)	236	(46.55)	99	(19.53)	0.572	0.428
					*P** 0.7650				*P*	0.4670

P value for Allele frequency difference, unadjusted
**P* value for genotype distribution, adjusted for recruitment site and age

Table 14 (cont'd).

			Genotype (%)						Alleles	
rs17876193		Total	CC		CG		GG		C	G
(intron 2)	SLE	408	332	(81.37)	74	(18.05)	4	(0.98)	0.902	0.1
	Controls	509	429	(84.28)	73	(14.34)	7	(1.38)	0.915	0.085
					*P** 0.4880				*P*	0.9323
rs17876116		Total	GG		GT		TT		G	T
(intron 2)	SLE	410	362	(88.29)	47	(11.46)	1	(0.24)	0.94	0.06
	Controls	511	473	(92.56)	37	(7.24)	1	(0.20)	0.962	0.038
					*P** 0.1400				*P*	0.0350
rs1639		Total	TT		TG		GG		T	G
(intron 3)	SLE	406	254	(62.56)	128	(31.53)	24	(5.91)	0.783	0.217
	Controls	497	329	(66.20)	149	(29.98)	19	(3.82)	0.812	0.188
					*P** 0.2460				*P*	0.1330
rs11545941		Total	CC		CG		GG		C	G
(exon5)	SLE	409	234	(57.21)	141	(34.47)	34	(8.31)	0.744	0.256
Ala148Gly	Controls	510	304	(59.61)	169	(33.14)	37	(7.25)	0.762	0.238
					*P** 0.9320				*P*	0.3940
rs987539		Total	TT		TC		CC		T	C
(intron 6)	SLE	404	102	(25.25)	183	(45.30)	119	(29.46)	0.479	0.521
	Controls	502	104	(20.72)	235	(46.81)	163	(32.47)	0.441	0.559
					*P** 0.8410				*P*	0.1090
rs3735586		Total	TT		TA		AA		T	A
(intron 7)	SLE	509	303	(59.53)	168	(33.01)	38	(7.47)	0.76	0.24
	Controls	410	250	(60.98)	130	(31.71)	30	(7.32)	0.768	0.232
					*P** 0.7860				*P*	0.6890
rs9641164		Total	AA		AT		TT		A	T
(intron 8)	SLE	411	261	(63.50)	126	(30.66)	24	(5.84)	0.788	0.212
	Controls	510	343	(67.25)	150	(29.41)	17	(3.33)	0.82	0.18
					*P** 0.1750				*P*	0.0930
rs6954345		Total	CC		CG		GG		C	G
(exon 9)	SLE	406	240	(59.11)	135	(33.25)	31	(7.64)	0.757	0.243
Ser311Cys	Controls	510	305	(59.80)	173	(33.92)	32	(6.27)	0.768	0.232
					*P** 0.8730				*P*	0.6090
rs13306702		Total	GG		GC		CC		G	C
after 3'-UTR	SLE	410	390	(95.12)	19	(4.63)	1	(0.24)	0.974	0.026
	Controls	507	486	(95.86)	19	(3.75)	2	(0.39)	0.977	0.023
					*P** 0.8820				*P*	0.6860
rs17876205		Total	GG		GC		CC		G	C
after 3'-UTR	SLE	407	394	(96.81)	13	(3.19)	0	(0.00)	0.984	0.016
	Controls	509	492	(96.66)	17	(3.34)	0	(0.00)	0.983	0.017
					*P** 0.6700				*P*	0.9030

P value for Allele frequency difference, unadjusted
**P* value for genotype distribution, adjusted for recruitment site and age

Table 15. Univariate analysis of *PON2* tagSNPs with SLE risk in blacks

			Genotype (%)						Alleles	
rs17876183		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(5'UTR)	**SLE**	**72**	72	(100.00)	0	(0.00)	0	(0.00)	1	0.000
	Controls	**57**	55	(96.49)	2	(3.51)	0	(0.00)	0.982	0.018
					*P** 0.0680				*P*	0.1536
rs2299267		**Total**	**AA**		**AG**		**GG**		**A**	**G**
(intron 1)	**SLE**	**72**	50	(69.44)	19	(26.39)	3	(4.17)	0.826	0.174
	Controls	**58**	43	(74.14)	13	(22.41)	2	(3.45)	0.853	0.147
					*P** 0.608				*P*	0.5524
rs10261470		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**SLE**	**72**	50	(69.44)	20	(27.78)	2	(2.78)	0.833	0.167
	Controls	**57**	39	(68.42)	17	(29.82)	1	(1.75)	0.833	0.167
					*P** 0.614				*P*	1.0000
rs4729189		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**SLE**	**71**	47	(66.20)	21	(29.58)	3	(4.23)	0.81	0.19
	Controls	**56**	30	(53.57)	25	(44.64)	1	(1.79)	0.759	0.241
					*P** 0.1050				*P*	0.3286
rs12534274		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**SLE**	**72**	46	(63.89)	22	(30.56)	4	(5.56)	0.792	0.208
	Controls	**57**	31	(54.39)	24	(42.11)	2	(3.51)	0.754	0.246
					*P** 0.4230				*P*	0.4788
rs11982486		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**SLE**	**72**	34	(47.22)	30	(41.67)	8	(11.11)	0.681	0.319
	Controls	**58**	31	(53.45)	22	(37.93)	5	(8.62)	0.724	0.276
					*P** 0.5800				*P*	0.4433
rs11981433		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**SLE**	**72**	32	(44.44)	35	(48.61)	5	(6.94)	0.688	0.313
	Controls	**55**	28	(50.91)	23	(41.82)	4	(7.27)	0.718	0.282
					*P** 0.6430				*P*	0.5950
rs2286233		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**SLE**	**72**	45	(62.50)	24	(33.33)	3	(4.17)	0.792	0.208
	Controls	**56**	32	(57.14)	21	(37.50)	3	(5.36)	0.759	0.241
					*P** 0.6460				*P*	0.5346
rs12704795		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 1)	**SLE**	**72**	32	(44.44)	35	(48.61)	5	(6.94)	0.688	0.313
	Controls	**56**	30	(53.57)	22	(39.29)	4	(7.14)	0.732	0.268
					*P** 0.4440				*P*	0.4331

P value for Allele frequency difference, unadjusted
**P* value for genotype distribution, adjusted for recruitment site and age

Table 15 (cont'd).

			Genotype (%)						Alleles	
rs17876193		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(intron 2)	**SLE**	**72**	66	(91.67)	6	(8.33)	0	(0.00)	0.958	0.042
	Controls	**56**	54	(96.43)	2	(3.57)	0	(0.00)	0.982	0.018
					*P** 0.2710				*P*	0.2530
rs17876116		**Total**	**GG**		**GT**		**TT**		**G**	**T**
(intron 2)	**SLE**	**72**	71	(98.61)	1	(1.39)	0	(0.00)	0.993	0.007
	Controls	**57**	57	(100.00)	0	(0.00)	0	(0.00)	1.000	0.000
					*P** 0.3860				*P*	0.3156
rs1639		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 3)	**SLE**	**65**	54	(83.08)	10	(15.38)	1	(1.54)	0.908	0.092
	Controls	**55**	50	(90.91)	4	(7.27)	1	(1.82)	0.945	0.055
					*P** 0.3580				*P*	0.2577
rs11545941		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon5)	**SLE**	**72**	40	(55.56)	27	(37.50)	5	(6.94)	0.743	0.257
Ala148Gly	**Controls**	**58**	32	(55.17)	20	(34.48)	6	(10.34)	0.724	0.276
					*P** 0.6050				*P*	0.7319
rs987539		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 6)	**SLE**	**72**	31	(43.06)	27	(37.50)	14	(19.44)	0.62	0.38
	Controls	**56**	21	(37.50)	27	(48.21)	8	(14.29)	0.62	0.38
					*P** 0.7600				*P*	0.97416
rs3735586		**Total**	**TT**		**TA**		**AA**		**T**	**A**
(intron 7)	**SLE**	**72**	35	(48.61)	26	(36.11)	11	(15.28)	0.67	0.33
	Controls	**58**	30	(51.72)	24	(41.38)	4	(6.90)	0.72	0.28
					*P** 0.4060				*P*	0.3145
rs9641164		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 8)	**SLE**	**72**	61	(84.72)	9	(12.50)	2	(2.78)	0.910	0.090
	Controls	**58**	55	(94.83)	3	(5.17)	0	(0.00)	0.974	0.026
					*P** 0.1020				*P*	0.0217
rs6954345		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon 9)	**SLE**	**71**	41	(57.75)	26	(36.62)	4	(5.63)	0.761	0.239
Ser311Cys	**Controls**	**58**	34	(58.62)	19	(32.76)	5	(8.62)	0.750	0.250
					*P** 0.6980				*P*	0.8445
rs13306702		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**SLE**	**72**	66	(91.67)	6	(8.33)	0	(0.00)	0.958	0.042
	Controls	**57**	50	(87.72)	7	(12.28)	0	(0.00)	0.939	0.061
					*P** 0.3209				*P*	0.4806
rs17876205		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**SLE**	**72**	72	(100.00)	0	(0.00)	0	(0.00)	1	0.000
	Controls	**56**	56	(100.00)	0	(0.00)	0	(0.00)	1	0.000
					*P** NA				*P*	NA

P value for Allele frequency difference, unadjusted
**P* value for genotype distribution, adjusted for recruitment site and age

5.5.2 Haplotype analysis of *PON2* variants with SLE risk in whites

In our combined (Pittsburgh+Chicago) white (cases+controls) sample, tagger analyses using r^2 cut off ≥ 0.8, identified a set of 15 not highly correlated tagSNPs of the 19 *PON2* variants that were selected in our study. Haplotype analysis with these 15 tagSNPs identified 16 haplotypes with a minimum frequency of ≥1% (Table16). Although, two of the haplotypes (# 6 and #16) showed statistically significant difference (*p* value 0.0404 and 0.0307) between cases and controls, after 1000 permutation tests, they were no longer significant (*p* value 0.242 and 0.163).

Table 16. Haplotype analysis of *PON2* variants with SLE risk in whites

# Haplotype	Haplotype Block	Haplotype Frequency	Case,Control Frequencies	*P*	Permutation *P* (#1000 permutations)
1	**GGCACGCACCGAGAG**	**0.308**	**0.309, 0.321**	**0.5659**	**1**
2	**GGGATGCATTAAGAG**	**0.174**	**0.185, 0.172**	**0.4695**	**1**
3	**GGCTTGGATTGAGGG**	**0.071**	**0.079, 0.068**	**0.3856**	**1**
4	**GGCACGCACTGTAAG**	**0.068**	**0.065, 0.073**	**0.5111**	**1**
5	**GGCACGCTTTGTGAG**	**0.053**	**0.053, 0.056**	**0.7881**	**1**
6	GGCACGCTTTGTAAG	0.045	0.035, 0.055	0.0404	0.242
7	GGCTTTCATTGAGGG	0.04	0.051, 0.033	0.0698	0.421
8	GGCTTGCATTAAGAG	0.038	0.037, 0.040	0.7149	1
9	GGCTTGCATTGAGGG	0.018	0.022, 0.016	0.3632	0.999
10	GGGATGCATTAAGGG	0.016	0.012, 0.020	0.2083	0.99
11	CGGATGCATTAAGAG	0.016	0.017, 0.015	0.7749	1
12	GGCTTGGATTGAGAG	0.014	0.015, 0.013	0.727	1
13	GGCACGCTTTGTGAA	0.014	0.013, 0.015	0.6378	1
14	GGGATGCATTGAGAG	0.013	0.016, 0.012	0.5233	1
15	GCCATGCATTGTGAG	0.013	0.014, 0.013	0.8811	1
16	GGCACGCACTGAGAG	0.011	0.005, 0.016	0.0307	0.163

5.6 ASSOCIATION OF *PON2* VARIANTS WITH LUPUS NEPHRITIS

Table 17 summarizes the single-site association analyses of *PON2* variants with lupus nephritis in Caucasian SLE cases. In white (Pittsburgh+Chicago) SLE patients (n=416) stratified by the presence (n=124) or absence (n=290) of lupus nephritis, five *PON2* variants (rs17876183, rs10261470, rs987539, rs9641164, and rs17876205) showed modest association (age and recruitment site adjusted *p* values ranging between 0.016-0.033 for genotype distribution) with lupus nephritis. Of these 5 variants, allele frequency difference was statistically significant for 2 variants, *PON2*/rs17876183(*P*=0.0267) and *PON2*/rs17876205(*P*=0.0475). For *PON2*/rs17876183G>A and rs17876205C>G SNPs, the minor allele frequencies (MAF) in white cases with nephritis *vs* white cases without nephritis was 4% *vs* 1% and 3% *vs* 1%, respectively. Genotype distribution for these two SNPs in SLE cases with nephritis/SLE cases without nephritis was 93.5%/98.26% for GG, 5.69%/1.74% for GA and 0.81%/0.0% AA genotypes of *PON2*/rs17876183G>A variant and 93.5%/98.24% for CC, 6.50%/1.76% for CG, 0%vs0% for GG genotypes for *PON2*/rs17876205C>G variant. Recruitment site and age adjusted odds ratio (OR) was 4.38 (95%CI=1.27-15.07, *P*=0.02) for GA *vs* GG genotype of *PON2*/rs17876183G>A variant. Similarly, OR was 4.08 (95%CI=1.26-13.21, *P*=0.02) for the GC *vs* GG genotype of *PON2*/rs17876205G>C variant.

