HELSINKI UNIVERSITY BIOMEDICAL DISSERTATIONS, No. 47

Department of Medical Genetics
University of Helsinki
Finland

MAPPING OF SUSCEPTIBILITY GENES FOR SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Sari Koskenmies

Academic dissertation

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in Biomedicum Lecture Hall 3, Haartmaninkatu 8, Helsinki, on the 12th of March 2004, at 12 noon.

Helsinki 2004

Supervised by:
Juha Kere, MD, PhD
Professor
Department of Biosciences at Novum
and Clinical Research Center
Karolinska Institute
Sweden
and
Department of Medical Genetics
University of Helsinki
Helsinki, Finland

Elisabéth Widen MD, PhD
Finnish Genome Center
University of Helsinki

Reviewed by:
Kimmo Aho, MD, PhD
Professor
National Public Health Institute
Helsinki

Maija Wessman, PhD
Docent
Finnish Genome Center
and
Folkhälsan Research Center
Helsinki

Official opponent:
Tom Pettersson, MD, PhD
Docent
Department of Medicine
University of Helsinki

ISBN 952-10-1676-0
ISBN 952-10-1677-9 (pdf version: http://ethesis.helsinki.fi)
ISSN 1457-8433
Yliopistopaino
Helsinki 2004

To Linnea, Elias and Jari-Pekka

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

I Koskenmies S, Widén E, Kere J and Julkunen H. Familial systemic lupus erythematosus in Finland. *The Journal of Rheumatology* 28: 758-760, 2001

II Koskenmies S, Vaarala O, Widén E, Kere J, Palosuo T and Julkunen H. The association of antibodies to cardiolipin, β2-glycoprotein I, prothrombin and oxidized low-density lipoprotein with thrombosis in 292 patients with familial and sporadic SLE. *Scandinavian Journal of Rheumatology* (in press).

III Koskenmies S, Lahermo P, Julkunen H, Ollikainen V, Kere J and Widén E**.** Linkage mapping of systemic lupus erythematosus (SLE) in Finnish families multiply affected by SLE. *Journal of Medical Genetics* 41: e2-5, 2004

IV Koskenmies S, Widén E, Onkamo P, Sevón P, Julkunen H and Kere J. Haplotype associations define target regions for susceptibility loci in systemic lupus erythematosus. *The European Journal of Human Genetics* (in press).

ABBREVIATIONS

aCL	anticardiolipin
ACR	American College of Rheumatology
ANA	antinuclear antibody
Anti- ß2-GPI	anti-ß2-glycoprotein I
APS	antiphospholipid syndrome
aPL	antiphospholipid antibody
CI	confidence interval
CR	complement receptor
DZ	dizygotic
dsDNA	double-stranded DNA
ESRD	end-stage renal disease
FcγR	Fc gamma receptor (a receptor for the Fc portion of immunoglobulin)
HLA	human leukocyte antigen
HPA	hypothalamo-pituitary-adrenal
HPM	haplotype pattern mining
HRT	hormone replacement therapy
HSA	human serum albumin
IC	immune complex
IDB	identity by descent
Ig	immunoglobulin
LA	lupus anticoagulant
LD	linkage disequilibrium
LDL	low-density lipoprotein
LE cell	lupus erythematosus cell
LOD	logarithm of odds
Mb	mega base
MBP	mannose binding protein
MHC	major histocompatibility complex
MPS	mononuclear phagocyte system
MZ	monozygotic
NPL	non-parametric linkage
ox-LDL	oxidised low-density lipoprotein
PARP	poly ADP-ribose polymerase gene
PBS	phosphate-buffered saline
PDCD1	programmed cell death 1gene
PCR	polymerase chain reaction
SLE	systemic lupus erythematosus
Sm	a ribonucleoprotein found in the cell nucleus
TDT	transmission disequilibrium test
λs	sibling recurrence risk

ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease with variable and diverse clinical manifestations. Its etiology is unclear, but based on epidemiological and animal studies both genetic and environmental factors are involved. Several candidate susceptibility loci have been identified in case-control association studies and in linkage studies (Kelly et al. 2002). Recent genome wide scans in different ethnic groups have suggested a number of susceptibility loci for SLE, but no gene has been identified as yet (Moser et al. 1998; Gaffney et al. 1998 and 2000; Shai et al.1999; Gray-McGuire et al. 2000; Lindqvist et al. 2000). These studies clearly indicate that multiple genes are involved in conferring susceptibility to SLE.

In this nationwide study, we first ascertained a large group of patients and their relatives for a genetic study, characterized their phenotypes, conducted a genome-wide scan for SLE susceptibility loci with Finnish multiplex families, and followed the linkage study with a genetic association study in the selected region. The clinical characteristics were evaluated for all identified families, 53 of which were multiply affected by SLE. There were no differences in clinical and laboratory findings between sporadic and familial cases, suggesting that familial and sporadic SLE are the same entity. As an attempt to stratify the families according to a restricted subphenotype, we investigated the presence of antiphospholipid antibodies (aPLs). However, this subphenotype did not cluster in any families, thus providing no tools to subdivide the pedigrees into more homogeneous subsets.

The genome-wide scan was performed on 35 multiplex families. We detected suggestive linkage in regions of chromosomes 6q and 14q, as well as to the HLA region on chromosome 6p. The 14q locus and a partially overlapping region on chromosome 6q have been implicated previously. To obtain additional evidence for susceptibility loci on 6q and 14q, and in order to refine their positions, we added 31 more families from a sub-isolate in central eastern Finland (Savo region), in which founder effects have been noted previously (Nyström-Lahti et al. 1994; Höglund et al. 1995). We performed fine-mapping, at 1 cM density, across the suggestive regions for linkage, and found evidence of excess sharing of a haplotype on chromosome 14q and excess transmission of a haplotype on chromosome 6q. These results confirmed the evidence for SLE susceptibility loci on chromosome 14q and 6q. Moreover, our results are compatible with the concept of a founder effect for susceptibility genes in SLE in central eastern Finland, and suggest a path to the identification of the putative susceptibility genes.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a rare autoimmune disease with female predominance. The prevalence of SLE in Finland is estimated as 28/100 000 (Helve 1985). Diagnosis of the disease is based on 11 American College of Rheumatology (ACR) classification criteria (Tan et al. 1982), of which four are sufficient. The clinical manifestations vary greatly from one person to another, and the course of the disease is characterized by periods of relapse and remission. The pathogenesis behind the disease remains unclear. The main immunological feature is uncontrolled formation of autoantibodies, leading to excess formation of immune complexes which deposit in tissues, causing inflammation and tissue damage.

Based on epidemiological studies, there is clear clustering of SLE patients in families and the risk for siblings or second degree relatives of an affected individual to develop the disease is markedly increased, both supporting the role of genetic components to SLE susceptibility (Hochberg 1987; Vyse and Todd 1996; Sestak et al. 1999). Moreover, the concordance rate of SLE in MZ (~ 25%) is over ten-fold compared to that in DZ (~ 2%) (Deapen et al.1992; Grennan et al.1997). This supports not only a genetic contribution in the development of SLE, but also indicates that multiple genes are involved in disease expression (Wanstrat and Wakeland 2001). The mode of inheritance of SLE is however unknown. The disease is tiggered by environmental factors, such as viruses, certain drugs and sun exposure (Wanstrat and Wakeland 2001).

Several candidate susceptibility loci for SLE have been identified in case-control association studies in man (including the HLA-region, Fcγ receptors, and complement components), and in linkage studies with mouse models (reviewed by Kelly et al. 2002). To date, six genome-wide scans (Moser et al. 1998; Gaffney et al. 1998 and 2000; Shai et al.1999; Gray-McGuire et al. 2000; Lindqvist et al. 2000), using SLE as a phenotype, have been conducted in different ethnic groups. Each revealed several suggestive loci, but only six surpassed the significance threshold of LOD $\geq$ 3.3. Recently, linkage to chromosome 2q37 (LOD 4.24) (Lindqvist et al. 2000), resulted in identification of a new susceptibility gene, programmed cell death 1 gene *(PDCD1)* (Prokurina et al. 2002).

Due to the heterogeneous nature of SLE, recent genome wide scans have focused on specific subphenotypes in order to achieve more homogeneous sets of families, and to increase the statistical power to detect susceptibility genes (Nath et al. 2001 and 2002; Kelly et al. 2002; Namjou et al. 2002a and 2002b; Quintero-Del-Rio et al. 2002; Scofield et al. 2003). In total, 17 regions have been significantly linked to SLE using both model-based and nonparametric approaches, 11 of them from such stratified studies. In addition, several other regions with suggestive linkage have been identified, but only some of those loci have been implicated in more than one study (Gaffney et al. 2000; Wakeland et al. 2001). These data, in addition to the twin studies, suggest that multiple genes are involved in conferring susceptibility to SLE.

The aim of this study was to asscertain a well-characterized group of SLE patients as the basis for genetic studies, i.e. to conduct a genome-wide scan for SLE susceptibility loci in Finnish multiplex families, and further, to fine-map those genomic regions identified in the genome-wide scan as possibly linked to SLE. For this purpose, we evaluated SLE patients from hospital registrations all over Finland, which resulted in identification of more than 80% of all Finnish SLE patients requiring hospital-based treatment. Of the 252 identified families, 53

were multiply affected by SLE. The clinical characteristics of all families were evaluated. We also studied the prevalence of different aPLs, associated with thrombotic events, as one of the subphenotypes (antiphospolipid syndrome) of SLE, in sporadic and familial patients. The aim of these clinical studies was to evaluate the phenotype differences between sporadic and familial SLE as a possible tool for further genetic studies.

REVIEW OF THE LITERATURE

1. Systemic lupus erythematosus

1.1. General

The term lupus (wolf) was already in use in the 13th century for the description of morbid cutaneous conditions, referring to erythematous (red) ulcerations about the face - a disease that eats away, bites, and destroys (Talbott 1993). Later, it was discovered that lupus erythematosus presents itself in two distinct forms: discoid lupus erythematosus, in which the manifestations are restricted only to skin, and systemic lupus erythematosus (SLE), in which many other organs are also affected. SLE, highly variable in its manifestations, is a potentially serious autoimmune disease of unknown cause. SLE is regarded as the prototype of autoimmune diseases, because of the wide variety of clinical and immunological manifestations. Approximately 90% of patients with SLE are women, and half of these are less than 30 of age when diagnosed (Cervera et al. 1993).

The prevalence and incidence rates of SLE vary considerably between different populations. The overall prevalence in the United States and Hawaii ranges between 14.6 to 122 per100 000 inhabitants (Rus et al. 2001). The prevalence of SLE in the United States is three to four times higher among African-Americans than among European-Americans, and manifests at a younger age and is more severe in this group (Fessel 1974; Kaslow and Masi 1978, Lawrence et al. 1998). The prevalence among African-Caribbeans and Asians is also much higher compared to Europeans (Rus et al. 2001).

In predominantly European populations, outside of the United States, the prevalence estimates range from as low as 12.5 /100 000 females in England (Hochberg 1987) to as high as 254/100 000 (both sexes combined) in Northern Ireland (Gourley et al. 1997). In Scandinavia, the prevalence of SLE ranges from 21.7/100 000 in Denmark to 35/100 000 in Iceland and 40/100 000 in Sweden (Voss et al. 1999; Gudmundsson and Steinsson 1990). In Finland the prevalence of SLE is estimated to be 28/100 000 (Helve 1985).