Table 17. Univariate analysis of *PON2* tagSNPs with lupus nephritis in white SLE patients

			Genotype (%)						Alleles	
rs17876183		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(5'UTR)	**Renal Involvement**	**123**	**115**	(93.50)	7	(5.69)	1	(0.81)	0.96	0.04
	No Renal Involvement	**287**	282	(98.26)	5	(1.74)	0	(0.00)	0.91	0.01
					*P** 0.0330				*P*	0.0267
rs2299267		**Total**	**AA**		**AG**		**GG**		**A**	**G**
(intron 1)	**Renal Involvement**	**123**	82	(66.67)	37	(30.08)	4	(3.25)	0.82	0.18
	No Renal Involvement	**287**	193	(67.25)	84	(29.27)	10	(3.48)	0.82	0.18
					*P** 0.977				*P*	0.9528
rs10261470		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**Renal Involvement**	**123**	100	(81.30)	18	(14.63)	5	(4.07)	0.89	0.11
	No Renal Involvement	**286**	216	(75.52)	68	(23.78)	2	(0.70)	0.87	0.13
					*P** 0.0160				*P*	0.6234
rs4729189		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**Renal Involvement**	**123**	79	(64.23)	35	(28.46)	9	(7.32)	0.79	0.22
	No Renal Involvement	**284**	182	(64.08)	91	(32.04)	11	(3.87)	0.80	0.20
					*P** 0.425				*P*	0.5957
rs12534274		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**Renal Involvement**	**123**	68	(55.28)	41	(33.33)	14	(11.38)	0.720	0.280
	No Renal Involvement	**283**	163	(57.60)	99	(34.98)	21	(7.42)	0.751	0.249
					*P** 0.3600				*P*	0.3551
rs11982486		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**Renal Involvement**	**123**	63	(51.22)	47	(38.21)	13	(10.57)	0.70	0.30
	No Renal Involvement	**287**	126	(43.90)	126	(43.90)	35	(12.20)	0.66	0.34
					*P** 0.3520				*P*	0.2042
rs11981433		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**Renal Involvement**	**122**	53	(43.44)	47	(38.52)	22	(18.03)	0.63	0.37
	No Renal Involvement	**285**	96	(33.68)	137	(48.07)	52	(18.25)	0.58	0.42
					*P** 0.0890				*P*	0.1806
rs2286233		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**Renal Involvement**	**124**	95	(76.61)	25	(20.16)	4	(3.23)	0.87	0.13
	No Renal Involvement	**288**	224	(77.78)	57	(19.79)	7	(2.43)	0.88	0.12
					*P** 0.9630				*P*	0.7013
rs12704795		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 1)	**Renal Involvement**	**121**	52	(42.98)	46	(38.02)	23	(19.01)	0.62	0.38
	No Renal Involvement	**284**	94	(33.10)	139	(48.94)	51	(17.96)	0.58	0.42
					*P** 0.2780				*P*	0.2389

P value for Allele frequency difference, unadjusted

**P* value for genotype distribution, adjusted for recruitment site and age

Table 17 (cont'd).

			Genotype (%)						Alleles	
rs17876193		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(intron 2)	**Renal Involvement**	**123**	104	(84.55)	17	(13.82)	2	(1.63)	0.92	0.09
	No Renal Involvement	**287**	228	(79.44)	57	(19.86)	2	(0.70)	0.89	0.11
					*P** 0.367				*P*	0.3414
rs17876116		**Total**	**GG**		**GT**		**TT**		**G**	**T**
(intron 2)	**Renal Involvement**	**124**	108	(87.10)	15	(12.10)	1	(0.81)	0.93	0.07
	No Renal Involvement	**286**	254	(88.81)	32	(11.19)	0	(0.00)	0.94	0.06
					*P** 0.402				*P*	0.5004
rs1639		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 3)	**Renal Involvement**	**121**	76	(62.81)	33	(27.27)	12	(9.92)	0.76	0.24
	No Renal Involvement	**285**	178	(62.46)	95	(33.33)	12	(4.21)	0.79	0.21
					*P** 0.058				*P*	0.4052
rs11545941		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon5)	**Renal Involvement**	**123**	69	(56.10)	40	(32.52)	14	(11.38)	0.72	0.28
Ala148Gly	**No Renal Involvement**	**268**	165	(57.69)	101	(35.31)	20	(6.99)	0.75	0.25
					*P** 0.245				*P*	0.3751
rs987539		**Total**	**CC**		**CT**		**TT**		**C**	**T**
(intron 6)	**Renal Involvement**	**122**	40	(32.79)	44	(36.07)	38	(31.15)	0.508	0.49
	No Renal Involvement	**282**	79	(28.01)	139	(49.29)	64	(22.70)	0.53	0.47
					*P** 0.0190				*P*	0.6309
rs3735586		**Total**	**TT**		**TA**		**AA**		**T**	**A**
(intron 7)	**Renal Involvement**	**124**	78	(62.90)	37	(29.84)	9	(7.26)	0.78	0.22
	No Renal Involvement	**286**	172	(60.14)	93	(32.52)	21	(7.34)	0.76	0.24
					*P** 0.9160				*P*	0.6543
rs9641164		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 8)	**Renal Involvement**	**124**	78	(62.90)	33	(26.61)	13	(10.48)	0.762	0.238
	No Renal Involvement	**287**	183	(63.76)	93	(32.40)	11	(3.83)	0.800	0.200
					*P** 0.0220				*P*	0.237
rs6954345		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon 9)	**Renal Involvement**	**120**	69	(57.50)	41	(34.17)	10	(8.33)	0.75	0.25
Ser311Cys	**No Renal Involvement**	**286**	171	(59.79)	94	(32.87)	21	(7.34)	0.76	0.24
					*P** 0.7640				*P*	0.6219
rs13306702		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**Renal Involvement**	**123**	114	(92.68)	8	(6.50)	1	(0.81)	0.96	0.04
	No Renal Involvement	**287**	276	(96.17)	11	(3.83)	0	(0.00)	0.98	0.02
					*P** 0.1210				*P*	0.1203
rs17876205		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**Renal Involvement**	**123**	115	(93.50)	8	(6.50)	0	(0.00)	0.97	0.03
	No Renal Involvement	**284**	279	(98.24)	5	(1.76)	0	(0.00)	0.99	0.01
					*P** 0.0170				*P*	0.0475

P value for Allele frequency difference, unadjusted

**P* value for genotype distribution, adjusted for recruitment site and age

5.7 SINGLE-SITE ANALYSIS OF *PON2* POLYMORPHISMS WITH PARAMETERS OF LDL OXIDATION

Table 18 shows the *PON2* genotype specific mean±SD values for the significant associations identified with the parameters of LDL oxidation (oxLDL) in Pittsburgh SLE cases. Data on all nine parameters of oxLDL that were evaluated in our study was available for 247/348 SLE cases for the Pittsburgh recruitment site. The associations that were indentified to be significant include *PON2*/rs13306702 with Ig(M) antibodies for copper-modified oxLDL (*P*= 0.0343), *PON2*/rs17876205 with E06 antibody (*P*= 0.031), *PON2*/rs10261470, *PON2*/rs4729189 and *PON2*/rs2286233 with Ig(M) antibodies for LDL immune complex with *p* values of 0.0193, 0.0282, and 0.0453, respectively, *PON2*/rs2286233 and *PON2*/rs11545941(Ala148Gly) with Ig(M) antibody for MDA modified LDL with *p* values of 0.0016 and 0.0282, respectively.

We also checked for association of oxLDL with PON activity, but could not detect any (data not shown).

Table 18. Genotype specific values of *PON2* associations with parameters of LDL oxidation*

PON2 SNP	Genotype [counts]	Mean	±	SD	OxLDL-Parameter	*P*
rs13306702						
	GG[281]	4927.31	±	4094.49	anti-cu-modified OxLDL (IgM)	0.0343
	GC[9]	7995.19	±	4519.51		
	CC[0]	NA		NA		
rs17876205						
	GG[275]	7136.17	±	3782.18	OxLDL-EO6 (oxPL/Apo B)	0.031
	GC[9]	9860.58	±	4496.91		
	CC[0]	NA		NA		
rs10261470						
	GG[224]	4289.00	±	2128.03	LDL immune complexes (IgM)	0.0193
	GA[64]	4980.12	±	2399.39		
	AA[3]	2063.86	±	1064.65		
rs4729189						
	AA[187]	4374.52	±	1961.31	LDL immune complexes (IgM)	0.0282
	AT[87]	4793.61	±	2717.48		
	TT[13]	2813.77	±	826.82		
rs22862333						
	AA[226]	4547.98	±	2139.32	LDL immune complexes (IgM)	0.0453
	AT[57]	4084.75	±	2510.59		
	TT[5]	2925.54	±	763.74		
rs22862333						
	AA[226]	15574.93	±	9301.73	anti-MDA-modified LDL (IgM)	0.0016
	AT[57]	12240.66	±	9207.90		
	TT[5]	7139.98	±	6743.77		
rs11545941(Ala148Gly)						
	CC[170]	13370.07	±	8615.02	anti-MDA-modified LDL (IgM)	0.0282
	CG[95]	16750.00	±	10172.49		
	GG[24]	16252.39	±	9568.80		

*Mean ±SD values are adjusted for age,BMI,smoking,HDL-C, total-cholesterol, triglycerides and high blood pressure. In parenthesis are the number of people for a paricular parameter of oxLDL for that genotype. NA--Not applicable for that paricular susbet. *P* values are based on the transformed data, adjusted for age,BMI,smoking, HDL-C, cholesterol,triglycerides.

5.8 ASSOCIATION *PON2* TAGSNPS WITH SUBCLINICAL CARDIOVASCULAR DISEASE

The subclinical cardiovascular disease measures that were examined in our study were carotid plaque and carotid IMT.