The average annual incidence of SLE in the United States is 1.8 to 7.6 cases per 100 000 persons per year (Rus et al. 2001). In Sweden, Iceland, and the United Kindom reported overall incidence rates for SLE are 4.8, 3.3 and 3.7 to 3.8 per 100 000 persons per year, respectively (Rus et al. 2001).

The diagnosis of SLE is based on both clinical symptoms and laboratory findings. As an aid for diagnosis, the American College of Rheumatology published SLE classification criteria (Tan et al. 1982), as presented in Table 1. While these were later updated (Hochberg 1997), we used the 1982 criteria. A diagnosis of SLE is made if the patient meets at least four of 11 criteria. Low serum complement level supports the diagnosis. A diagnosis of SLE is unlikely in the absence of antinuclear antibodies (ANA), as these are detected sporadically in up to 2% of the female population over the age of 40 (Wakeland et al. 2001).

Table 1. The 1982 systemic lupus erythematosus classification criteria from the American College of Rheumatology (Tan et al. 1982). *

Category	Symptom
Skin criteria	1. Malar (butterfly) rash (over the cheeks and nose)
	2. Discoid rash (scarring rash in sun-exposed areas)
	3. Photosensitivity
	4. Oral ulcerations
Systemic criteria	5. Arthritis (non-erosive)
	6. Serositis (pleurisy or pericarditis)
	7. Renal disorders (proteinuria or cellular casts)
	8. Neurological disorders (seizure or psychosis with no other etiology
Laboratory criteria	9. Hematologic: hemolytic anemia or leukocytopenia or lymphocytopenia or thrombocytopenia
	10. Immunologic: Positive LE cell preparation or anti-dsDNA antibodies or anti-Sm-antibodies or false positive serologic test for syphilis
	11. ANA (antinuclear antibody)

*Any four of these 11 criteria, present serially or simultaneously during any interval of observation, establish the diagnosis of SLE. Table adapted from Wakeland et al. (2001).

1.2. Etiology

1.2.1. Hormonal and environmental factors

The strongest risk factor for developing SLE is being female. The hormonal hypothesis for the development of the disease is supported by the lower female to male sex ratio in early onset (age 0-9; ratio 2:0) and late onset (age 60+, ratio 2.3) SLE, compared to 8:1 among females and males in young adulthood (20-39 years) (Hochberg 1993). Studies of sex hormones in patients with SLE have implicated hormonal aberrations, including high estrogen levels, low androgens, and abnormalities in the prolactin-gonadal axis (Walker and Jacobson 2000; McMurray 2001; McMurray and May 2003). These findings are supported by mouse models of lupus (Roubinian et al. 1977). Moreover, hormones, especially those used as estrogen replacement therapy, have been suggested as etiological factors for SLE (Sanchez-Guerrero et al.1995 and 1997; Meier 1998). Both the past use of oral contraceptive pills (Sanches-Guerrero et al. 1995) and hormone replacement therapy (HRT), especially when used for longer periods, are associated with a slightly increased risk of developing SLE (Sanches-Guerrero et al. 1997; Meier et al. 1998).

There is preliminary evidence that a defective hypothalamo-pituitary-adrenal (HPA) axis is present in both mouse and human lupus (Gutierrez et al. 1998; Lecner et al. 2000). The HPA axis is the chief component of the stress response. In stress situations, increased concentration of serum glucocorticoids are essential for the prevention of autoreactive or unrestrained amplification of the immune response, which may result in self-injury and autoimmunity

(Mok and Lau 2003). In SLE, the dysregulated HPA axis may be responsible for disease susceptibility and progression.

Allthough genetic factors and the hormonal milieu may create a predisposition to SLE, initiation of the disease probably results from several environmental triggers. The Epstein Barr virus infection is clearly associated with SLE in young adults and children (James 1997). Ultraviolet radiation often exacerbates the manifestations of established SLE, but can also trigger the disease, especially when a person is exposed to sun after viral or bacterial infection (Gilliam and Sontheimer 1982). Aromatic amines and hydrazines and their derivates can induce SLE-like disease, and exist in a variety of compounds used in agriculture, industry, and commercial applications, as well as in drugs (Mok and Lau, 2003). Such drugs include hydralazine and procainamide, the best-characterized drugs for the development of SLE in humans (so called drug induced lupus) (Pramatarov 1998). Finally, some dietary factors and smoking are also associated with the development of SLE (Prete 1985; Harbige 1996; Chaussy et al. 2003; Formica et al. 2003).

1.2.2. Genetic factors

The genetic predisposition for SLE was first suggested by the familial occurrence of the disease and by analyses of concordance rates in monozygotic twins. The concordance rate for MZ (~25%) relative to DZ (~ 2%) is over ten fold, supporting not only the genetic contribution in the development of the disease but also indicating that multiple genes are involved in disease expression (Deapen et al. 1992; Grennan et al. 1997). Based on family studies, approximately 10 to 12 % of SLE patients have a 1^{st} or 2^{nd} degree relative with the disease (Hochberg 1987; Lawrence et al. 1987). Moreover, the calculation of λs, the ratio of the risk of disease among the siblings of affected individuals compared to disease prevalence in the general population, also supports a potential role for genetic predisposition in disease susceptibility (Risch 1987). If λs is close to 1.0, there is no evidence for familial clustering (Vyse and Todd 1996). In SLE, the estimated λs is 20, which means that a sibling of an affected individual has a 20 times greater risk of developing SLE than a member of the general population (Vyse and Todd 1996). As a comparison, the λs for a monogenic Mendelian disease, such as cystic fibrosis, is over 10 times higher ($\lambda s = 500$). The higher the λs, the easier it is to map susceptibility genes (Lindqvist and Alarcón-Riquelme 1999).

Case-control association studies in humans have implicated a number of susceptibility loci, including the HLA region, Fcλ receptors, and complement components, as evidence for a genetic contribution in the development of SLE (Kelly et al. 2002). In addition, SLE studies using inbred mouse strains suggest multiple susceptibility loci (Kelly et al. 2002).

2. Pathogenesis

The exact pathogenesis of SLE remains elusive. A complicated and multifactorial interaction of various genetic, environmental, hormonal, and immunoregulatory factors are probably involved (Mok and Lau 2003). Immunologically, SLE is characterized by loss of self-tolerance leading to T- and B-cell hyperactivity, cytokine dysregulation, and uncontrolled production of autoimmune antibodies. Secreted autoantibodies can be deposited in tissues, causing inflammation and tissue damage (Hahn 1993). They may also form circulating immune complexes (ICs) with various antigens, which are released in excess due to abnormal apoptosis of various cell types (Robson and Walport 2001; Tsokos 2001). Moreover, defects in the clearance system of apoptotic cells and ICs favour the deposition of ICs in tissues causing inflammation (Hahn 1993; Robson and Wahlport 2001).

Autoantibodies are directed to intracellular (e.g. dsDNA, histones), cell-surface (red cells, white cells, platelets) and extracellular constituents (Emlen 1993; Malide et al. 1993; Stockl et al. 1994). The hallmark of SLE is elevated serum level of immunoglobulin G (IgG) antibodies to nuclear constituents (so called antinuclear antibodies, ANA).

The decreased clearance of ICs is caused by a deficiency of complement components and/or defects in the receptors for the complement (CRs) and Fc portion of immunoglobulin (FcγR). Complement is required for fixing ICs to erythrocytes (solubilization), which are then together transported from the circulation to mononuclear phagocyte system (MPS) in liver or spleen. Without solubilization, the ICs are deposited in tissues (Emlen 1993; Salmon 1993).

In order to bind the ICs to complements and further to erythrocytes, FcγRs and CRs are needed. They are also needed in MPS, where erythrocyte-fixed immune complexes are bound to fixed-tissue phagocytes, stripped from the erythrocytes, phagocytozed and degraded (Emlen 1993; Salmon 1993).

Immune complexes can also be formed *in situ*, as antibodies bind directly to tissue antigens. Complement activation is not required for the degradation of *in situ* ICs, and these are cleared from tissue primarily by FcγRs on fixed tissue magrophages, with binding of the Fc portion in IgG containing ICs (Emlen 1993).

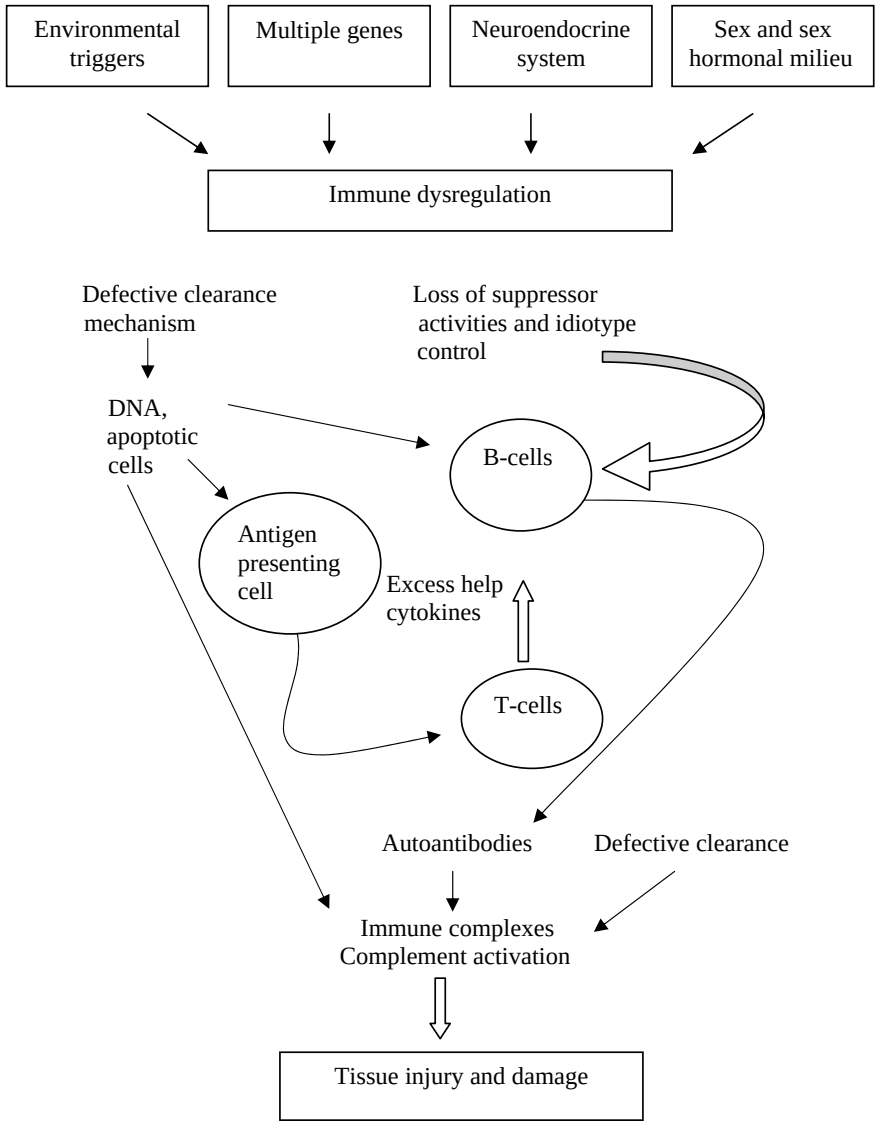


Fig. 1 The hypothetical scheme for pathogenesis of systemic lupus erythematosus, adapted from Mok and Lau (2003).