5.8.1 Single-site analysis for carotid plaque

Table 19 shows the univariate analysis of *PON2* tagSNPs with carotid plaque in combined (Pittsburgh +Chicago) white SLE cases (n=416) which was stratified by the presence (n=102) or absence (n=211) of carotid plaque. Single-site analyses of *PON2* variants with carotid plaque identified *PON2*/rs11981433 and *PON2*/rs12704795 variants with significant association with both allele frequency difference (*p* value 0.0127 and 0.0122) and genotype distribution (*P* = 0.03 and 0.01). The MAF of *PON2*/rs11981433T>C variant was 33% in white SLE cases with plaque and 43% in white SLE cases without plaque while the genotype distribution was 42.42%/35.71% for TT, 49.49%/42.38% for TC and 8.08% /21.90% for CC genotype in white SLE cases with plaque/white SLE cases without plaque. Similarly, MAF of *PON2*/rs12704795T>G variant was 33% in white SLE cases with plaque and 43% in white SLE cases without plaque while the genotype distribution was 40.59%/35.75% for TT, 52.48%/41.55% for TG and 6.93% /22.71% for GG genotype in white SLE cases with plaque/white SLE cases without plaque. Based on biological evidence, these two SNPs were evaluated under recessive model (*p* value 0.016-0.0099 for genotype distribution). The covariate adjusted odds ratio (OR) calculated under this recessive

model was 0.32 (95% CI= 0.12-0.81,*P*=0.016) for CC*vs*TT+TC genotype for *PON2*/rs11981433T>C variant. Similarly, OR was 0.28 (95%CI=0.10-0.73,*P*=0.0099) for GG*vs*TT+TG genotypes for *PON2*/rs12704795T>C variant. For another SNP rs11982486, only the genotype distribution was statistically significant with a *p* value of 0.03 (Table 19).

Table 19. Univariate analysis of *PON2* tagSNPs with carotid plaque in SLE whites

SNP		Total	Genotype (%)						Alleles	
rs17876183		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(5'UTR)	**With Plaque**	**101**	97	(96.04)	4	(3.96)	0	(0.00)	0.98	0.02
	Without plaque	**210**	204	(97.14)	5	(2.38)	0	(0.00)	0.98	0.02
					*P** 0.39				*P*	0.7874
rs2299267		**Total**	**AA**		**AG**		**GG**		**A**	**G**
(intron 1)	**With Plaque**	**101**	68	(67.33)	27	(26.73)	6	(5.94)	0.81	0.19
	Without plaque	**211**	141	(66.82)	63	(29.86)	7	(3.32)	0.82	0.18
					*P** 0.80				*P*	0.7518
rs10261470		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**With Plaque**	**102**	78	(76.47)	23	(22.55)	1	(0.98)	0.88	0.12
	Without plaque	**210**	164	(78.10)	43	(20.48)	3	(1.43)	0.88	0.12
					*P** 0.76				*P*	0.8324
rs4729189		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**With Plaque**	**100**	65	(65.00)	29	(29.00)	6	(6.00)	0.80	0.21
	Without plaque	**209**	136	(65.07)	66	(31.58)	7	(3.35)	0.81	0.19
					*P** 0.67				*P*	0.6925
rs12534274		**Total**	**GG**		**GA**		**AA**		**G**	**A**
(intron 1)	**With Plaque**	**101**	53	(54.08)	36	(36.73)	9	(9.18)	0.724	0.276
	Without plaque	**207**	120	(57.97)	70	(33.82)	17	(8.21)	0.749	0.251
					*P** 0.85				*P*	0.5266
rs11982486		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**With Plaque**	**100**	48	(48.00)	47	(47.00)	5	(5.00)	0.72	0.29
	Without plaque	**211**	97	(45.97)	81	(38.39)	33	(15.64)	0.65	0.35
					*P** 0.03				*P*	0.1084
rs11981433		**Total**	**TT**		**TC**		**CC**		**T**	**C**
(intron 1)	**With Plaque**	**99**	42	(42.42)	49	(49.49)	8	(8.08)	0.67	0.33
	Without plaque	**210**	75	(35.71)	89	(42.38)	46	(21.90)	0.57	0.43
					*P** 0.03				*P*	0.0127
rs2286233		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 1)	**With Plaque**	**102**	76	(74.51)	21	(20.59)	5	(4.90)	0.85	0.15
	Without plaque	**211**	168	(79.62)	39	(18.48)	4	(1.90)	0.89	0.11
					*P** 0.46				*P*	0.1679
rs12704795		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 1)	**With Plaque**	**101**	41	(40.59)	53	(52.48)	7	(6.93)	0.67	0.33
	Without plaque	**207**	74	(35.75)	86	(41.55)	47	(22.71)	0.57	0.43
					*P** 0.01				*P*	0.0122

P value for Allele frequency difference, unadjusted
**P* value for genotype distribution, adjusted for recruitment site,age,BMI,smoking and lipid profile

Table 19 (cont'd).

SNP	Group	Total	Genotype (%)						Alleles	
rs17876193		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(intron 2)	**With Plaque**	**100**	72	(72.00)	27	(27.00)	1	(1.00)	0.86	0.15
	Without plaque	**211**	176	(83.41)	33	(15.64)	2	(0.95)	0.91	0.09
					*P** 0.23				*P*	0.0439
rs17876116		**Total**	**GG**		**GT**		**TT**		**G**	**T**
(intron 2)	**With Plaque**	**101**	94	(93.07)	7	(6.93)	0	(0.00)	0.97	0.04
	Without plaque	**211**	183	(86.73)	27	(12.80)	1	(0.47)	0.93	0.07
					*P** 0.31				*P*	0.0558
rs1639		**Total**	**TT**		**TG**		**GG**		**T**	**G**
(intron 3)	**With Plaque**	**99**	55	(55.56)	37	(37.37)	7	(7.07)	0.74	0.26
	Without plaque	**210**	137	(65.24)	60	(28.57)	13	(6.19)	0.80	0.21
					*P** 0.38				*P*	0.1511
rs11545941		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon5)	**With Plaque**	**100**	55	(55.00)	37	(37.00)	8	(8.00)	0.73	0.26
Ala148Gly	**Without plaque**	**210**	122	(58.10)	70	(33.33)	18	(8.57)	0.74	0.25
					*P** 0.87				*P*	0.7380
rs987539		**Total**	**CC**		**CT**		**TT**		**C**	**T**
(intron 6)	**With Plaque**	**100**	23	(23.00)	46	(46.00)	31	(31.00)	0.46	0.54
	Without plaque	**208**	66	(31.73)	90	(43.27)	52	(25.00)	0.53	0.46
					*P** 0.44				*P*	0.0860
rs3735586		**Total**	**TT**		**TA**		**AA**		**T**	**A**
(intron 7)	**With Plaque**	**101**	60	(59.41)	31	(30.69)	10	(9.90)	0.75	0.25
	Without plaque	**210**	128	(60.95)	68	(32.38)	14	(6.67)	0.77	0.23
					*P** 0.46				*P*	0.51
rs9641164		**Total**	**AA**		**AT**		**TT**		**A**	**T**
(intron 8)	**With Plaque**	**101**	58	(57.43)	36	(35.64)	7	(6.93)	0.750	0.240
	Without plaque	**211**	139	(65.88)	59	(27.96)	13	(6.16)	0.790	0.200
					*P** 0.55				*P*	0.2016
rs6954345		**Total**	**CC**		**CG**		**GG**		**C**	**G**
(exon 9)	**With Plaque**	**100**	59	(59.00)	31	(31.00)	10	(10.00)	0.75	0.26
Ser311Cys	**Without plaque**	**208**	124	(59.62)	70	(33.65)	14	(6.73)	0.76	0.24
					*P** 0.33				*P*	0.6014
rs13306702		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**With Plaque**	**101**	98	(97.03)	3	(2.97)	0	(0.00)	0.99	0.98
	Without plaque	**211**	201	(95.26)	10	(4.74)	0	(0.00)	0.02	0.02
					*P** 0.97				*P*	0.4330
rs17876205		**Total**	**GG**		**GC**		**CC**		**G**	**C**
after 3'-UTR	**With Plaque**	**99**	94	(94.95)	5	(5.05)	0	(0.00)	0.98	0.02
	Without plaque	**210**	205	(97.62)	5	(2.38)	0	(0.00)	0.99	0.01
					*P** 0.20				*P*	0.2790

P value for Allele frequency difference, unadjusted

**P* value for genotype distribution, adjusted for recruitment site,age,BMI,smoking and lipid profile

5.8.2 Single-site analysis for carotid IMT

Table 20 shows the univariate analysis of *PON2* tagSNPs with carotid IMT in combined (Pittsburgh +Chicago) white SLE cases (n=416) of which we had data for only 312 individuals with carotid IMT. In single-site *PON2* variants rs12704795 and rs17876205 showed modest association with carotid IMT, with *p* values of 0.037 and 0.017, respectively under codominant model. Under recessive model, *p* value for the first SNP (*PON2*/rs12704795) was 0.01, while *p* value for another variant *PON2*/rs11981433 was reported to be statistically significant (0.03).

We also checked a possible relation between the parameters of LDL oxidation and subclinical CVD measures (carotid plaque and carotid IMT) but did not detect any association (results not shown). Similar to oxidized LDL parameters we also checked for any association between carotid plaque or carotid IMT with PON activity, but did not detect any (data not shown).

Table 20. Carotid IMT measurements (Mean ±SD) by *PON2* genotype in white SLE cases *

PON2 SNP	Genotype[counts]	Mean	±	SD
rs17876183	GG[272]	0.687	±	0.108
	GA[8]	0.678	±	0.076
	AA[1]	0.668	±	NA
		P 0.82022		
rs2299267	AA[187]	0.682	±	0.108
	AG[83]	0.692	±	0.093
	GG[12]	0.724	±	0.164
		P 0.23027		
rs10261470	GG[217]	0.682	±	0.096
	GA[61]	0.701	±	0.138
	AA[4]	0.749	±	0.096
		P 0.50097		
rs4729189	AA[176]	0.686	±	0.098
	AT[91]	0.687	±	0.124
	TT[12]	0.705	±	0.084
		P 0.95597		
rs12534274	GG[161]	0.682	±	0.097
	GA[94]	0.696	±	0.126
	AA[22]	0.684	±	0.088
		P 0.594		
rs11982486	TT[129]	0.695	±	0.123
	TC[118]	0.683	±	0.085
	CC[34]	0.667	±	0.104
		P 0.20874		
rs11981433	TT[103]	0.693	±	0.111
	TC[128]	0.689	±	0.110
	CC[49]	0.663	±	0.082
		P 0.08528		
rs2286233	AA[219]	0.686	±	0.109
	AT[56]	0.694	±	0.099
	TT[8]	0.635	±	0.091
		P 0.1456		
rs12704795	TT[101]	0.694	±	0.112
	TG[128]	0.692	±	0.108
	GG[49]	0.661	±	0.080
		P 0.0372		
rs17876193	CC[225]	0.681	±	0.100
	CG[53]	0.716	±	0.128
	GG[3]	0.621	±	0.052
		P 0.0517		

PON2 SNP	Genotype [counts]	Mean	±	SD
rs17876116	GG[250]	0.685	±	0.105
	GT[31]	0.692	±	0.120
	TT[1]	0.749	±	0.000
		P 0.63808		
rs1639	TT[172]	0.680	±	0.102
	TG[90]	0.694	±	0.107
	GG[18]	0.709	±	0.150
		P 0.35364		
rs11545941	CC[167]	0.685	±	0.102
(Ala148Gly)	CG[93]	0.685	±	0.121
	GG[20]	0.700	±	0.068
		P 0.59723		
rs987539	CC[82]	0.675	±	0.085
	CT[124]	0.687	±	0.114
	TT[72]	0.702	±	0.112
		P 0.12911		
rs3735586	TT[175]	0.684	±	0.100
	TA[86]	0.689	±	0.126
	AA[20]	0.702	±	0.072
		P 0.31959		
rs9641164	AA[176]	0.680	±	0.100
	AT[88]	0.693	±	0.106
	TT[18]	0.720	±	0.157
		P 0.2613		
rs6954345	CC[170]	0.686	±	0.100
(Ser311Cys)	CG[88]	0.692	±	0.124
	GG[20]	0.696	±	0.072
		P 0.59723		
rs13306702	monomorphic	0.687	±	0.107
		0.664	±	0.105
		NaN		
		P 0.14405		
rs17876205	GG[273]	0.684	±	0.105
	GC[6]	0.784	±	0.153
	CC[0]	Na		NA
		P 0.01671		

*Mean ±SD, and genotype counts in parenthesis are adjusted for recruitment site, age, BMI, smoking and lipid profile. NA--not apllicable for that particular subset.*P* values are based on the transformed data, adjusted for recruitment site, age, BMI,smoking,lipid profile

5.9 CORRELATION OF *PON2* GENETIC VARIATION WITH PON ACTIVITY

Tables 21 shows the mean ± SD levels of PON activity in blacks and whites, further stratified by case-control status for both the recruitment sites (Pittsburgh and Chicago). Since serum PON activity was measured at different time points with slightly different protocols for Pittsburgh and Chicago and their mean values stratified by race and case–control status differed for each locale, therefore, analyses were done separately for each site.

For Pittsburgh, the overall mean ± SD values for PON activity was significantly higher in blacks (1096.8071 ± 595.7473 units/liter) than in whites (671.4365 ± 466.8715 units/liter) ($P<0.0001$). A similar trend was observed for the Chicago recruitment samples (whites *vs* blacks, 351.4333±292.5718 *vs* 727.2024±440.1286 units/liter, $P<0.001$). Association of each *PON2* variant with PON activity was first analyzed in single-site and then in a multiple regression model along with the known modulators of PON activity (*PON1* and *PON3* SNPs) to determine the independent contribution of *PON2* variants with PON activity.

Table 21. Mean ± SD values of PON Activity in Pittsburgh and Chicago site

Mean ± SD values of PON/ paraxon activity	Pittsburgh white			(n*)	Pittsburgh black			(n)
cases	613.877	±	412.9438	[291]	1022.2	±	518.358	[38]
controls	709.854	±	496.3924	[436]	1164.3	±	656.933	[42]
		P = 0.00653				*P* = 0.29		
Overall (between whites and blacks)	671.437	±	466.8715	[725]	1096.8	±	595.747	[79]
				P < 0.0001				

Mean ± SD values of PON/ paraxon activity	Chicago white			(n*)	Chicago black			(n)
cases	326.53	±	248.0744	[68]	711.87	±	470.289	[25]
controls	381.142	±	338.0211	[57]	751.16	±	402.106	[16]
		P = 0.3				*P* =0.7843		
Overall (between whites and blacks)	351.433	±	292.5718	[93]	727.2	±	440.129	[73]
				P < 0.0001				

*[n]-number of subjects with PON activity data

5.9.1 Association of PON activity with *PON2* genotype in Pittsburgh cohort

Table 22 for whites and 23 for blacks show the association (*p*) values and the PON activity (mean ±SD) values of each *PON2* variant with PON activity for the Pittsburgh recruitment site. Within each ethnic group for the Pittsburgh sample, cases and controls were analyzed together, adjusting for disease status in addition to other covariates (age, BMI and smoking).

In Pittsburgh whites, PON activity data was available for 291/346 SLE cases and 434 /454 controls. Single-site analyses in combined (cases+controls) Pittsburgh Caucasian sample identified eleven SNPs [*PON2*/rs10261470, rs4729189, rs12534274, rs11982486, rs11981433, rs12704795, rs11545941(Ala148Gly), rs987539, rs3735586, rs6954345(Ser311Cys), rs2299267]

with significant association (p value ranging between 0.027-2.2x10^{-16}) with PON/paraoxon activity after adjusting for disease status, age, BMI and smoking.

In blacks, PON activity data was available for 38/48 SLE cases and 42/42 controls, where single-site analyses in the combined (cases+controls) sample reported modest association with 5 variants [*PON2*/rs11545941(Ala148Gly), rs3735586, rs6954345(Ser311Cys), rs9641164 and rs1639] (p value ranging between 0.01-0.036) with PON activity, after adjusting for disease status, age, BMI and smoking.

5.9.2 Association of PON activity with *PON2* genotype in Chicago cohort

Table 22 for whites and 23 for blacks show the association (p) values and the PON activity (mean ±SD) values of each *PON2* variant with PON activity for the Chicago recruitment site. PON activity analyses for the Chicago recruitment site were performed similar to that of Pittsburgh recruitment site, where blacks and whites were analyzed separately, and within each ethnic group the cases and controls were analyzed together (adjusted for disease status, age, BMI and smoking). For the recently collected Chicago samples, we had data for all 68 SLE cases and 57 controls that were included in this study. In univariate analyses, 4 SNPs [*PON2*/rs6954345(Ser311Cys), rs12534274, rs11545941(Ala148Gly), rs3735586] showed significant association (p values ranging from 0.002-0.0001) with PON activity after adjusting for disease status, age, BMI and smoking.

In Chicago blacks with data available for all 16 SLE cases and 25 controls, only *PON2/*rs987539 SNP showed modest association (p value 0.01) with PON activity in combined (cases+controls) blacks, after adjusting for disease status, age, BMI and smoking.

Table 22. PON activity, by *PON2* genotype in whites *

	Pittsburgh whites(cases+controls)			Chicago whites(cases+controls)		
	Genotype [Counts]	Mean*	± SD	Genotype [Counts]	Mean	± SD
rs2299267	AA[443]	716.059	± 508.048	AA[78]	358.2837	± 277.081
	AG[177]	591.322	± 366.774	AG[30]	294.1194	± 232.752
	GG[21]	618.678	± 442.365	GG[4]	121.4493	± 36.5229
		0.027[#]			**0.08094**	
rs10261470	GG[472]	712.076	± 493.132	GG[92]	362.8103	± 300.923
	GA[147]	582.648	± 396.808	GA[16]	332.8144	± 234.123
	AA[13]	450.395	± 316.044	AA[4]	72.37211	± 69.2638
		0.0036			**0.0815**	
rs4729189	AA[384]	745.129	± 511.293	AA[72]	377.5407	± 323.301
	AT[209]	582.501	± 395.509	AT[36]	314.5768	± 209.117
	TT[33]	507.432	± 349.935	TT[5]	82.33868	± 69.3882
		0.00006121			**0.05224**	
rs12534274	GG[356]	542.125	± 380.158	GG[64]	255.526	± 211.089
	GA[208]	786.472	± 478.366	GA[37]	419.7143	± 335.109
	AA[59]	1109.8	± 607.821	AA[9]	637.8863	± 357.035
		2.20E-16			**0.0001**	
rs11982486	TT[303]	743.109	± 499.471	TT[45]	374.3762	± 287.923
	TC[242]	639.447	± 445.61	TC[53]	316.0525	± 290.698
	CC[86]	551.103	± 419.672	CC[14]	319.7593	± 288.616
		6.12E-05			**0.5244**	
rs11981433	TT[229]	785.407	± 489.971	TT[38]	423.5202	± 299.357
	TC[278]	661.08	± 467.505	TC[50]	350.8275	± 301.643
	CC[126]	517.301	± 402.545	CC[20]	244.5782	± 229.98
		6.12E-05			**0.06986**	
rs12704795	TT[227]	789.143	± 490.473	TT[36]	413.961	± 293.156
	TG[275]	659.678	± 470.226	TG[55]	329.0813	± 292.134
	GG[125]	519.011	± 404.101	GG[21]	236.3891	± 232.225
		6.96E-08			**0.05069**	
rs11545941(Ala148Gly)	CC[387]	595.213	± 419.246	CC[61]	260.2942	± 213.049
	CG[192]	738.126	± 469.039	CG[40]	453.0547	± 354.073
	GG[52]	1062.22	± 631.004	GG[11]	439.4769	± 278.873
		4.487E-11			**0.00246**	
rs987539	TT[147]	821.803	± 513.86	TT[27]	421.1806	± 294.424
	CT[272]	705.68	± 474.82	CT[50]	360.2296	± 309.075
	CC[202]	525.872	± 381.208	CC[34]	249.3817	± 224.268
		3.38E-09			**0.05066**	
rs3735586	TT[386]	585.724	± 399.476	TT[65]	259.4186	± 202.317
	TA[196]	758.265	± 493.191	TA[35]	485.7498	± 356.921
	AA[49]	1061.04	± 655.052	AA[12]	414.4739	± 327.407
		1.43E-11			**0.0009299**	

*Values are the mean ± SEM units/ litre for paraoxon, adjusted for disease, age , BMI and smoking. n values in parenthesis adjusted for, disease, age , BMI and smoking. [#]Values are *P* values based on square root transformed data adjusted for disease, age, BMI, and smoking . NA-- not apllicable for that subset.

Table 22 (cont'd).

	Pittsburgh whites (cases+controls)				Chicago whites (cases+controls)			
	Genotype [Counts]	Mean	±	SD	Genotype [Counts]	Mean	±	SD
rs17876183	GG[607]	677.4925	±	475.1301	GG[107]	350.977	±	294.877
	GA[24]	660.9257	±	439.4486	GA[4]	134.9	±	64.1465
	AA[1]	503.9238	±	NA	AA[0]	NA	±	NA
		0.9405				**0.1092**		
rs17876193	CC[526]	697.5648	±	486.9874	CC[91]	348.29	±	307.024
	CG[96]	572.9768	±	380.8751	CG[21]	303.808	±	186.626
	GG[9]	597.5932	±	445.5286	GG[0]	NA	±	NA
		0.066007				**0.7192**		
rs17876116	GG[577]	682.8981	±	479.1594	GG[99]	354.868	±	293.836
	GT[55]	631.1826	±	402.7563	GT[13]	281.339	±	267.033
	TT[5]	71.04268	±	NA	TT[1]	130.011	±	NA
		0.220123				**0.3431**		
rs1639	TT[398]	670.7315	±	498.6099	TT[73]	353.766	±	308.551
	TG[193]	671.5979	±	412.0905	TG[31]	343.558	±	264.379
	GG[31]	701.4885	±	428.2126	GG[5]	292.578	±	219.208
		0.831782				**0.8655**		
rs17876205	GG[609]	679.9885	±	474.2099	GG[110]	343.793	±	287.024
	GC[20]	606.3972	±	458.1739	GC[2]	74.5235	±	40.3412
	CC[0]		±		CC[0]		±	
		5.33E-01				**0.1623**		
rs13306702	GG[612]	676.2834	±	474.3334	GG[98]	336.944	±	272.63
	GC[19]	633.2168	±	381.9003	GC[11]	285.717	±	204.193
	CC[2]	1450.874	±	422.1571	CC[0]		±	
		0.098513				**0.7086**		
rs9641164	AA[409]	676.136	±	505.0579	AA[72]	350.054	±	303.708
	AT[194]	671.076	±	407.3522	AT[36]	336.969	±	271.995
	TT[30]	736.3305	±	427.3337	TT[4]	362.511	±	216.775
		0.673384				**0.9538**		
rs2286233	AA[478]	699.1022	±	498.9292	AA[87]	338.344	±	283.647
	AT[145]	608.8927	±	380.2248	AT[20]	309.616	±	246.46
	TT[9]	571.532	±	299.4656	TT[6]	504.809	±	449.332
		2.80E-01				**0.4952**		
rs6954345(ser311Cys)	CC[387]	585.1964	±	399.6148	CC[69]	256.615	±	203.596
	CG[195]	757.8552	±	492.4825	CG[39]	471.177	±	355.927
	GG[48]	1082.87	±	649.7644	GG[8]	460.935	±	322.859
		3.19E-12				**0.0009928**		

*Values are the mean ± SEM units/ litre for paraoxon, adjusted for disease, age , BMI and smoking. n values in parenthesis adjusted for, disease, age , BMI and smoking. #Values are *P* values based on square root transformed data adjusted for disease, age, BMI, and smoking . NA-- not apllicable for that subset.