3. Clinical characteristics

The clinical manifestations of SLE are extremely variable among patients, and the course of the disease is characterized by periods of remission and relapse. Almost any organ can be involved, including joints, skin, kidneys, heart, lungs and brain (Gripenberg-Gahmberg and Konttinen 2002). A typical patient is a young woman in her child-bearing years, presenting with intermittent fatigue, joint pain and swelling, rashes (butterfly rash), low white blood cell count, anaemia, and chest pain due to pleuritis. Around 40% of patients develop nephritis (Cervera et al. 1993). Vasculitis (an inflammation of blood vessels) and serositis (pleurisy and/or pericarditis) are present in 20 to 40% of patients, and neuropsychiatric manifestations, most frequently seen as headaches and depression, in 25 to 80% (Gripenberg-Gahmberg and Konttinen 2001). Moreover, coronary artery disease and myocardial infarction are frequently seen, and in fact, nowadays are the leading causes of death of SLE patients (Urowitz et al. 1976; Abu-Shakra and Lee 1995; Manzi et al. 1997). Around 30% of patients have antiphopholipin antibodies (aPL) (Love and Santori 1990; Cervera et al. 1993), predisposing these patients to thrombosis, recurrent fetal loss and/or thrombocytopenia, a syndrome called antiphospholipid syndrome (APS).
A severe renal failure can be the first symptom of the disease. End-stage renal disease (ESRD) requiring hemodialysis or renal transplantation, develops in 5 to 22% of patients with lupus nephritis (reviewed by Mojcik and Klippel 1996). The overall 10-year survival rate for SLE patients has been reported to be between 75 to 92%, with more than 90% of patients surviving longer than 5 years (Ruiz-Irastorza et al. 2001; Cervera and Asherson 2003). Lower survival rates are seen in patients with nephropathy. The outcome of renal transplantations in SLE patients is good, however, and the survival and nephritis recurrence rate does not differ from those of other patients with ESRD (Mojick and Klippel 1996).

4. Antiphospholipid syndrome (APS)

Antiphospholipid syndrome (APS) is defined as the association of aPLs with a variety of clinical phenomena, including arterial and venous thrombosis, thrombocytopenia, and obstetric complications in the form of recurrent miscarriages, fetal death, or fetal growth retardation (Hughes 1993, Branch 1994). APS is probably the most common hypercoagulable disorder (Thomas 2001). Thrombotic events occur in approximately 30% of patients with aPLs (Galli and Barbui 1999). APS can be present without any underlying disease (primary or idiopathic APS) (Asherson et al. 1989), or it can be related to systemic lupus erythematosus, to other autoimmune or neoplastic diseases, or other pathological conditions (secondary APS) (Alargon-Segovia et al. 1989). An international consensus of classification criteria for APS has been recently published (Wilson 2001).

The aPLs are a large and heterogeneous family of immunoglobulins, directed against anionic phospholipids or specific plasma proteins with affinity to anionic (phospholipid) surfaces (Galli et al. 1998; Keswani and Chauhan 2002). The clinically most relevant and best-studied aPLs are lupus anticoaculant (LA) and anticardiolipin antibodies (aCL), both associated with arterial and venous thrombosis with variable specificities and sensitivities (Levine et al. 2002). aCL antibodies are also detected in various non-thrombotic conditions, such as infections (HIV, Lyme disease, and syphilis), and associated with the use of certain drugs (Triplett et al. 1988). Moreover, these ”benign” antibodies are seen in some proportions in the general population, especially in older groups, probably as a response to common viral illnesses or with coexistent chronic diseases (Vila et al. 1994; Petri 2000).

In the 1990´s it was found that aCLs do not bind directly to phospholipids, but rather to plasma proteins, with affinity to anionic (phosphpolipid) surfaces. Of these plasma proteins, *β2*-glycoprotein (*β2*-GPI) and prothrombin are the most studied (Galli and Barbui 1999). Anti-*β2*-GPI antibodies are said to be more closely associated with thrombosis than is aCL. Moreover, "pathogenic" aCLs, associated with APS and thrombosis, are distinguished from "benign" aCLs, since they are *β2*-GPI-dependent. In non-thrombotic contexts, aCLs are *β2*-GPI-independent (Galli et al. 1990; McNally et al.1995). Antiprothrombin antibodies have been associated with pregnancy loss in patients with APS (von Landenberg 2003), but whether they increase risk of thromboemolic events remains unknown.

In addition to the thrombogenic nature of different aPLs, there is evidence that aPL antibodies to ox-LDL, anti- *β2*-GPI, and anti-prothrombin antibodies would have a role in premature atherosclerosis in SLE and APS. Patients with both diseases have a high risk for atherosclerotic cardiovascular events (Witztum et al. 1991; Vaarala et al. 1993; Witztum and Horkko 1997; Jara et al. 2003).

Familial clustering of raised antibody levels (Goldberg et al. 1995; Hellan et al. 1998), and HLA linkages suggests probably that the antibodies occur in genetically susceptible hosts in response to some antigenic challenge (Sebastiani et al. 1996; Wilson and Gharavi 1996; Granados 1997).

Several hypotheses have been proposed to explain the pathogenesis of thrombosis in patients with APS (Levine et al. 2002). To date, there is no definitive association between specific clinical manifestations and particular subgroups of antiphospholipid antibodies. Therefore, multiple tests for these antibodies are recommended, since patients may be negative according to one test yet positive in another (Levine et al. 2002).

5. Genetics of complex autoimmune diseases

5.1. Penetrance and genetic heterogeneity

The phenotype in complex traits is most likely caused by the interaction of environmental factors and multiple genes (MHC and non-MHC genes), which may be common in the general population and alone are insufficient or even necessary for disease expression. This means that each single gene as such has a small effect on the risk for the disease (low penetrance) (Lander 1996; Chakravarti 1999). Furthermore, even in the presence of a full set of susceptibility alleles at multiple loci, overt disease may not always develop (incomplete penetrance), and thus environmental factors may be needed to trigger the disease (Vyse and Kozin 1996). Complex diseases are traits that do not follow Mendelian law of inheritance, and the pathogenesis is often poorly understood, hence candidate genes are difficult to determine, especially when the functions of most genes remain poorly understood.

Genetic heterogeneity refers to the presence of multiple combinations of genes within the genome that are capable of causing a similar or identical disease phenotype (Wanstrat and Wakeland 2001). For example, two or three different combinations of genes within one family, or in different populations, can result in identical disease phenotypes. This genetic heterogeneity is created by various forces during generations, including the chance effects of genetic drift and population bottlenecks, and also selection.

Genetic heterogeneity in complex diseases is apparent from association and linkage studies in human and mouse models (Kelly et al. 2002). Association studies for candidate susceptibility genes in both type I diabetes and in SLE, for example, have revealed significant variations between different ethnic groups in the association of specific alleles and disease phenotypes (Leech et al. 1995; Salmon et al. 1996). This ethnic diversity is also apparent in many linkage studies in humans, with associations of specific genomic intervals only in specific ethnic groups (Moser et al. 1998; Lindqvist et al. 2000). Genetic heterogeneity has also been observed in linkage studies in mice, where for example, lupus susceptibility was mediated in the lupus-prone BXSB strain by loci that were not involved in diseases susceptibility in the NZM2410, NZB/NZW or MRL/*lpr* mouse strains (Hogart et al. 1998).

5.2. Epistatic interactions

Epistastis is defined as the interaction between different genes, where the effect of one locus linked to the trait is altered or masked by effects at another locus (Cordell 2002). This means that the power to detect the first locus is likely to be reduced, and elucidation of the joint effects at the two loci is hindered by their interaction (Cordell 2002). In complex diseases like SLE or type I diabetes, where there are more than two loci involved, the situation is even more complicated due to possible interactions between some or all contributing loci (Cordell 2002).

Evidence for epistatic interactions among susceptibility alleles have been reported in both mouse models and human linkage studies for many complex autoimmune diseases (Prins et al. 1993; Gray Mc-Guire et al. 2000; Morel et al. 2000). Although the extent of epistasis in human is unknown, many functional polymorphisms influencing immune recognition and responsiveness are probably caused by epistatic interactions, and will therefore be a component of autoimmune disease susceptibility in most species (Wanstrat and Wakeland 2001).

6. Identifying disease susceptibility genes

6.1. The role of phenotyping

The basis for identification of susceptibility genes for any disease is to collect families having several affected individuals. Moreover, these families should be clinically well defined, and show intergenerational transmission of the disease. In complex diseases, however, the phenotype is often very broad, complicating the diagnosis. In SLE, identification of any four of 11 classification criteria in an individual is enough for diagnosis (Tan et al. 1982). However, these four criteria can overlap or be totally different in different individuals. Moreover, the onset of the disease and the severity of manifestations vary greatly from one patient to another; one person may have only mild pain in their joints, while another can suffer from severe kidney, central nervous system, or cardiac manifestations. Hence, there are not only variations between different phenotypes, but also variations within phenotypes, both reflecting the broad clinical diversity and the extent of genetic heterogeneity in SLE patients. Finally, the diagnosis may be biased due to the course of the disease; SLE is characterized by periods of remission and relapse and hence the disease can be missed, and patients with milder symptoms can be undiagnosed.
Grouping patients and families according to clinical covariates has been a successful strategy for gene identification, at least in some complex diseases. Association with lower age at onset

has led to gene identification by classical positional cloning of the breast-ovarian cancer gene *BRCA1* (Miki et al. 1994; Wooster and Stratton 1995). Similarly, stratification has been successful in studies with thyroid carcinoma and psoriasis, with convincing evidence of linkage to chromosome 2q22 and HLA regions, respectively (Samuelsson 1999; McKay et al. 2001). Recent genetic studies employing a genome-wide scan approach for SLE have focused on stratification of patients with subphenotypes, with convincing linkage results (Nath et al. 2001 and 2002; Kelly et al. 2002; Namjou et al. 2002a and 2002b; Quintero-Del-Rio et al. 2002; Scofield et al. 2003). Stratification often requires either large pedigrees with multiple affected family members with the same subphenotypes or large numbers of nuclear families with the same phenotypes, however, both of which can be difficult to ascertain in small populations with rare complex diseases.

6.2. The advantage of founder population

Another way to decrease the risk of genetic heterogeneity is to choose a study population with a founder effect. This means that the increased frequency of some rare genetic disorders within a population can be assumed to have occurred through enrichments of a single ancestral disease-causing allele (de la Chapelle and Wright 1998; Kere 2001). The founder chromosome with the disease-causing gene is transferred over time through healthy carriers to apparently unrelated patients. Despite recombination, which tends to shorten the shared common chromosomal region in each meioisis throughout generations, the ancestral genetic constitution in the present-day patients is likely to have been preserved in close vicinity to the disease gene. When the disease-associated chromosomes are genotyped with microsatellite markers, certain alleles for each marker show remarkable overrepresentation when compared to the allele frequencies in the general population. Disease associated alleles are said to be in linkage disequilibrium, whereas random alleles are present at more distant loci, and thus have reached an equilibrium (Kere 2001; Jorde 1995).

Examples of populations with founder effects include Finland, Sardinia, Iceland, and Japan (Wright et al. 1999). All of these countries are geographically isolated, population expansions have occurred by growth rather than immigration. In these countries, allelic heterogeneity may have been diminished by genetic drift before expansion (Wright et al. 1999). Founder populations have proven to be very useful for finding genes in monogenic diseases, which follow the Mendelian law of inheritance. For example, in Finland there are over 30 rare recessive diseases, for most of which the gene has been successfully mapped utilizing linkage disequilibrium in positional cloning (de la Chapelle and Wright 1998; Peltonen et al. 1999). These diseases, also termed the “Finnish disease heritage”, are typical to Finland and rarely seen in other countries, indicating an isolated gene pool for the Finns.