Table 23. PON activity, by *PON2* genotype in blacks*

	Pittsburgh whites (cases+controls)				Chicago whites (cases+controls)			
	Genotype [Counts]	Mean*	±	SD	Genotype [Counts]	Mean	±	SD
rs2299267	AA[50]	1117.35	±	545.502	AA[26]	763.929	±	467.436
	AG[20]	1077.9	±	705.970	AG[5]	787.047	±	462.298
	GG[1]	335.846	±	NA	GG[2]	438.821	±	80.4196
			0.4014#				**0.8109**	
rs10261470	GG[44]	1118.7	±	600.711	GG[24]	777.76	±	455.64
	GA[22]	1088.91	±	616.943	GA[8]	738.971	±	432.356
	AA[2]	873.624	±	24.611	AA[1]	97.0406	±	NA
			0.8748				**0.1952**	
rs4729189	AA[37]	1131.9	±	524.152	AA[18]	854.584	±	468.526
	AT[28]	1115.7	±	702.618	AT[15]	619.503	±	409.288
	TT[3]	820.825	±	476.355	TT[0]	NA	±	NA
			0.542				**0.1277**	
rs12534274	GG[41]	966.358	±	549.118	GG[21]	715.74	±	501.099
	GA[26]	1282.2	±	644.465	GA[11]	853.971	±	333.855
	AA[3]	1381.98	±	238.554	AA[1]	715.74	±	NA
			0.103				**0.3074**	
rs11982486	TT[35]	1114.38	±	572.285	TT[17]	686.598	±	283.492
	TC[30]	1101.26	±	652.150	TC[15]	800.433	±	602.154
	CC[6]	953.364	±	459.111	CC[1]	996.385	±	NA
			0.916				**0.805**	
rs11981433	TT[32]	1128.26	±	595.122	TT[15]	756.822	±	372.864
	TC[33]	1080.22	±	644.842	TC[16]	727.223	±	548.332
	CC[4]	1072.94	±	143.573	CC[1]	994.934	±	NA
			0.978				**0.6934**	
rs12704795	TT[32]	1128.26	±	595.122	TT[17]	765.598	±	354.682
	TG[33]	1080.22	±	644.842	TG[15]	710.9	±	562.441
	GG[4]	1072.94	±	143.573	GG[1]	996.385	±	NA
			0.978				**0.5944**	
rs11545941(Ala148Gly)	CC[37]	920.224	±	564.850	CC[21]	752.255	±	503.494
	CG[31]	1275.36	±	595.694	CG[8]	773.875	±	403.109
	GG[3]	1392.21	±	247.411	GG[4]	671.67	±	312.26
			0.0205				**0.9235**	
rs987539	TT[23]	1271.77	±	545.241	TT[18]	799.52	±	445.351
	CT[33]	1106.58	±	603.007	CT[9]	912.814	±	358.233
	CC[13]	805.249	±	593.986	CC[6]	344.727	±	415.394
			0.0699				**0.01261**	
rs3735586	TT[37]	939.979	±	560.870	TT[17]	629.061	±	453.422
	TA[27]	1179.84	±	592.200	TA[13]	888.709	±	396.405
	AA[7]	1589.47	±	486.431	AA[3]	809.262	±	649.969
			0.0187				**0.2499**	

*Values are the mean ± SEM units/ litre for paraoxon, adjusted for disease, age , BMI and smoking. n values in parenthesis adjusted for, disease, age , BMI and smoking. #Values are *P* values based on square root transformed data adjusted for age, BMI, and smoking . NA-- not apllicable for that subset.

Table 23 (Cont'd).

	Pittsburgh whites (cases+controls)				Chicago whites (cases+controls)			
	Genotype [Counts]	Mean*	±	SD	Genotype [Counts]	Mean	±	SD
rs17876183	GG[69]	1105.507	±	597.963	GG[32]	762.433	±	450.835
	GA[1]	823.8448	±	NA	GA[1]	277.173	±	NA
	AA[0]		±		AA[0]	NA	±	NA
			0.701#				**0.271**	
rs17876193	CC[65]	1122.503	±	574.165	CC[30]	741.797	±	464.601
	CG[6]	799.737	±	772.895	CG[1]	462.773	±	NA
	GG[0]	NA	±	NA	GG[0]	NA	±	NA
			0.0777				**0.739**	
rs17876116	GG[69]	1110.67	±	593.894	GG[33]	747.729	±	451.704
	GT[1]	467.3668	±	NA	GT[0]	NA	±	NA
	TT[0]	NA	±	NA	TT[0]	NA	±	NA
			0.3426				**NA**	
rs1639	TT[60]	1165.924	±	593.045	TT[24]	686.410	±	432.132
	TG[7]	581.8786	±	442.863	TG[3]	807.886	±	197.813
	GG[1]	561.5474	±	NA	GG[1]	610.929	±	NA
			0.0174				**0.827**	
rs17876205	GG[70]	1101.483	±	594.568	GG[32]	757.281	±	453.546
	GC[0]	NA	±	NA	GC[0]	NA	±	NA
	CC[0]	NA	±	NA	CC[0]	NA	±	NA
			NA				**NA**	
rs13306702	GG[64]	1110.254	±	599.636	GG[28]	764.920	±	466.053
	GC[6]	1007.934	±	580.047	GC[5]	651.456	±	390.511
	CC[0]	NA	±	NA	CC[0]	NA	±	NA
			0.7445				**0.586**	
rs9641164	AA[64]	1148.419	±	586.746	AA[30]	763.871	±	469.346
	AT[7]	608.8998	±	434.344	AT[2]	495.686	±	206.291
	TT[0]	NA	±	NA	TT[1]	631.613	±	NA
			0.01107				**0.963**	
rs2286233	AA[38]	1166.023	±	533.417	AA[19]	748.606	±	448.896
	AT[26]	1089.439	±	694.531	AT[13]	752.372	±	491.105
	TT[6]	744.9265	±	419.596	TT[0]		±	
			0.218				**0.913**	
rs6954345(Ser311Cys)	CC[38]	935.7802	±	549.982	CC[23]	739.836	±	487.192
	CG[29]	1306.135	±	595.324	CG[6]	940.929	±	356.099
	GG[4]	1080.888	±	654.597	GG[3]	562.693	±	293.168
			0.0365				**0.439**	

*Values are the mean ± SEM units/ litre for paraoxon, adjusted for disease, age , BMI and smoking. n values in parenthesis adjusted for, disease, age , BMI and smoking. #Values are *P* values based on square root transformed data adjusted for disease, age, BMI, and smoking . NA-- not apllicable for that subset.

5.9.3 Multiple regression analyses of *PON2* SNPs with PON activity

Our previous studies on the SLE cohort have identified *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 to be the significant contributors to PON activity variation. Therefore, before performing multiple regression of *PON2* variants along with these known contributors, pairwise LD was analyzed between all the selected 19 *PON2* SNPs and *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 variants. This pairwise LD was computed separately for each ethnic group, at the two recruitment sites (Pittsburgh and Chicago).

5.9.3.1 LD pattern of *PON2* SNPs with *PON1* and *PON3* variants in whites

Figures 17 and 18 show the LD structure of the selected 19 *PON2* variants with *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 for the Pittsburgh whites and Chicago whites, respectively. Tagger analysis (r^2 cut-off $\geq$ 0.7) of all these variants in Pittsburgh whites (cases+controls) was similar to that of Chicago whites (cases+controls), results summarized in Table 24. *PON2*/Ser311Cys(rs6954345) showed significant LD with *PON3*/10340 in Pittsburgh whites (cases+controls) [D'=0.91, r^2=0.78) and Chicago whites (cases+controls) [D'=0.88, r^2=0.74]. This SNP *PON2*/rs6954345(Ser311Cys) also tagged three other *PON2* variants [(*PON2*/rs3735586, *PON2*/rs12534274 and *PON2*/rs11545941(Ala148Gly)]. Another *PON3* polymorphism, *PON3*/2115 was also identified to be in significant LD with *PON2*/rs17876193 in Pittsburgh whites (cases+controls) [D'=91, r^2=0.82] and in Chicago whites (cases+controls) [D'=93, r^2=0.73]. Two other highly correlated *PON2* SNP pairs were *PON2*/rs11981433 & *PON2*/rs12704795. Of these highly correlated SNP pairs, the *PON2* variants that were included in linear multiple regression analyses for PON activity were *PON2*/rs6954345(Ser311Cys), rs11981433, rs9641164 and rs17876193.

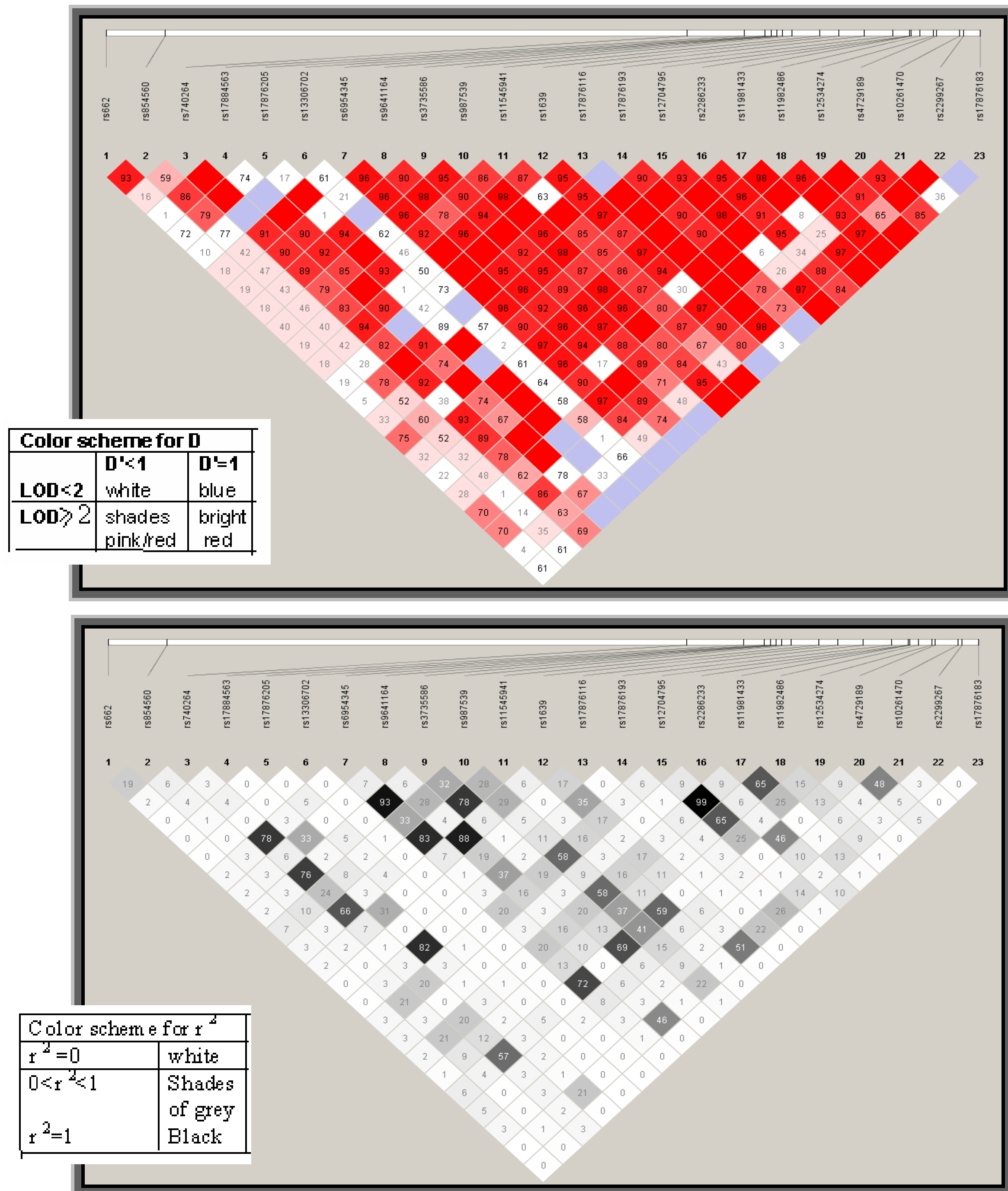


Figure 17. LD plot of 19 *PON2* SNPs with *PON1*/192(rs662), *PON1*/55(rs854560), *PON3*/10340(rs740264) and *PON3*/2115(rs178764563 in Pittsburgh whites (cases+controls).