Lessons from mapping monogenic disorders have encouraged researchers to use founder populations to study the genetically more complicated complex diseases. Indeed, the reduced genetic heterogeneity and the ability to utilize linkage disequilibrium in founder populations favor their use over more heterogeneous populations in mapping susceptibility genes for complex diseases. Moreover, Finland has a good administrative infrastructure, initially created during the regime of King Gustavus of Vasa in 1523-1560. At this time church records were created; these have been a major source of information for genetic studies today. The church records reported births and deaths, marriages, and movement of families, and hence today provide a highly reliable source of genealogical information, especially since >90% of the Finnish population still belongs to the Evangelic Lutheran State Church (Peltonen et al. 1999).

Encouraging results mapping genes for complex diseases in Finland include asthma, psoriasis, schizophrenia, multiple sclerosis, and familial combined hyperlipidemia (Laitinen et al. 2001; Asumalahti et al. 2002; Hovatta et al. 1999; Kuokkanen et al. 1997; Pajukanta et al. 1998).

7. Mapping susceptibility genes

7.1. Linkage analysis

Linkage analysis is a practical way to approach the identification of disease susceptibility genes that are unknown or unsuspected, without prior knowledge of the function of those genes. With a genome-wide scan we can identify possible susceptibility loci, then narrow down the region with additional genetic markers (using association), and finally clone the gene. The markers used in linkage studies are short tandem repeats, "microsatellites", those DNA sequences that show considerable variability among individuals (polymorphism) but that are selectively neutral. Recently, single nucleotide polymorphisms (SNPs), single base pair variations which account for approximately 90% of human DNA polymorphism, are increasingly used in studies of complex diseases (Collins et al. 1998). Investigating genetic linkage depends on having families. The main hypothesis is that alleles from two loci tend to segregate together in a family if they are located physically close to each other on a chromosome (Risch 2000). The easiest way to identify linkage is to use affected sibpairs, where allele sharing in excess of 50% (the expectation of no linkage) is sought (Risch 2000). However, linkage analyses using sibpairs are powerful only if there is a large number of sibpairs available (Kruglyak and Lander 1995).

The extent of linkage is determined as a function of the distance between two loci, measured by the number of crossovers between the two loci among the observed meioses, i.e., recombination fraction (theta,) (Xu et al. 1998). The recombination fraction and the evidence for linkage can be estimated by a LOD score (logarithm of odds), which is a likelihood-based parametric linkage approach. The LOD score represents log10 of the ratio of the likelihood that two loci (marker and disease) are linked at a certain recombination fraction (θ) compared to the likelihood that they are not ($\theta = 0.5$) (Ott and Bhat 1999; Terwilliger and Ott 1994).

In parametric linkage analyses the mode of inheritance is known. It is widely used in Mendelian traits, where parameters such as penetrance and disease allele frequencies are usually estimated by epidemiological surveys (Ott and Bhat 1999). Linkage analyses can be performed either by two-point or multipoint linkage analyses (Xu et al.1998). Lander and Kruglyak (1995) introduced criteria for the significance of genome scans. In Mendelian traits LOD > 3.6 ($p = 2 \times 10^{-5}$) and LOD > 2.2 ($p = 7 \times 10^{-4}$) are regarded as thresholds for significant and suggestive linkage, respectively. The definition, however, is based on highly dense marker maps and complete information.

In complex diseases where the mode of inheritance is unknown, the LOD score may still be calculated. Because the parameters in complex traits are difficult to define, both dominant and recessive inheritance models are used to calculate LOD scores with different gene frequencies and penetrance values to determine, which model gives the highest scores. Recommendations for LOD scores in complex diseases are LOD > 3.3 and LOD > 1.9 for significance and suggestive linkage, respectively (Lander and Kruglyak 1995), again with complete information and highly dense marker maps. Another way to measure linkage in complex diseases is to use non-parametric linkage (NPL) analysis. This is a model-free method,

requiring no knowledge of the inheritance pattern of the disease. This method considers alleles shared by affected individuals, and ignores those unaffected. For example, the program package GENEHUNTER (Kruglyak et al 1996) calculates NPL in extended pedigrees, assessing identity by-descent (IBD), allele sharing among affected relatives within pedigrees.

7.2. Allelic association and linkage disequilibrium

Allelic association is another nonparametric approach to map disease genes. In genetically complex traits association can be used for evaluation of candidate loci as well as for fine-mapping of a region, once a region of interest has been identified (Lander and Schork 1994; Pericak-Vance, 1998). Allelic association can be due to either association or linkage. In association, the frequencies of marker alleles are significantly increased or decreased, and hence deviate from a random occurrence with respect to the disease phenotype. Allelic association can, however, occur by chance. This may be the case if the population is substructured, as occurrs in recent admixture of populations. Here alleles may show statistical association simply by chance, due to differences in allele frequencies in the two mixing populations (Lander and Schork 1994; Pericak-Vance 1998).

Two types of association studies exist, *case-control* and *family based* studies. In *case-control* studies, allele frequencies are compared between unrelated affected individuals and a set of matched (sex, age, onset of disease) healthy controls. The advantage of case-control studies are that cases and controls are easy to obtain and genotype. Isolated populations with genetic homogeneity are suggested as good case-control sample sets (Peltonen et al. 2000). As mentioned above, however, population substructure has to be taken into account (Kere 2001).

The advantage of *family-based* association studies is that this approach eliminates the concern that population substructure may be the cause of the association (Pericak-Vance 1998). Family-based studies compare frequencies of alleles transmitted from healthy parents to an affected child. The transmission disequilibrium test (TDT) is the most widely used test to measure association. It is also very powerful for detecting linkage in genetically complex traits, where the genetic effect is small (Spielman et al. 1993; Risch and Merikangas 1996).

In linkage disequilibrium (LD), two marker alleles lie so close to the disease susceptibility allele that they are inherited together over many generations. Thus the same allele will be detected in affected individuals in multiple, apparently unrelated families. The physical extent of the chromosomal region preserved around the mutation is dependent on the number of generations (reflecting the number of meiotic recombinations) since the disease allele was transmitted from a common ancestor (de la Chapelle and Wright 1998). LD is conceptually based on the same phenomenon as linkage analyses but considers the accumulation of recombinations over many generations, so that the recombination distances measured are very small (< 1cM), and the recombination events can only be inferred based on the level of sharing of the same allele (Pericak-Vance 1998).

LD genome-wide scans have been regarded as the method of choice for mapping complex diseases (Lander et Schork 1994; Kruglyak 1999; Reich et al. 2001).They have been very useful for mapping susceptibility genes within genetically young isolates (<200 generations) (Schaid 1998). Recently, however, it was discovered that LD is not restricted to within only one population but also exists extensively between populations. Association studies with dense single nucleotide polymorphic markers (SNPs) led to the discovery that the human genome is organized into haplotype blocks, regions which for some reason have avoided

historical recombination, and in which LD is extensive. These haplotype blocks show limited diversity. In fact, only a few common haplotypes account for over 80% of all haplotypes in the block (Daly et al. 2001; Patil et al. 2001; Gabriel et al. 2002).

The length of the haplotype blocks vary somewhat between different populations. In African and African-American populations the mean size of a block is around 22 kb, compared to 44 kb in European and Asian populations (Gabriel et al 2002). Over 50% of all haplotypes are shared by these four populations, and boundaries of the blocks are highly correlated across different populations, strongly supporting the common African origin of all human populations (Reich et al 2001; Gabriel et al.2002).

The major advantage of these blocks is that over 80% of the haplotypes are defined by less than 10% of the SNPs in the block. Therefore, instead of genotyping all SNPs within the block, only a small fraction, defined as tagging SNPs, are needed to capture most of the haplotypes. Before this can be done, however, a haplotype map covering the whole genome must first be constructed. This probably involves genotyping millions of SNPs in order to find the tagging SNPs, but after this work is done, only a subset of SNPs are required for whole-genome LD mapping (Daly et al.2001; Patil et al. 2001; Gabriel et al. 2002; Reich et al. 2002).

7.3. Simulation

Simulation is an appropriate method to estimate the power to detect linkage or LD between alleles at the disease and marker loci for a given population (Laitinen et al. 2001). One can generate genotypes with random artificial data, constructed with the same marker density, informativeness and exact pedigree structure as those of a real study. The data are simulated over N iterations, giving the threshold for suggestive linkage (NPL score exceeded once per genome scan at random) and significant linkage (NPL score exceeded once per 20 genome scans at random), as suggested by Lander & Kruglyak (1995).

8. SLE genetics

8.1. Mouse genetics of SLE

Genetic studies with mouse models have, over the years, revealed valuable information concerning the pathogenesis and genetics of many complex diseases, with results serving as a possible guide to reveal the syntenic regions in humans (i.e. chromosomal regions with homologous loci in human and mice) in the search for susceptibility genes. In SLE, the most useful animal model for the investigation of genetic contributions to disease have included those mouse strains, which develop lupus-like disease spontaneously. These include hybrids of New Zealand black (NZB) and white (NZW) mice (Drake et al. 1995), MRL mice, homozygous for lymphoproliferation (lpr) mutation (Cohen and Eisenberg 1991), and BXS mice, which carry a SLE disease-accelerating gene on the Y chromocome (Izui et al. 1995). These strains are primary models for severe lupus-like glomerulonephritis. The use of mice in genetic studies has many advantages. Directed breeding of backcross or F2 intercross mice allows the generation of large numbers of affected offspring, which can then be mapped for contributing genes. The phenotypes of different mice strains is much more uniform than that of humans, and environmental factors can be more carefully monitored (Vyse and Kotzin 1996).

To date, numbers of chromosomal regions containing genes for mouse lupus susceptibility have been identified (Table 2). There are several susceptibility loci, which are mapped to similar regions across multiple mouse strains, specifically on chromosomes 1, 4, 7, and the MHC region on chromosome 17. This suggests that at least some susceptibility genes may be shared between different lupus-prone mouse strains, yet the majority of the loci are strain specific. This indicates that each lupus-prone mouse strain is susceptible in part due to a unique set of disease genes, reflecting the genetic heterogeneity in susceptibility to mouse lupus.

Despite the advantages mouse models can offer to studies of susceptibility genes in humans (e.g. large number of offspring, monitoring of environmental factors), it must be emphasized that one mouse model may be representative only for one patient, and that the same genetic mutations may not underlie both mouse and human diseases (Vyse and Kotzin 1996).

Table 2. Mouse susceptibility loci and their equivalent human syntenic regions (if known). Adapted from Kelly et al. 2002

Mouse chromosome	Approxi-mate placement (cM)[a]	Identified mouse loci	Mouse model	Human syntenic region[b]	Reference
1	7.7	*Bxs4/Sle10*	BXSB	8	Haywood et al. 2000
	33	*Bxs1/Sle7*	BXSB	2q33-qter	Hogarth et al. 1998
	71	*Bxs2/Sle8*	BXSB	2q11-13	Hogarth et al. 1998
	88	*Sle1*	NZM	1q22-2	Morel et al. 1994
	90	*Lbw7*	NZB/NZW	1q21-2	Kono et al.1994
	95	*Nba2*	NZB/NZW	1q21-2	Vyse et al.1997
	100	*Bxs3/Yaa4*	BXSB	1q41-4	Hogarth et al. 1998
3	35	*Sles3*	NZM	3q25	Morel et al. 1999
	40	*Bxs5/Sle11*	BXSB		Haywood et al. 2000
4	45	*Sle2*	NZM		Morel et al. 1994
	56	*Lbw2*	NZB/NZW	1p35-34	Kono et al.1994
	58	*Lmb1*	MRL	1p35-34	Vidal et al. 1998
	58	*Sles2*	NZM	1p35-34	Morel et al. 1999
	62	*Nba1*	NZB/NZW	1p36-35	Drake et al. 1994
5	20	*Sle6*	NZM	4p16	Morel et al. 1999
	41	*Lmb2*	MRL	4p13-12	Vidal et al. 1998
	81	*Lmb3*	NZB/NZW		Kono et al.1994
6	65	*Lbw4*	NZB/NZW	12p13-12	Kono et al.1994
7	15	*Sle3*	NZM	19q13.1-13.2	Morel et al. 1994
	23	*Lbw5*	NZB/NZW	19q13.3-13.4	Kono et al.1994
	28	*Lmb3*	MRL		Vidal et al. 1998
	51	*Nba3*	NZB/NZW		Drake et al. 1995
9	1	*Sles4*	NZM	11q22	Morel et al. 1999
10	41	*Lmb4*	MRL	12q22-24	Vidal et al. 1998
11	29	*Lbw8*	NZB/NZW	5q23-35	Kono et al.1994
17	19	*Lbw1*	NZB/NZW	6p21.3	Kono et al.1994
	19	*Sles1*	NZM	6p21.3	Morel et al. 1999
	23	*H-2*	NZM	6p21	Morel et al. 1994
18	47	*Lbw6*	NZB/NZW		Kono et al.1994

[a]Locations and [b]syntenic regions identified from the Mouse Genome Database (MGD), Mouse Genome Informatics Web Site, The Jackson Laboratory, Bar Harbor, Maine. World Wide WebURL:http://www.informatics.jax.org/). [October 1].

8.2. Human genetics of SLE

8.2.1. Association studies in SLE

8.2.1.1. HLA association

The first report of an association between SLE and HLA in man was published by Grumet et al. in 1971. Since then, several studies have confirmed this observation and indicated that HLA-DR2 and HLA-DR3 separately confer 2- to 3-fold relative risk for the development of SLE in European-Caucasian populations. On the other hand, in African-Americans HLA-DR2 and HLA-DR7 have been associated with SLE. There are also established associations between SLE and HLA-B7, -B18, -DQAi, -DQB1, -DQ3 and –DR7 (Arnett 1997). Variation among associated alleles and haplotypes (HLA often shows LD) between populations, and within a population with different ethnics groups, exist. In different subphenotypes of SLE, there are strong associations between the production of specific autoantibodies and various HLA–DQ alleles (Bell and Maddison 1980); the heterozygous combination DQw2.1/DQw6, for example, has been shown to correlate closely with the production of autoantibodies to Ro/SS-A and La/SS-B, and importantly this association is present in both European and African –American populations. Related to these findings, the production of low levels of anti-Ro (in the absence of anti-La) in Europeans was associated with DR2 and DQw6, which are in linkage disequilibrium, whereas the same LD haplotype was associated to production of anti-Sm in African-American SLE patients. HLA-DQ allelic associations have also been associated with antiphospholipid antibodies (anticardiolipin antibodies and/or the lupus anticoagulant), anti-dsDNA, and in a subset of patients with lupus nephritis. (Vyse and Kotzin 1998) Interestingly, some HLA alleles may be protective; HLA-B40 among an Australian population and HLA-DR4 in a Japanese population are suggested to decrease the risk of developing SLE (Kameda et al. 1982; Whittingham et al. 1983).

8.2.1.2. Complement components

The complement system consists of ~20 plasma proteins that function in defending the body against infections and mediate the clearance of necrotic and apoptotic cell debris from the body.

Deficiency states within the complement system increase the risk of SLE. Around 90% of individuals with C1q deficiency and 75% with C4 deficiency develop SLE, while less than 20% of C2 and C3 deficient individuals develop this disease (Sturfelt 2002). C1q deficiency is a very rare disorder causing a particularly severe form of SLE, with glomerulonephritis and skin manifestations, beginning in the first or second decade of life (Bowness et al. 1994). Some patients with C1q deficiencies die from meningitis or recurrent infection. Both complete and partial C1q deficiencies have been reported among SLE patients (Kelly et al. 2002).

Deficiencies in complements C2 and C4 are associated with a mild form of lupus (Vyse and Kotzin 1998). These patients show allelic variations in the form of C2A/C2B and C4A/C4B. The deficiency of these complement components can be either partial or complete. Partial C4 deficiency is common; the C4A null allele (C4A*Q0) is associated with almost every SLE population studied today, with increased frequencies specifically in patients of the African-American and European–American origin (Arnett and Reveille 1992). The C4A null allele is in linkage disequilibrium with HLA-DR3, but is also seen in populations without HLA-DR3

association, hence indicating that the C4 allele may be an independent risk factor for SLE (Fielder et al. 1983; Kelly et al. 2002) Complete C4 deficiency (four null alleles) is rare, and such cases manifest an early age of SLE onset, renal disease, and anti-Ro without anti-DNA autoantibodies (Ratnoff 1996).

C2 deficiency is the most frequent complement deficiency seen in man (Kelly et al. 2002). Patients with this deficiency manifest a mild form of the disease, with cutaneous and articular involvement. These patients often lack pleuropericardial, neurologic or renal manifestations. Photosensitivity or the presence of anti-Ro autoantibodies can be associated to C2 deficiency (Kelly et al. 2002).

8.2.1.3. Fcγ receptors

The Fcγ-receptors FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) function to bind and clear IgG antibodies and IgG-containing immune complexes from the circulation. Each of these receptor families contains multiple genes and alternative splicing variants. In addition, there are allele variants with distinct functional capacities to phagocytes (Salmon et al. 1992). FcγRIIA association with SLE has been directly demonstrated (Salmon et al. 1996). FcγRIIA occurs in two allelic forms, with either an arginine (R131) or a histidine (H131) at position 131 in the second Ig-like domain. The H131 allele can only efficiently bind IgG2 on monocyte-magrophages with optimal clearance of IgG2-immune complexes in the homozygous state (Salmon et al. 1992). The skewed distribution toward a homozygous R131/R131 genotype has been associated with SLE, especially with nephritis, both in European (Duits et al. 1995; Dijstelbloem et al. 2000) and among African-American patients (Salmon et al. 1996). However, controversial results, reporting no association with the R131/R131 genotype, in these ethnic groups also exist (Botto et al. 1996; Salmon et al 1996; Manger et al. 1998, Oh et al. 1999). This can be due to either genetic variation in different ethnic groups, to admixture or to artifact.

Similar to FcγRIIA, skewing of the frequencies of allelic variants of FcγRIIIA and FcγRIIIB have been observed among SLE patients (Kelly et al. 2002). The FcγRIIIA receptors contribute in cell death and are found on the surface of natural killer cells, mononuclear phagocytes, and renal mesangial cells (Radeke et al. 1994). Various FcγRIIIA alleles are associated with lupus nephritis, but are also seen in patients with arthritis and serositis. FcγRIIIB are selectively expressed on neutrophils and have low affinity binding to IgG. Individuals with a N2/N2 genotype have decreased capacity to mediate phagocytosis than those with a N1/N1 genotype (Salmon et al. 1990).

The genes encoding the Fcγ receptors in humans are located on chromosome 1q22-24. Interestingly, the loci linked to nephritis in New Zealand hybrid mice (*Nba2/Sle1*) is also loated on distal mouse chromosome 1, and includes the mouse orthologue of human FCGR2A gene, *Fcgr2*.

8.2.1.4. Other genes

Weak association with cytokines, mannose binding protein (MBP), and apoptosis genes have been identified in patients with SLE, but additional studies are needed to further determine these genes as susceptibility loci. The susceptibility genes associated with human SLE and their location are listed in Table 3.

MBP is a complement regulatory protein structurally analogous to C1q, and is capable of activating the classical complement pathway (Kelly et al. 2002). Cytokines are important in modulating humoral and cellular immunity, and various abnormalities in interleukin (IL) production have been described in patients with SLE (Horowitz 1997). The apoptosis genes *Fas* and Fas ligand (*FasL*) are involved in programmed cell death or apoptosis. Mutations in these genes are suggested to prevent self-reactive B- and T-cells from undergoing apoptosis, thereby leading to autoimmunity (Tan and Arnett 1998). Conversely, *Bcl-2* appears to prolong the survival of B- and T-cells, leading to the development of SLE-like disease in transgenic mice (Strasser et al. 1990).

Table 3. Genes associated with systemic lupus erythematosus

Gene	Chromosomal location
Major histocompatibility complex class II (DR and DQ)	6p21.3
Complement components	
C1q (homozygous deficiency)	1q36.3
C1r (homozygous deficiency)	12p13
C1s (homozygous deficiency)	12p13
C4 (partial deficiency; C4A null alleles, C4B null alleles)	12p13
C2 (homozygous deficiency)	6p21.3
Complement receptor-1 (CR1)	1q23
Mannose binding protein (MBP)	10q11.2
Cytokines	
Tumor necrosis factor (TNFα)	6p21.3
IL (interleukin)-10	1q31-32
IL-6?	7p21-15
IL-1 receptor antagonist	2q14.2
FcγRII	1q23-24
FcγRIII	1q23-24
Apoptosis genes	
Bcl-2	18p21.33
FasL	1q23
Fas?	10q24.1

Adapted from Tan and Arnett 1998

8.3. Linkage studies in SLE

8.3.1 Unstratified studies

To date, six genome-wide scans using SLE as the phenotype, without stratification for subphenotype, have been conducted in different ethnic groups using different analytical approaches (Gaffney et al. 1998 and 2000; Moser et al. 1998; Shai et al. 1999; Gray-McGuire et al. 2000, Lindqvist et al. 2000). Six susceptibility loci (Table 4) surpassed the threshold for significance (LOD $\geq$ 3.3). In addition, more than 50 suggestive susceptibility loci have been identified. Moreover, a number of targeted studies have been performed based on studies of mouse lupus (candidate regions), or to refine results obtained in genome wide scans in rodents.

1q22-q24 (FcγRIIA)

The first two genome wide scans in SLE were published in 1998 by two separate groups (Gaffney et al. 1998; Moser et al. 1998). Moser et al (1998) found significant linkage (LOD 3.45) to chromosome 1q23, a region containing a candidate gene for Fc gamma receptor (*Fcγ*RIIA). The study population consisted of 31 African-American and 55 European-American families, which were analyzed separately as well as combined into a total collection of 94 pedigrees. The linkage to chromosome 1q23 was mainly detected in the African-American pedigrees. No other linkage studies have confirmed this result. Linkage to chromosome 1q23-q24, however, is supported by two independent studies (Shai et al. 1999; Tsao et al. 2000), with evidence for linkage at marker D1S484 (Z = 2.56, p = 0.006 and p = 0.019 respectively). This marker lies 3 cM distal to *Fcγ*RIIA. In addition, Gray-McGuire et al. (2000), using many of the same pedigrees (Moser et al. 1998), found evidence of the suggestive linkage to chromosome 1q23-q24 (LOD = 2.47, p = 0.01) applying different methods. Case-control association studies in human SLE and linkage studies with mice (discussed earlier) both corroborate the observed linkage to *Fcγ*RIIA.