Total: 797 individuals (after 3 individuals missing >50% genotype are excluded)

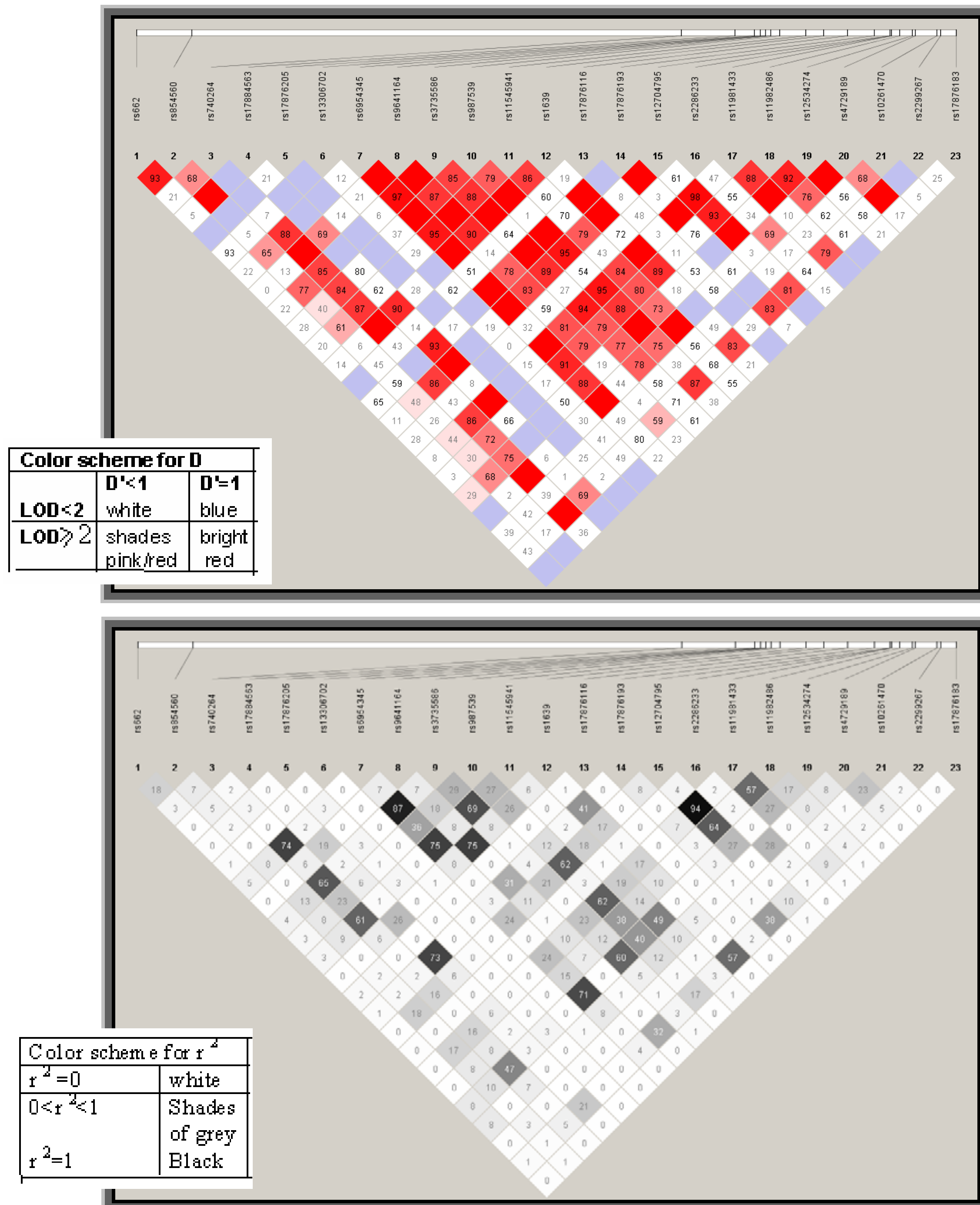


Figure 18. LD plot of 19 PON2 SNPs with PON1/192(rs662), PON1/55(rs854560), *PON3*/10340(rs740264) and PON3/2115(rs178764563 in Chicago whites (cases+controls).

Total: 124 individuals (after 3 individuals missing >50% genotype are excluded)

Table 24. Tagger analyses ($r^2 \geq 0.7$) of *PON2* SNPs with *PON1 and *PON3*** in Pittsburgh whites[#] and Chicago whites[##]**

Test	Alleles Captured
rs6954345 (Ser311Cys)	rs3735586, rs11545941 (Ala148Gly), rs12534274,***PON3*/rs740264 (10340)**
rs17876193	***PON3*/rs17884563 (2115)**
rs1639	rs9641164
rs12704795	rs11981433
rs11982486	
rs10261470	
rs17876183	
rs987539	
***PON1*/rs854560 (L55M)**	
rs2299267	
rs17876116	
rs4729189	
rs2286233	
***PON1*/rs662 (Q192R)**	
rs13306702	
rs17876205	

**PON1* SNPS are *PON1*/rs662 (Q192R), *PON1*/rs854560 (L55M), ***PON3* SNPs are *PON3*/rs740264(10340) and *PON3*/rs17884563 (2115) SNPs
797 individuals in Pittsburgh white (cases+ controls)
##124 individuals in Chicago white (cases+ controls)

5.9.3.2 LD pattern of *PON2* SNPs with *PON1* and *PON3* variants in blacks

Table 25 shows the tagger analyses (cut off $r^2 \geq 0.07$) of *PON2* variants with *PON1(PON1/192* and *PON1/55)* and *PON3 (PON3/10340* and *PON3/55)* SNPs in Pittsburgh blacks (cases+controls). The black sample in Chicago was considerably small therefore, LD pattern was analyzed only for Pittsburgh blacks (Figure 19). In Pittsburgh blacks (cases+controls) significant LD was observed between *PON2*/rs6954345 (Ser311Cys) and SNPs [*PON2*/rs3735586, *PON2/*rs12534274], *PON3*/2115 and SNPs [*PON2*/rs17876193 and *PON2/*rs9641164] and *PON2*/ rs12704795 and SNPs [*PON2*/rs11981433, rs11982486].

Table 25. Tagger analyses ($r^2 \geq 0.7$) of *PON2* SNPs with *PON1 and *PON3*** in Pittsburgh blacks**

Test	Alleles Captured
rs6954345 (Ser311Cys)	rs3735586, rs11545941 (Ala148Gly),rs12534274
PON3/rs740264 (10340)	
***PON3*/rs17884563 (2115)**	rs17876193,rs9641164
rs1639	
rs12704795	rs11981433,rs11982486
rs10261470	
rs17876183	
rs987539	
***PON1/*rs854560 (L55M)**	
rs2299267	
rs17876116	
rs4729189	
rs2286233	
***PON1/*rs662 (Q192R)**	
rs13306702	
rs17876205--monomorphic not included	

**PON1* SNPS are *PON1*/rs662 (Q192R), *PON1*/rs854560 (L55M), ***PON3* SNPs are *PON3*/rs740264(10340) and *PON3*/rs17884563 (2115) SNPs

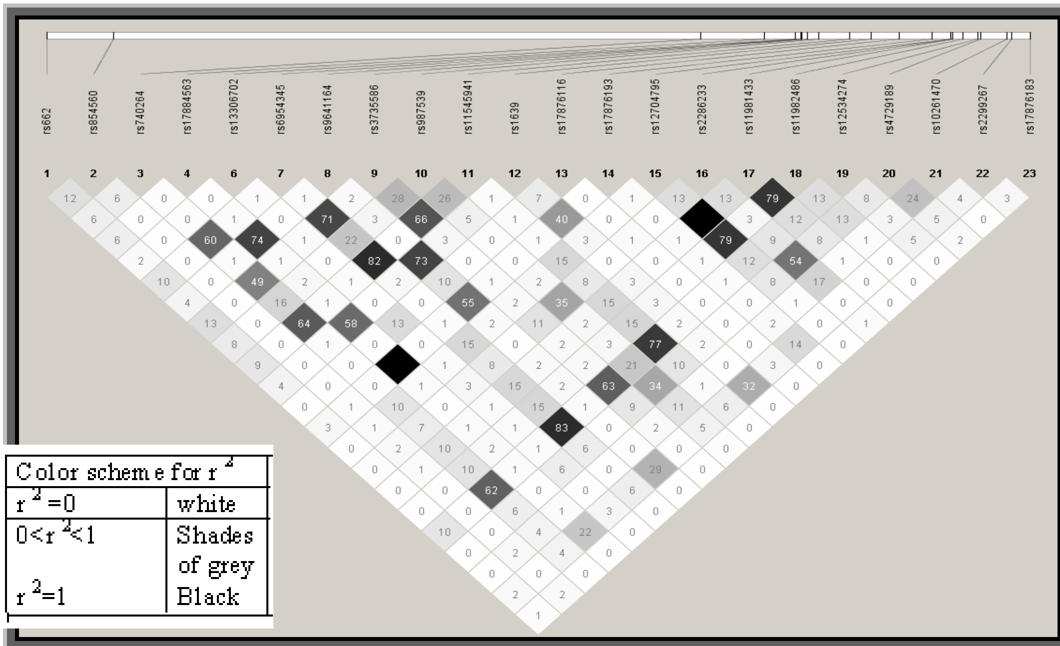


Figure 19. LD plot of 19 *PON2* SNPs with *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 in Pittsburgh blacks (cases+controls).

5.9.3.3 Multiple regression analyses for PON activity in whites

Table 26 summarizes the results of multiple regression analyses of *PON2* SNPs (along with *PON1* and *PON3*) with PON activity in Pittsburgh whites (cases+controls).

For the Pittsburgh recruitment site in combined (cases+controls) whites, linear multiple regression analyses with PON activity-associated but not strongly correlated (r^2 < 0.7) SNPs which included 7 *PON2* SNPs along with *PON1*/192, *PON1*/55 and *PON3*/2115, identified 2 *PON2* associations, *PON2*/rs6954345(Ser311Cys) and *PON2*/rs987539 with a *p* value of $8.732x10^{-6}$ and 0.046, respectively with PON/paraoxon activity, in addition to *PON1* and *PON3*. The overall contribution of these two *PON2* SNPs rs6954345(Ser311Cys) and *PON2*/rs987539 to the variation in PON activity were 1.3% and 0.3%, respectively.

Table 26 summarizes the results of multiple regression analyses of *PON2* SNPs (along with *PON1* and *PON3*) with PON activity for Chicago whites (cases+controls). For the Chicago recruitment site in combined (cases+controls) whites, when PON activity-associated but not strongly correlated (r^2< 0.7) SNPs [*PON1*/192, *PON1*/55, *PON3*/2115, *PON2*/rs6954345(Ser311Cys)] were analyzed in a multiple regression model, no association was observed with *PON2*.

5.9.3.4 Multiple regression analyses for PON activity in blacks

Table 26 shows the results of multiple regression analyses of *PON2* SNPs (along with *PON1* and *PON3*) with PON activity in Pittsburgh blacks (cases+controls). In combined (cases+controls) blacks when PON activity-associated but not strongly correlated (r^2 < 0.7) SNPs [*PON1*/192, *PON1*/55, *PON3*/2115, *PON3*/10340, *PON2*/rs6954345(Ser311Cys), *PON2*/rs1639] were

evaluated in a multiple regression model, no association was observed with any of the *PON2* variants.

Table 26. Multiple Linear regression of *PON2* variants with *PON1* and *PON3**

Pittsburgh (white)			Chicago (white)			Pittsburgh (black)		
FACTORS	***P***	**R2**	**FACTORS**	***P***	**R2**	**FACTORS**	***P***	***R2***
disease	6.12E-10		disease	0.270755		**disease**	0.026637	
age	0.18901		age	0.119893		age	0.13728	
bmi	0.03749		bmi	0.814689		bmi	0.102469	
smokever	0.11464		smokever	0.065752		smokever	0.168158	
PON1/192	< 2.2e-16	0.4148	PON1/192	< 2.2e-16	0.4151	PON1/192	5.93E-08	0.3400
PON1/55	1.15E-08	0.0214	PON1/55	0.002538	0.0250	PON1/55	0.330154	
PON3/2115	1.79E-05	0.0214	PON3/2115	0.154909		PON3/2115	0.809229	
PON2/rs6954345(Ser311Cys)	8.73E-06	0.0128	PON2/rs6954345(Ser311Cys)	0.231924		PON2/rs6954345(Ser311Cys)	0.199363	
PON2/rs987539	0.04607	0.0028				PON2/rs1639	0.556426	
PON2/rs11981433	0.32783					PON3/10340	0.003333	0.0500
PON2/rs2299267	0.06136							
PON2/rs4729189	0.55033							
PON2/rs11982486	0.16634							
PON2/rs10261470	0.68259							

* *PON1* and *PON3* SNPs -*PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115

6.0 DISCUSSION

The Lupus Foundation of America estimates that approximately 1.5 million US residents and more than 5 million people all over the world are suffering from SLE with the prevalence varying between 20-60 cases per 100,000 (Danchenko et al. 2006), which is more pronounced in women than men. Females in their child bearing ages (15-44) years are reported to have the highest incidence rates and within the same gender, African American women show 3 times higher risk of SLE than Caucasians (Danchenko et al. 2006). Majority of deaths in SLE patients are attributed to CHD, which is seemingly high and even exceeds the mortality rates from all types of cancer (Manzi et al. 2000). SLE poses a significant public health problem, since the disease symptoms are similar to many other common disorders and often people with SLE go unnoticed in their early stages. Medical care by use of immunosuppressive drugs has increased the life span of lupus patients, but lack of absolute cure is troubling. Given the heterogeneity in SLE symptoms, continuing efforts through research work to understand the underlying mechanism for this autoimmune disorder and its related complications would help in better prognosis of the disease.