6p11-p21 (HLA region) and 16q13

Gaffney et al. (1998) published their first genome wide scan using 105 sib-pair families (cohort 1), mostly of Caucasian origin (84 families). By utilizing multipoint non-parametric methods, they were able to demostrate significant linkage of SLE to HLA loci at 6p11-p21 (D6S257, LOD = 3.9), and to 16q13 (D16S415, LOD = 3.64). Later, the same group recruited 82 additional sib-pair families (cohort 2), again mainly Caucasian (Gaffney et al. 2000). The two patient cohorts were first analyzed separately. Different linkage results were obtained for each patient cohort. Nevertheless, combined multipoint analyses again demostrated evidence for linkage at D6S426 (HLA region), with an overall LOD score of 4.19 (resulting purely from cohort 1) and 16q13 (D16S415, LOD = 3.85), hence suggesting the contribution of these two loci to SLE susceptibility.

Two independent genome wide scans (Lindqvist et al. 2000; Shai et al. 1999) demonstrated linkage to HLA region at markers D6S273 (LOD = 1.54) and D6S276 (NPL = 1.6). Moreover, as discussed earlier, association studies in man (HLA association, complement components) and linkage studies in mice, have indicated evidence for HLA contribution in the development of SLE. Finally, the human HLA region is synthetic with mouse MHC, in the proximity of which lies the most potent suppressive modifier gene, *Sles1*, which suppresses the autoimmune phenotype of murine *Sle1* (Wakeland et al. 2001).

Linkage of SLE to the 16q13 region is supported by Shai et al. (1999), who observed linkage at marker D16S3136 (NPL = 2.14, p = 0.017). The 16q13 region contains a susceptibility gene, nucleotide-binding oligomerization domain (NOD2), for Crohn´s disease, which is an autoimmune inflammatory disease (Hugot et al. 2001). In addition to SLE, there is evidence for linkage to 16q13 in other autoimmune diseases, including type I diabetes (Becker et al. 1998), suggesting that apparent sharing of susceptibility loci between various autoimmune disorders may be important for genetic predisposition to autoimmunity in general.

1q41-q44 (PARP)

This locus was originally identified by Tsao et al. (1997) as a candidate region homologous to mouse lupus susceptibility. A significant increase in allele sharing (over 0.5) in 52 SLE-affected sibpairs from three ethnic groups (Caucasians, Asians and African-Americans) provided evidence for linkage at 1q41-q42. This region contains several candidate genes, of which poly ADP-ribose polymerase (PARP) gene was identified to be that most associated with SLE among affected offspring in 124 families (Tsao et al. 1999). The 1q41-q42 locus showed also strong evidence for linkage also in the primary genome-wide scan by Moser et al. (1998) (D1S3462, LOD = 3.5), with a dominant inheritance model. Recently, Graham et al. (2001) localized a candidate gene (estrogen-related receptor gamma, ESRRG) in this region centromeric to PARP. The patients were primarily those analyzed in the combined genome-wide scan by Gaffney et al. (2000), where only a weak linkage result from cohort 1 at 1q41(D1S299, LOD = 1.33) was identified. With the addition of 24 sibpairs (total of 210 sibpairs) and 122 trio families, Graham et al. (2001) demonstrated significant linkage at marker D1S490 (p = 0.0091), suggesting that a human SLE susceptibility locus is located centromeric to PARP, near this marker at 1q41. Finally, in a genome-wide scan, (Shai et al. 1999) significant linkage was found to region 1q44 (D1S2785) with NPL score 3.33 (p = 0.0006). That study comprised 80 mixed families, multiply affected by SLE, of which linkage was clearly found only among Mexican-Americans. Even though the linked locus resides distal to 1q, it supports the importance of this chromosomal region as one of the candidate regions for a susceptibility gene for SLE, at least in some ethnic groups.

2q37 (PDCD1)

Significant linkage for SLE susceptibility to chromosome 2q37 (D2S125, LOD = 4.24) was identified in a genome-wide scan using a homogeneous population from Iceland and Sweden (Lindqvist et al. 2000). The two populations were first analyzed separately. The strongest evidence for linkage in Icelandic families occurred at 4p13-p15 (D4S1627, Z = 3.2), and in Swedish families at 2q37 (D2S125, Z = 2.18). When the families were combined, the LOD at D2S125 increased to 4.24. The LOD with the combined data set for chromosome 4 remained unchanged. Although no other studies have confirmed the linkage at 2q37, this region contains a locus for programmed cell death 1 gene (*PDCD1*), which is considered a good candidate gene for SLE. In fact later, in a large multinational study (2,510 individual) (Prokurina et al. 2002), one intronic single nucleotide polymorphism (SNP) in *PDCD1* was found to associate with development of SLE in Europeans (p = 0.0001) and Mexicans (p = 0.009). The *PDCD1* gene is an immunoreceptor, a member of the immunoglobulin family, which is known to regulate peripheral self-tolerance in T-and B-cells. Mice lacking *Pdcd1* develop a SLE-like disease (Prokurina et al. 2002). The SLE associated SNP in *PDCD1* alters a binding site for runt-related transcription factor1 (RUNX1, also called AML1), suggesting a mechanism through which it can contribute to the development of SLE in humans (Prokurina et al. 2002).

4p15.2-p16

Evidence for moderate or suggestive linkage to 4p15-p16 has been obtained at least in three independent genome-wide scans. Moser et al. (1998) identified suggestive linkage at D4S403 (LOD = 2.18) in European-American families with a dominant model of inheritance. The same marker had moderate linkage (LOD = 1.31) in the combined data set (cohort 1 and 2) of Gaffney et al. (2000). Finally, Lindqvist et al. (2000) presented evidence of linkage to D4S1627 (LOD = 3.2) in six Icelandic families. The 4p15.2-p16 region contains a number of interesting candidate genes, including human lymphocyte differentiation antigen (*CD38*), and bone marrow stromal cell antigen 1 (*BST1*) (Wakeland et al. 2001).

Recently, a genome-wide scan of 126 multiplex pedigrees, including 469 sibling pairs and 175 affected relative pairs, revealed supporting evidence for linkage on chromosome 4p (Gray-McGuire et al. 2000). The pedigrees used in this study were essentially the same as those included in the original genome wide scan of Moser et al. (1998). The maximun LOD of 3.84 (p = 0.003) was identified at marker D4S2366 in European-American families.

We have here described those identified regions from six independent genome wide scans that have shown a significant evidence of linkage in patients with SLE (Table 4). In addition to these reported regions, there are several additional loci that show weaker, but suggestive, evidence for linkage in SLE. Those regions with at least nominal evidence for linkage in at least two independent family collections are listed in Table 5.

Table 4. Regions showing significant linkage to SLE in six genome wide scans

Locus	LOD/Z	Marker	No. of families, SLE patients	Origin of families	Reference
1q22-q24 1q41-q43	LOD = 3.45[a] LOD = 3.5[a]	(FcγRIIA) D1S3462	94 families 220 patients	55 EA families 31 AA families 8 Other	Moser et al. 1998 (OMRF)
1q44	NPL = 3.33[b] (p = 0.0006)	D1S2785	80 families 188 patients	43 MA families 37 EA families	Shai et al. 1999 (USC)
2q37	Z = 4.24[a]	D2S125	17 families 44 patients	11 Swedish 6 Icelandic	Lindqvist et al. 2000 (Uppsala)
4p15-p16	LOD = 3.84[c]	D4S2366	126 families 175 ARPs	112 EA families 52 AA families 11 Other	Gray McGuire et al. 2000 (OMRF)
6p11-p21 16q13	LOD = 4.19[b] LOD = 3.85[b]	D6S426 D16S415	187 families 399 patients	148 EA families 17 AA families 13 Hispanic 9 Other	Gaffney et al 1998; Gaffney et al. 2000 (Minnesota)

Analytical methods used for established linkages: [a] model-based two-point linkage, [b] non- parametric multipoint linkage, [c] conditional logistic regression technique with affected relative pairs (ARP). EA = European-American, AA = African-American, MA = Mexican-American

Table 5. Regions demonstrating "suggestive"* linkage in SLE in at least two independent studies†. Adapted from a study by Gaffney et al. (2000)

Locus	LOD (marker) Minnesota	LOD (marker) OMRF	LOD (marker) USC	LOD (marker) Uppsala
1q31		2.04		1.61 (D1S1660)
2q32-35	1.45 (D2S126)	2.09 (D2S1391)		
4p13-15	1.31 (D4S403)	2.18 (D4S403)		
6q26-27		2.04 (D6S1027)		1.35 (D6S503)
9p24-21		2.08 (D9S925)		2.27 (D9Sgata62f03)
11q23		2.10 (D11S2002)		1.15 (D11S1998)
13q31-32	1.02 (D13S170)	2.50 (D13S779)		
14q11-23	2.81 (D14S276)	2.21 (D14S742)	2.02 (D14S258)††	1.15 (D14S592)
15q26	1.07 (D15S127)			1.95 (D15S657)
19q13.1		2.05 (D19S246)		2.06 (D19S246)
20p12-13	2.62 (D20S186)		1.13 (D20S115)‡	
20q11-13	1.64 (D20S119)	2.49 (D20S481)		

*Recommended criteria for suggestive linkage in genome-wide scan for a complex disease (LOD>1.9 for complex pedigrees, LOD > 2.2 for sib pairs (Lander and Kruglyak, 1995). † shown are markers meeting criteria for each interval. Supporting evidence (LOD > 1.5) from an independent family collections is also shown if present. ‡ Z scores were converted to LOD scores by equitation: LOD = A^2/Zln10 (Kong and Cox, 1997). Lod = Logarithm of odds. OMRF = Oklahoma Medical Research Foundation. USC = University of Southern California. ††= NPL (non-parametric linkage) score.

8.3.2. Stratified studies

In recent genome-wide scan studies, pedigrees have been stratified according to clinical manifestations. The aim is to achieve genetically and clinically homogeneous sets of families, and to increase the power to detect susceptibility genes for different subphenotypes of SLE. At least 11 regions have been significantly linked to SLE using both model-based and nonparametric approaches with different phenotypes (Table 6). The families analyzed in these stratified studies were mostly the same as those genotyped in the primary genome wide scan using the SLE phenotype (Moser et al. 1998; Gaffney et al. 1998 and 2000; Shai et al.1999; Gray-McGuire et al. 2000; Lindqvist et al. 2000).

Table 6. Regions showing significant linkage in genome-wide scans with different SLE subphenotypes.

Subphenotype	Locus	LOD/NPL	Marker	Candi-date gene	Material	Author
Vitiligo	17p13	LOD = 3.64 (NPL = 4.02)	D17S974-D17S1298	*SLEV1*	16 EA families	Nath et al. 2001
Neuropsychiatric Manifestations (Seizures and psychosis)	4p16	LOD = 5.19 (EA) (NPL = 3.12 (EA))	D4S3007	*SLEB3*	23 EA families 20 AA families	Nath et al. 2002
Self-reported rheumatoid arthritis	5p15.3	LOD = 6.2 (EA) (NPL = 5.64 (EA))	D5S2505	*SLER1*	14 EA families 19 AA families 3 OE families	Namjou et al. 2002
Anti-double stranded DNA (dsDNA) antibodies	19p13.2 18q21.1	LOD = 4.93 (EA) LOD = 3.40 (AA)	D19S714 D18S858	*SLED1* *SLED2*	37 EA families 29 AA families 5 OE families	Namjou et al. 2002
Hemolytic anemia	11q14 1q24	LOD = 4.5 (AA) LODPAL = 4.0 * (EA)	D11S 2002 D1S1589	*SLEH1*	17 EA families 16 AA families 2 HP families	Kelly et al. 2002
Renal disease	10q22.3 11p15.6	LOD = 3.16 (EA) LOD = 2.58 (AA)	D10S2470 D11S1984	*SLEN1* *SLEN3*	31 EA families 40 AA families	Quintero-Del-Rio et al. 2002
Nucleolar antinuclear antibodies (ANA)	11q14	LOD = 5.62 (AA)	D11S2002	*SLEH1*	12 AA families 14 EA families 1 NA family	Sawalha et al.2002
Thrombo-cytopenia	1q22-23 11p13	LOD = 3.71 (All) LOD = 5.72 (AA)	D1S1677 D11S1392		38 mixed pedigrees 13 AA families	Scofield et al. 2003

LODPAL= multipoint conditional logstic regression on affected relative pairs (Olson 1999),
* = surpasses the suggested threshold for significant linkage (LOD > 3.3, Lander et Kruglyak 1995).
AA = African-American; EA = European-American; OE = other ethnic families; HP = Hispanic; NA = Native American

8.4. Overlap with other autoimmune disorders

SLE belongs to the group of autoimmune diseases which are caused by an immune response, humoral or cell mediated, against self antigens. Complex interactions of genetic and environmental factors play a role in their background. Collectively, autoimmune diseases are estimated to affect 4 to 5% of the population, and females are generally more often affected than males (Vyse and Todd 1996). Examples of autoimmune diseases include type I diabetes, rheumatoid arthritis, Hashimoto´s thyreoiditis, and myastenia gravis. There is evidence for both familial clustering of different autoimmune diseases (Lin et al. 1998), and for coexistence of several autoimmune diseases within the same individual (Lorber et al. 1994). Moreover, recent genome mapping studies in complex diseases have led to identification of susceptibility loci for a number of autoimmune diseases. The non random clustering of identified loci has been observed in both animal models (Vyse and Todd 1996) and human autoimmune diseases (Becker et al. 1998), suggesting that clinically distinct autoimmune diseases may be controlled by a common set of susceptibility genes (Becker et al 1998).

AIMS OF THE STUDY

1. To find multiplex SLE families in Finland and to clinically characterize the affected patients as compared to patients with sporadic SLE.

2. To study the associaton of aPL with thrombotic events in patients with SLE.

3. To conduct a genome-wide search for SLE susceptibility loci in multiply affected pedigrees using linkage mapping.

4. Fine-map the genomic regions identified in the genome wide scan in patients with SLE.

MATERIALS AND METHODS

1. Patient recruitment

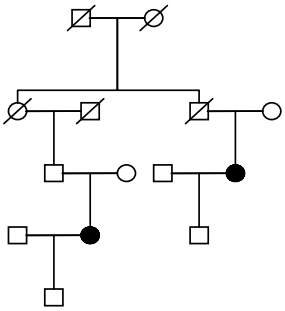
The recruitment of patients began in 1995 at two University hospitals; Helsinki and Kuopio (in central eastern Finland, Fig. 4). Patients treated in these hospitals during 1992 to 1995 were identified from the hospital registries and were contacted personally or by mail. Additionally, patients registered by the Lupus Foundation of Finland, and patients answering an advertisement published in patient bulletins, were recruited. The second recruitment phase in 1996 targeted families multiply affected by SLE. Physicians treating SLE patients (mainly rheumatologists) from of 21 hospitals in Finland were contacted. A letter was sent to all patients treated in these hospitals during 1993 to 1996. All patients were asked whether they had relatives or family members diagnosed with SLE or a related connective tissue disease. Patients with a positive family history were asked to participate in the study and informed consent was obtained. According to the prevalence of SLE in Finland (Helve 1985), we succeeded in contacting roughly 1200 of 1500 available patients by phone or by mail during the two phases of recruitment, accounting for approximately 85% of all Finnish SLE patients requiring hospital-based treatment. In Finland the diagnosis of SLE is practically always assessed in hospital by a specialist. In most of the identified families there was only a single SLE patient (sporadic cases) and these could not be used for a linkage study.

Blood samples from patients and their relatives were obtained from a total of 252 families, of which 53 were multiply affected by SLE (multiplex families), and the rest was families of sporadic patients. Patients were interviewed by the same physician (H.J.) either personally or by phone, and the case records from the hospitals were reviewed. All patients met the American College of Rheumatology criteria for the diagnosis of SLE (Tan et al. 1992).

2. Study subjects

In Study I the clinical characteristics of 113 patients from 53 families were evaluated. Forty-six out 53 multiplex families had 2 affected members. The relationships in 2 affected families were: 17 sibpairs, 10 parent/offspring, 7 aunt/niece or aunt/nephew-pairs, three female cousin-pairs, two female monozygotic twin pairs, one dizygotic twin pair. In 6 families, affected pedigree members were distant relatives. Examples of such families are shown in Fig. 2.

Fam A



Fam B

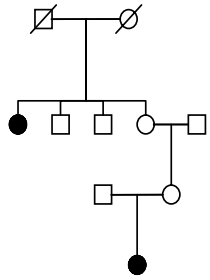


Fig.2 Examples of relationships in families where the 2 affected members were more distantly related to each other. ● = affected female; □ = non-affected male; ⊘ = deceased

Families with three affected pedigree members

Seven of 53 multiplex families had 3 affected pedigree members. Patients in these families were mostly first-degree relatives (Fig.3, Fam 1, 2 and 3). Two of the families had both first-degree and more distant relatives (Fig.3, Fam. 4 and 5).

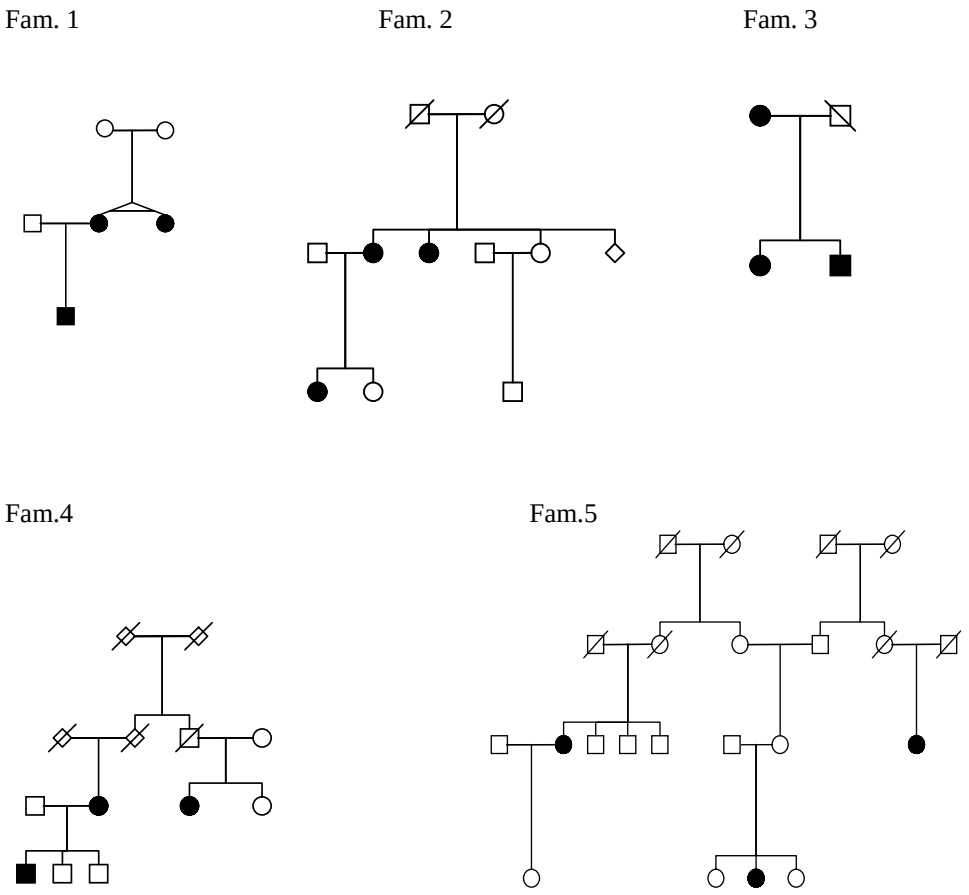


Fig 3. Examples of relationships in families with three SLE patients.

■ = affected male; ● = affected female; □ = non-affected; ⊘ = deceased

In Study II, we investigated one SLE subphenotype, antiphospolipid syndrome, using serum samples from 89 patients with familial SLE (45 out of 53 multiplex families, described above) and from 203 sporadic patients.

From a total of 53 multiplex families, 35 were informative for a linkage study, and were included in a primary genome wide scan (study III). Parents were recruited to facilitate phasing of chromosomes, and if unavailable, an unaffected sibling was sampled to allow reconstruction of parental genotypes. In total, 73 SLE patients and 96 healthy relatives (169 individuals altogether) were included in this linkage study. In 32 of 35 families there were 2 affected family members. The most common combination was sister/sister (16 families),

followed by aunt/niece (8 families). Three families had 3 SLE patients. In 2 of these families, the affected family members were first-degree relatives (Fig 3. Fam 3), whereas in one family there were both second-degree (a cousin) and first-degree (mother/son) relatives (Fig 3. Fam 4). Seven of the 73 familial cases (10%) were male, corresponding to the sex distribution of SLE in the general population (Hochberg 1987).

For the haplotype association study (IV), 34 families originating from a restricted region in Savo in central eastern Finland (Fig. 4) were recruited and genotyped together with multiplex families using dense marker maps. According to taxation records, the Savo area (Fig. 4) was settled in late 1600´s by no more than 1000 people (229 houses), whereafter the population has grown, virtually without immigration, to its current size of 200 000. Due to the population history of Northern Savo, we anticipated reduced genetic heterogeneity of the probands in these families, ideal for a linkage disequilibrium study. Moreover, this region is already known for a previously characterized founder effect, for the *MLH1* susceptibility gene for colon cancer (Nystrom-Lahti et al. 1994).

Three of the finemapped Savo families were multiply affected by SLE, and hence had been already in the primary genome-wide scan. The remainder of the 31 families were simplex trios, mostly including mother, father and offspring (in 13 families), or with only one of the parents available (8 families) for haplotype reconstruction. Consanguinity was investigated from population records back the late 18th century (in most families back to the mid 19th century), and none was observed between the families. Altogether, 66 families with 262 individuals were included in this fine-mapping and association study.

Fig 4. The location of Savo region in central eastern Finland

3. Control individuals

In Study I, patients from sporadic SLE families were used as a control for comparison of clinical manifestations and laboratory findings. Control patients were chosen in alphabetical order and matched by sex, age and duration of SLE symptoms.

In Study II, serum samples from 111 people (mean age 56.6 years, SD 19.9 years, range 14 to 93 years, males 44%) with no previously diagnosed chronic autoimmune diseases were used as controls. The cut-off levels for positivity were set at the 95th percentile for IgG-, IgA-, and IgM-aCL, anti-protrombin, and anti β2-GPI. For antibodies to ox-LDL, the upper reference value was set at the 95th percentile for 200 healthy blood donors attending the Finnish Red Cross Blood Transfusion Service.

For the association study (IV) healthy spouses within families were used as controls.

4. PCR amplification

Genomic DNA (20 ng) prepared from peripheral blood samples, was amplified in 5 μl PCR assays using 0.33 μM of fluorescently labeled primers and 0.2 units of AmpliTaq Gold DNA Polymerase. Prior to the PCR assays, the DNA was distributed to 384-well microtiter plates using a Hydra-96 microdispenser (Robbins Scientific, Sunny Vale), whereafter the DNA solution was dried. The PCRs were assembled using a TECAN Genesis 150/8 robotic sample processor (TECAN Ag, Hombrechtikon, Switzerland), and run in a Dual 384-Well GeneAmp PCR System 9700 (PE Biosystems, Foster City, CA). Following an initial heating step of 12 min at 95°C, 30 cycles of PCR were performed (10 cycles of 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C, and 20 cycles of 30 sec at 89°C, 30 sec at 55°C and 30 sec at 72°C).