Like other complex disorders, SLE predisposition is determined by the combined effects of genetic and environmental factors. Several studies have established that genetic elements strongly influence SLE risk with possible involvement of multiple genes on several chromosomes (Deapen et al. 1992, Sestak et al. 1999, Lawrence et al. 1987). A linkage peak with

a LOD score of 2.40 on chromosome 7q21.1 at 77.5 Mb for SLE was identified by Gaffney et al. (2000). *PON* multigene family (*PON1*, *PON2* and *PON3*), which clusters on 7q21-22 at 94.5-94.6 Mb, is in close vicinity to this linkage peak for SLE and hence *PON2* qualifies as a positional candidate for SLE. *PON2*, the oldest member of the *PON* cluster shares high degree of sequence and structural and functional similarity with the rest of its members, as all of them arose by gene duplication (Primo-Parmo et al. 1996). Although our earlier studies have not found any clear cut association with *PON1* (except for few modest associations with *p* value ranging between 0.03-0.04) (Tripi et al. 2006), or *PON3* (Sanghera et al. 2008) SNPs and SLE risk, we and others demonstrated a significant association between SLE risk and PON/paraoxon activity (Tripi et al. 2006, Alves et al. 2002, Kiss et al. 2007). In this study we have evaluated the role of the genetic variation of the remaining member of this gene family, *PON2,* with SLE risk and its related phenotypes.

PON activity is under strong genetic influence with a major contribution from the *PON1* genetic variation (Tripi et al. 2006, Carlson et al. 2006). Studies have shown that PON/paraoxon activity is a better predictor than *PON* genotypes for CHD as well as for SLE risk (Durrington et al. 2001, Mackness et al. 2004, Ayub et al. 1999, Jarvik et al. 2000, Mackness et al. 2001, Jarvik et al. 2003, Rozek et al. 2005, Tripi et al. 2006). *PON1*/192, *PON1*/55, *PON3*/10340 and *PON3*/2115 genetic variants were found to explain ~ 30 % of the variation in PON activity in our SLE sample (Tripi et al. 2006, Sanghera et al. 2008). Since role of *PON2* genetic variation in relation to PON activity has not been reported, another purpose of this study was to evaluate the relation of *PON2* genetic variation with PON activity regardless of the SLE status. In our study sample PON activity was measured using paraoxon, commonly used in human and animal models to measure PON activity.

CHD develops from atherosclerosis, where LDL oxidation plays a pivotal role. One of the major counter forces to this LDL oxidation is credited to the anti-oxidant function of the *PON* gene cluster (Ng et. al. 2005), established both *in vivo* (Ng et al. 2006) and *in vitro* (Ng et al. 2001). Not only *PON1*, *PON2* and *PON3* differ in their expression profiles, but they also differ in their anti-oxidant properties. Unlike *PON1* and *PON3,* which are mainly expressed in liver and secreted in blood where they get bound to HDL, *PON2* is not HDL bound rather it is ubiquitously expressed and remains intracellular associated with membrane components (Ng et al. 2001, Ng et al. 2005). *In vitro* studies have shown PON2 expression in cells (smooth muscle cell, macrophages and endothelial cells) that take part in the atherosclerotic process (Ng et al. 2001, Horke et al. 2007). Therefore *PON2* is proposed to provide intracellular protection against oxidative stress in arterial wall (Reddy et al. 2008). Rabbit *PON3* is found to inhibit LDL oxidation 100 times more efficiently than rabbit *PON1* (Dragonov et al. 2000), although *PON3* is found in very low levels compared to *PON1* in rabbit blood (Dragonov et al. 2000). On the other hand, human *PON2* shows similar ability to that of rabbit *PON3* in inhibiting LDL oxidation (Rosenblat et al. 2003). Functional studies showing anti-oxidant property of *PON2* have revealed that over expression of *PON2* in HeLa cells can inhibit LDL lipid peroxide formation, invert oxidation of mildly oxidized LDL (MM-LDL), and thereby inhibit monocyte chemotaxis by MM-LDL (Ng et al. 2001). Although PON2 protein is not detected in human serum, LDL isolated from mice serum treated with adenoviral mediated *PON2* (Ad*PON2*) showed significantly lower levels of oxidation (Ng et al. 2006). Both PON activity and *PON2* genetic variation have been reported to be associated with CHD risk (Sanghera et al. 1998, Chen et al. 2003, Leus et al. 2001). Therefore *PON*2 is a good biologic candidate gene for CHD risk in SLE. In this study we have also evaluated the impact of *PON2* polymorphisms with different

parameters of LDL oxidation and subclinical carotid vascular disease measures (carotid plaque and carotid IMT) in our SLE cases.

A total of 19 *PON2* tagSNPs, including two coding SNPs *PON2*/Ala148Gly (rs11545941) and *PON2*/Cys311Ser(rs6954345), were selected from two databases (HapMap and Seattle) in order to maximize the information that would allow covering almost all the common genetic variation within the entire *PON2* gene span of 30 Kb. All tagSNPs, except *PON2*/rs17876183 (MAF=3%, located at 5'-UTR) and *PON2*/rs17876205 (MAF=2%, located at 3'-flanking region) had MAF≥ 5% in either database. Even though all these 19 variants were reported as tagSNPs in at least one of the two databases, a total of 15 tagSNP bins were identified in our white (Pittsburgh+Chicago) sample using tagger analysis ($r^2 \geq 0.8$). In our black sample (Pittsburgh+Chicago), tagger analysis identified a total of 18 tagSNPs ($r^2 \geq 0.8$), though some similarities in LD pattern have been noted between blacks and whites. One SNP was not included in LD analysis because it was monomorphic (*PON2*/rs17876205) in blacks. The minor allele frequencies in whites and blacks for the analyzed SNPs were similar to those reported in HapMap database. Genotype distribution in almost half of the selected *PON2* tagSNPs were significantly different ($P<0.01$) between Caucasians (n=927) and African Americans (n=131), therefore all association analyses were performed separately in blacks and whites. Because our black population was small, SLE related sub-phenotype analyses were not performed as it would not be statistically meaningful.

PON/paraoxon activity at the Pittsburgh recruitment site was 59 % higher for blacks than whites and within each ethnic group higher in controls than cases (17% in each ethnic group) (Tripi et al. 2006). Similarly PON/paraoxon activity at the recently collected Chicago sample was higher in blacks (twice as much) and within each ethnic group higher in controls than cases

(~16% higher in whites, ~5% higher in blacks). In combined (cases+controls) Pittsburgh Caucasian sample, 11 SNPs [*PON2*/rs10261470, rs4729189, rs12534274, rs11982486, rs11981433, rs12704795, rs11545941(Ala148Gly), rs987539, rs3735586, rs6954345 (Ser311Cys), rs2299267] showed significant association with PON/paraoxon activity after adjusting for disease status, age, BMI and smoking (p value ranging between 0.027-2.2x10^{-16}). These 11 SNPs belonged to 7 tagSNP bins according to our tagger analysis using r^2 cut-off $\geq$ 0.7. The most strongly associated tagSNP bin included 4 SNPs (p value ranging between 1.43x10^{-11}-2.2x10^{-16}) of which two were coding SNPs [*PON2*/Ala148Gly(rs11545941), *PON2*/Ser311Cys(rs6954345), rs3735586, rs12534274]. One of the *PON3* SNPs (*PON3*/10340) that was reported to be strongly associated with PON activity, was in high LD with this most strongly associated tagSNP bin of 4 *PON2* SNPs (r^2 ranging between 0.78-0.57). Among these, *PON2*/rs6954345(Ser311Cys) showed the highest correlation with *PON3*/10340 (D'=0.91,$r^2 \geq$ 0.78). When all PON activity-associated but not strongly correlated (r^2 <0.7) *PON1*, *PON2* and *PON3* SNPs [*PON1*/192, *PON1*/55, *PON3*/2115, *PON2*/rs6954345(Ser311Cys), *PON2*/rs987539, *PON2*/rs11981433, *PON2*/rs2299267, *PON2*/rs4729189, *PON2*/rs11982486, *PON2*/rs10261470] were included in a multiple regression model, only two *PON2* SNPs remained significant [*PON2*/rs6954345(Ser311Cys),P =8.732x10^{-6}, *PON2*/rs987539,P=0.046], in addition to *PON1* and *PON3* SNPs. The overall contribution of these two *PON2* SNPs to the variation in PON activity were 1.3% and 0.3%, respectively. The only noteworthy highly significant *PON2* SNP rs6954345(Ser311Cys), was the same SNP that was highly correlated with the strongly associated *PON3* SNP (*PON3*/10340). In combined (cases+controls) Chicago Caucasian sample, 4 SNPs [*PON2*/rs6954345 (Ser311Cys), rs12534274, rs3735586, rs11545941(Ala148Gly),] showed significant association (p values ranging from 0.002-0.0001)

with PON/paraoxon activity after adjusting for disease status, age, BMI and smoking. These 4 SNPs belonged to the same bin that showed highly significant association with PON activity in Pittsburgh site and was also in high LD with the *PON3* SNP (*PON3*/10340) that is known to modulate the PON activity. Because the pairwise tagging of *PON2* SNPs with *PON3* and *PON1* in Chicago Caucasian sample were similar to that of Pittsburgh Caucasian sample, again the same *PON2* SNP rs6954345(Ser311Cys) showed the highest correlation with *PON3*/10340 (D'=0.88, $r^2 \geq 0.74$). However, unlike Pittsburgh whites (cases+controls), *PON2*/rs6954345 (Ser311Cys) was no longer significant in Chicago whites (cases+controls) when all PON activity-associated but not strongly correlated ($r^2 < 0.7$) *PON1, PON2* and *PON3* variants [*PON1*/192, *PON1*/55, *PON3*/2115, *PON2*/rs6954345(Ser311Cys)] were analyzed in a multiple regression model. This lack of *PON2* association with PON activity in white Chicago sample could be attributed to lack of power due to their considerable small size to that of Pittsburgh Caucasians, even though few *PON2* variants showed significant association in the Chicago Caucasian combined (cases+controls) sample. Similarly in Pittsburgh blacks, *PON2* variants were no longer significant in multiple regression model again probably due to lack of power in a small sample size. Unlike PON1 and PON3 enzymes, that are bound to circulating HDL in the blood, PON2 enzyme is known to be predominantly intracellular and membrane bound (Ng et al. 2006, Levy et al. 2007, Horke et al. 2007). Therefore, one would not expect a major contribution of *PON2* towards serum PON activity. However, one cannot exclude the possibility of a low level background secretion of PON2 enzyme into blood (Ng et al. 2001) and / or the presence of a specific secreted isoform which might have been overlooked in previous studies. Although recent studies mainly focused on two PON2 isoforms (Shamir et al. 2005, Horke et al. 2007), Mochizuki et al. (1998) reported that alternative splicing of PON2 transcript results in several

PON2 splice forms. Nevertheless, the fact that we did not identify an independent major effect of *PON2* variation on serum PON activity argues against the presence of a secreted PON2 isoform or even such an isoform exists, it does not seem to exhibit an independent activity for paraoxon substrate. A study that used recombinant human *PON1*, *PON2* and *PON3* found that *PON2* had minimal contribution to the paraoxonase or arylesterase activity (Dragonov et al. 2005). Because the only *PON2* tagSNP bin that showed strong association with serum PON activity is in high LD with a strongly associated *PON3* SNP, the observed *PON2* association is very likely an indirect result from this high LD. When the mean PON/paraoxon activities of the *PON2*/rs6954345(Ser311Cys) were compared to the *PON3*/10340 *SNP*, both showed an increasing PON activity associated with their minor alleles. Unlike the *PON2/*rs6954345 (Ser311Cys) SNP, *PON3*/10340 SNP resides within an intron. However, the latter tags several putative functional *PON3* promoter SNPs as well as a synonymous *PON3* coding SNP. Overall, our results support that circulatory HDL-bound *PON1* and *PON3* are the main modulators of serum PON activity, with a major contribution from *PON1* (Tripi et al. 2006, Sanghera et al. 2008, Carlson et al. 2006). Lack of complete knowledge about the natural substrate for *PON2* is a major limiting factor in evaluating the role of *PON2* genetic variation with PON2 enzymatic activities.

Although several studies linked *PON2* variants to various diseases like amyotrophic lateral sclerosis (Valdmanis et al. 2008), stroke (Slowik et al. 2007), Alzheimer's disease (Janka et al. 2002), CHD (Sanghera et al. 1998,Chen et al. 2003, Martinelli et al. 2004), and metabolic traits (Boright et al. 1998) with some controversial findings (Wheeler et. al. 2004) there is no published study that links *PON2* with SLE risk. The results of our study do not suggest a major contribution of *PON2* genetic variation with SLE risk neither in single-site or haplotype

analyses. Similarly only modest contribution of the *PON1* or *PON3* variation to SLE risk has been found for the SNPs that have been analyzed to date (Tripi et al. 2006, Sanghera et al. 2008).

Lupus nephritis and premature cardiovascular disease are the two major causes of mortality in SLE patients (Foster et al. 2007, Manzi et al. 1997). Some studies have reported associations of *PON2* genetic variation and or PON/paraoxon activity with renal dysfunction/failure or various nephropathies (Calle et al. 2006, Pinizzotto et al. 2001, Hasselwander et al. 1998). Our group has previously reported modest allele and genotype associations of 3 *PON1* promoter polymorphisms with Caucasian lupus nephritis patients (Tripi et. al. 2006). Similarly, we also observed some modest associations of lupus nephritis with *PON2* genetic variation in white cases (rs17876183, rs10261470, rs987539, rs9641164, and rs17876205, p values ranging between 0.016-0.033 for genotype distribution). Of these five SNPs, only two (*PON2*/rs17876183 and rs17876205) were significant for both genotype distribution and allele frequency difference between Caucasian SLE cases with and without nephritis, with their minor alleles associated with increased risk. Because our lupus nephritis sample size was relatively small and associations were modest and would not survive multiple testing corrections, our findings await replication in larger SLE patient samples. Unlike SLE disease status, no association was reported between PON/paraoxon activity and lupus nephritis in our sample (Tripi et al. 2006). These five SNPs that showed association with lupus nephritis were different from the *PON2* SNP that showed association with nephropathy in Type 1 and Type 2 diabetes (Calle et al. 2006).

Given the important role of LDL oxidation in atherosclerosis and CHD risk, and the known effect of *PON2* on LDL-oxidation, we next evaluated whether *PON2* genetic variation had any impact on the parameters of LDL oxidation. The data on LDL oxidation were available

on nine parameters [antibody (IgG) for copper-modified oxLDL , antibody for (IgM) for copper-modified oxLDL , antibody (IgG) for MDA-modified oxLDL, anti-MDA-modified LDL (IgM)], E06 antibody for oxLDL-EO6 epitope, antibody (IgG) for LDL immune complexes , antibody Ig(M) for LDL immune complexes , and antibodies for Lp(a) and Lp(a) Mg] in our Pittsburgh SLE cases only. Six SNPs that showed significant association with different parameters of LDL oxidation were *PON2*/rs13306702 with Ig(M) antibodies for copper-modified oxLDL (*P* = 0.0343), *PON2*/rs17876205 with E06 antibody (*P*=0.031), *PON2*/rs10261470, *PON2*/rs4729189 and *PON2*/rs2286233 with Ig(M) antibodies for LDL immune complex (*P*= 0.0193, 0.0282, and 0.0453, respectively), *PON2*/rs2286233 and *PON2*/rs11545941(Ala148Gly) with Ig(M) antibody for MDA modified LDL (*P*=0.0016 and 0.0282, respectively). One of these SNPs [*PON2*/rs11545941(Ala148Gly)] was reported to be associated with susceptibility to LDL oxidation in another study that evaluated *PON2* tagSNPs along with *PON1* and *PON3* (Carlson et al. 2006). Again our population size was small and many of those associations were modest and would not survive corrections for multiple testing. Perhaps the most noteworthy was the association of *PON2*/rs2286233 with two oxLDL parameters [*P*=0.0016 for Ig(M) antibodies against MDA modified LDL and *P*= 0.0453 for Ig(M) antibodies against LDL immune complex]. We did not find any association between oxLDL parameters and PON activity, consistent with others (Kiss et al. 2007, Carlson et al. 2006). Carlson et al. (2006) found association of PON1 genetic variation but not PON activity with oxidized LDL. There are conflicting reports about association of oxLDL with atherosclerosis in SLE patients (Frostegard et al. 2005, Hayem et al. 2001). Therefore we also checked a possible relation between the parameters of LDL oxidation and subclinical CVD measures (carotid plaque and carotid IMT) but did not detect any association. Oxidation of LDL

creates antigenic epitopes on the oxLDL particle which leads to antibody production against oxLDL. Even though oxidation of LDL is considered to be a key factor in atherosclerosis, not all studies support the idea that these antibodies to oxLDL help in accelerating atherosclerosis (Shoenfeld et al. 2002, Frostegard et al. 2007, Frostegard et al. 2007). There are conflicting reports about the relation between oxLDL parameters and the subclinical CVD measures (Hulthe et al. 2002, Wallenfeldt et al. 2004). A recent report by multiethnic study of atherosclerosis (MESA) concluded that association of oxLDL with subclinical CVD (P=0.0002) was through its relationship with other cardiovascular risk factors, (total cholesterol, HDL-cholesterol and triglycerides) as the adjustment for those other factors attenuated this association (P= 0.026) (Holvoet et al. 2007).

Next we wanted to check the association of *PON2* SNPs directly with subclinical CVD measures. In our study 3 *PON2* SNPs (rs11982486, and rs11981433, and rs12704795) showed modest association with carotid plaque under codominant model (p value 0.03, 0.03 and 0.01 for genotype distribution) in combined (Pittsburgh +Chicago) white SLE sample after adjusting for recruitment site, age, BMI, smoking and lipid profile. Two of these 3 *PON2* SNPs (*PON2*/rs11981433 and *PON2*/rs12704795) showed association for both allele frequency difference (P= 0.0127 and 0.0122) and genotype distribution (P= 0.03 and 0.01). These two variants (*PON2*/rs11981433 and rs12704795) were also in high LD (D'=0.99, r^2=0.98). Based on the biological model where *PON2* deficient mice developed significantly larger atheromatosus lesions (Ng et al. 2006) as compared to their wild and heterozygous counterparts, suggesting a recessive model, associations of these SNPs were further checked under recessive model. Under recessive model two of these SNPs rs11981433, and rs12704795 showed higher significance for their association with carotid plaque (0.016 and 0.0099, respectively) with minor alleles

providing a protective effect. The covariate adjusted OR under recessive model, was 0.32 (95% CI=0.12-0.81, *P*=0.016) for *PON2*/rs11981433T>C SNP, and 0.28 (95%CI=0.10-0.73,*P*=0.0099) for *PON2*/rs12704795T>G. Carotid IMT analysis in the combined (Pittsburgh+Chicago) white SLE cases revealed modest association with 2 SNPs (*PON2*/rs12704795 and rs17876205) under codominant model with *p* values 0.037 and 0.017, respectively. When analyzed under recessive model, rs12704795 showed increased significance with a *p* value of 0.01, while for *PON2*/rs17876205 no *p* value could be computed as no individuals were homozygous for the minor allele. The latter SNP was associated with increased IMT although there were only 6 heterozygous individuals with IMT information, because of the low MAF (1.6%). Next we checked whether the three SNPs that showed association with carotid plaque would be associated with carotid IMT. In fact one of these two SNPs (*PON2*/rs11981433) was found to be associated with carotid IMT (*P*=0.03) under recessive model in addition of being protective for carotid plaque. The 2 SNPs (*PON2*/rs11981433 and rs12704795) that showed association with both protection of plaque and decreasing IMT were in high LD (D'=0.99, r^2=0.98). Consistent association of these 2 SNPs (*PON2*/rs11981433 and rs12704795) with carotid IMT and plaque suggest that *PON2* genetic variation might have a genuine effect (protective) effect on subclinical carotid vascular disease. Both of these SNPs are located in intron1 suggesting that they may not be the true causative variants, but rather capturing effects of the functional variants. Because those two SNPs (rs11981433 and rs12704795) are different than the *PON2* SNPs that are found to be associated to oxLDL, we may speculate that they may be exercising their effect through mechanisms that are different than LDL oxidation. Recent functional studies have reported changes in *PON2* expression in human carotids during progression of atherosclerosis or in the macrophages in patients with hypercholesterolemia (Fortunato et al. 2007, Rosenblat et al.

2003). Unlike *PON1* and *PON3*, *PON2* is expressed in several cells that contributed to atherosclerotic process (Ng et al. 2001, Horke et al. 2007). It is possible that *PON2* protects those cells against oxidative stress and impairment of *PON2* activity may change the behavior of those cells in favor of the atherosclerotic event. Similar to oxLDL parameters, our studies did not find any association between carotid plaque or carotid IMT with PON activity.

The limitations of our study include the lack of a control group with subclinical CVD measures (analyses were performed only in SLE cases) that would have allowed us to establish SLE related and non-related effects on CVD risk, as well as a lack of a number of elderly individuals (>60 yrs of age) that would allow us to distinguish between premature and age-related CVD risk.

In conclusion, our results suggest that *PON2* may have influence on LDL oxidation and subclinical CVD risk. Given the small size of our sample this initial results will need replication in larger samples.

In summary, the main findings from our study where we analyzed the impact of *PON2* tagSNPs with PON activity, SLE risk, lupus nephritis, parameters of LDL oxidation and subclinical carotid vascular disease (carotid IMT and carotid plaque) in a large biracial population of >1000 individuals are:

1. Distributions of *PON2* SNPs significantly differ between blacks and whites.
2. Among Pittsburgh whites, *PON2*/rs6954345(Ser311Cys), and rs987539 genetic variants showed significant association with PON/paraoxon activity explaining 1.3 %, and 0.3, respectively of the variation.
3. *PON2* tagSNPs are not obvious contributors to SLE risk, both in whites and blacks.
4. Some modest associations were detected between *PON2* SNPs (rs17876183, rs10261470, rs987539, rs9641164, and rs17876205) and lupus nephritis in white (Pittsburgh+Chiacago) cases, which warrant further studies with large sample size.
5. Among Pittsburgh white SLE cases 6 SNPs (*PON2*/rs11545941(Ala148Gly), rs13306702, rs2286233, rs10261470, rs17876205, rs4729189) showed significant association with different parameters of LDL oxidation (oxLDL).
6. Among white (Pittsburgh+Chicago) SLE cases, 3 SNPs (*PON2*/rs12704795, rs11981433, rs11982486) showed significant association with carotid plaque while 3 SNPs (*PON2*/rs12704795, rs11981433, and rs17876205) showed modest associations with carotid IMT. Two highly correlated SNPs (*PON2*/rs11981433 and *PON2*/rs12704795) showed association with both carotid plaque and IMT.

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