5. Genotyping of microsatellite markers

The amplified PCR fragments were multiplexed (on average 13 markers in a pool), and then separated using capillary array electrophoresis (MegaBACE 1000, Amersham Pharmaceuticals, Amersham, UK). The capillary runs were analyzed using the Genetic Profiler 1.1 software (Amersham Pharmaceuticals), which performs automatic sizing and allele calling. All electropherograms were manually reviewed prior to analyses.

Microsatellite markers from the Linkage mapping set MD-10 (PE Biosystems) were used in the primary genome-wide scan. Of the 400 markers in the linkage mapping set, we retrieved data from 388 markers. The average intermarker distance was 9.73 cM. The data contained six gaps of 20 to 26 cM. To cover the gaps, and to fine-map the susceptibility region for SLE obtained in Study III, a total of 73 extra markers were genotyped, combined with the primary genome wide scan and re-analysed. The final density between markers in fine mapping regions (Study IV) was $\leq$ 1cM. All markers were selected from Marshfield genetic maps (http://research.marshfieldclinic.org/genetics/) and the marker order for fine mapping was verified using the NCBI sequencing database (http://www.ncbi.nlm.nih.gov) and UCSC human genome assembly (http://genome.ucsc.edu). The marker order was in accordance with the deCode genetic linkage map (Kong 2002). For multiplex families, Mendelian errors were checked using the Pedmanager and PedCheck software (O´Connel et al. 1998), and overall

genotype frequencies were tested for Hardy-Weinberg equilibrium. The results were analyzed using non-parametric multipoint linkage analysis with the Genehunter software (Kruglyak et al. 1996).

6. Assays for aPL antibodies and ox-LDL

6.1. Anti-cardiolipin ELISA

Anti-cardiolipin antibodies were detected according to a previously decribed ELISA method, with the exception that 10% bovine serum was used as the blocking solution (Vaarala et al. 1993)

6.2. Anti-protrombin ELISA

A method for detection of anti-prothrombin has been previously described in detail (Puurunen et al. 1996). Briefly, irradiated polystyrene plates (Combiplate® Enchanced Binding, Labsystems, Helsinki, Finland) were coated with human prothrombin (Sigma Chemical Co., St.Louis, MO) at a concentration of 10µg/ml in PBS, 100µl per well, and incubated overnight at +4^{o}C. The plates were washed with 0,5% Tween-PBS before blocking with 3% gelatin in PBS for 1 h at room temperature. Serum samples were diluted 1:50 in 0.3% gelatin-PBS and incubated for 1.5 h at room temperature. Alkaline phosphatase-conjugated rabbit F(ab´)2 fragment anti-human IgG antibodies (Jackson Immunoresearch Laboratories, West Grove, USA), diluted 1:2000 in 0.2% human serum albumin (HAS)-PBS, was added and incubated for 1 h at room temperature. P-nitrophenyl phosphate (Sigma Chemical Co), 1mg/ml in carbonate buffer, pH 9.8, was used as a substrate and incubated for 30 min at room temperature. The absorbance was measured by an optical reader at 405 nm.

6.3. Anti-ß2-glycoprotein I (GPI)

The assay was performed similarly to the anti-prothrombin ELISA except that the concentration of *ß*2-GPI used for coating was 5 mg/ml and the samples were diluted 1:200.

6.4. Antibodies to ox-LDL

IgG class antibodies to malondialdehyde-modified LDL were measured using an ELISA method described previously (Vaarala et al. 1993).

7. Statistical analyses

7.1. Linkage analysis

Linkage analyses were carried out using non-parametric multipoint analysis (NPL) with the Genehunter software (v.s. 2.0 for chromosomes 1-22, and v. 1.3 for the X-chromosome) (Kruglyak et al. 1996)

7.2. Simulation

The thresholds of significant and suggestive linkage are defined by Lander and Kruglyak (1995) as the NPL score levels corresponding to the random occurrence of one false positive hit per 20 genome scans (p = 0.05 per genome scan), and one false positive occurrence per genome scan, respectively. For any given set of families and markers, the exact levels of significant and suggestive NPL scores can be determined by computer simulation (Laitinen et al. 2001). To get an estimate of the global p-value, we performed allele dropping simulations under a null hypothesis of no linkage. The simulations consisted of 200 iterations (Laitinen et al. 2001). In each iteration, founder alleles were drawn from the estimated allele frequency distributions, and missing genotypes were introduced to the same positions as in the real data. As a result, we obtained an empirical distribution for the NPL scores corresponding to suggestive and significant linkage (Lander and Kruglyak, 1995).

7.3. Haplotype association analysis

Haplotype Pattern Mining (HPM) is an association analysis based on data mining methodology (Toivonen et al. 2000, Sevon et al. 2001). It is ideal for complex diseases, as no inheritance model for the disease is required. The statistical model is non-parametric. The method searches for combinations of marker alleles which are more frequent in disease-associated chromosomes than in control chromosomes. The method allows gaps, missing, and erroneous data in haplotypes, maximizing the length of the shared haplotype. The number and the length of gaps can be set by the user. The capacity to handle high proportions of phenocopies makes the method promising for complex disease mapping.

In the present study, independent trios from the pedigrees were haplotyped using an in-house software tool designed for this purpose (Petteri Sevon, unpublished software). The maximum length of pattern (markers), maximum number of gaps and minimum X^2 for a pattern were defined. To compensate for variable marker densities and marker information contents, a total of 10,000 permutations were run to obtain empirical p-values (Toivonen et al. 2000).

7.4. Transmission Disequilibrium Test (TDT)

The TDT was introduced as a method for identification of linkage between complex disease and a genetic marker in the presence of association (linkage disequilibrium) (Spielman et al. 1993). TDT uses data from families, and evaluates the transmission of an associated marker alleles or haplotypes from a heterozygous parent to an affected offspring. In our study, TDT was conducted with GENEHUNTER 2.1 (Kruglyak et al. 1996); transmissions from homozygous parents are excluded and cases where one parent is missing are used only when the genotyped parent and the proband are both distinct heterozygotes (Curtis and Sham 1995).

7.5. Hardy-Weinberg equilibrium

The overall genotype frequencies for multiplex families were tested for Hardy-Weinberg equilibrium using the program developed by Guo and Thompson (1992). Hardy-Weinberg equilibrium means that alleles in a population are assorting randomly among individuals in each generation, i.e. according to the formula $p^2 + 2pq + q^2 = 1$, where p and q are the frequencies of alternative alleles and p^2 and q^2 refer to the proportion of homozygotes for each allele and 2pq to the proportion of heterozygotes. This calculation forms an essential quality control procedure for genotyping.

7.6. Other tests

The statistical tests used in evaluating the aPLs in patients with SLE were as follows:
The sensitivity, specificity, positive predictive values and negative predictive values were calculated using a SPSS program. Spearman`s rank correlation was applied to study correlations between the different assays. The X^2 test and the Fischer´s exact test, when appropriate, were used for the analysis of two-by-two tables.

RESULTS

1. Characterization of clinical manifestations in patients with familial and sporadic SLE (Study I)

The clinical manifestations and laboratory findings of 113 patients from 53 multiplex families and 113 patients with sporadic SLE are presented in Table 7.

When multiple comparisons (Bonneferoni corrections) were taken into account, there were no statistical differences in clinical manifestations or laboratory findings between familial and sporadic cases of SLE. The mean number of ACR criteria fulfilled by familial cases was 5.2 (range 4-9), and by sporadic cases 5.4 (range 4-9). The mean ages at onset of SLE in familial and sporadic cases were 30.7 years (1-66) and 29.0 years (7-59). There were no differences in clinical characteristics between men with familial SLE and respective controls with sporadic SLE. Additionally, there were no significance difference in occurrence of clinical manifestations between those SLE patients who were close relatives (34 affected siblings and 20 cases of parent-offspring combinations) compared to those who were more distantly related (32 affecteds).

Table 7. Comparison of clinical characteristics between familial and sporadic cases of SLE

Clinical manifestation	Familial SLE N= 113 (%)	Sporadic SLE N= 113 (%)	Difference, *P*
Butterfly rash	52/113 (46)	57/113 (50)	0.51
Discoid rash	6/113 (5)	13/113 (12)	0.09
Photosensitivity	78/113 (69)	72/113 (64)	0.40
Mouth ulcers	19/11 (17)	13/113 (12)	0.23
Arthritis	89/113 (79)	99/113 (88)	0.08
Pleuritis	17/113 (16)	28/113 (25)	0.07
Pericarditis	11/113 (10)	22/113 (20)	0.036
Nephritis	30/113 (27)	33/113 (29)	0.66
Convulsions	10/113 (9)	4/113 (4)	0.10
Psychosis	2/113 (2)	3/113 (3)	0.65
AIHA	4/107 (4)	4/109 (4)	0.98
Leukopenia	78/110(71)	74/110 (67)	0.56
Thrombocytopenia	17/108 (16)	26/109 (24)	0.13
dsDNA-antibodies	67/96 (70)	93/112 (83)	0.024
FP-STS	7/42 (17)	12/48 (25)	0.33
Antinuclear antibodies	109/111 (98)	110/112 (98)	1.0
Deep venous thromboses	15/113 (13)	8/113 (7)	0.12
Stroke or TIA	9/113 (8)	2/113 (2)	0.034

AIHA = autoimmune haemolytic anaemia; dsDNA = double-stranded DNA; FP-STS = false positive serologic test for syphilis; TIA = trans ischemic attack.

2. Evaluation of one subphenotype of SLE, antiphospholipid syndrome (Study II)

Study I revealed a tendency for venous thrombosis and TIA/stroke to occur in patinets with familial SLE. However, the differences of those between familial and sporadic SLE was not statistically significant. Since aPLs are known to associate with thrombosis in patients with SLE, we evaluated the overall prevalence of different aPLs and their association with thrombosis and coronary heart disease in Finnish patients with SLE and APS. Moreover, since the familial occurrence of aPL and APS has been described earlier (Mackie et al. 1987; Ford et al. 1990; Dagenis et al 1992), our particular interest was to investigate whether there was any clustering of aPLs within multiplex families where at least two patients had had a thrombosis, reflecting genetic susceptibility to the development of APS.

From a total of 292 SLE patients (89 familial and 203 sporadic cases), 153 (52%) had antibodies detected by at least one method (Table 8). The sensitivity and specificity of each individual antibody assay to detect the history of thrombosis are also shown. Since it is known that antibodies tend to cross-react with each other, the combinations of different antibodies are also presented. All, except IgM-aCL, correlated rather well with each other. The correlation was highest between IgG-aCL and anti-prothrombin ($r = 0.464$) and the lowest between IgM-aCL and anti-ox-LDL ($r = 0.032$).

All antibody assays, except IgM-aCL, were significantly associated with a history of any thrombosis (Table 9). Arterial thrombosis was clearly associated with IgG-aCL, anti-b2-GPI and anti-prothrombin, whereas venous thrombosis correlated significantly with all other assays except IgM-aCL and anti-prothrombin. Because of a small number of affected patients with CHD, no significant association of any antibodies and the occurrence of CHD were seen.

Multiplex families

The proportion of patients with familial SLE and a history of thrombosis (15/89) was not statistically different from that in families with sporadic SLE cases (25/203) (OR 1.4; 95% CI 0.7-2.9). Of the 45 of 53 multiplex SLE families, there were 5 families with 3 affected pedigree members. In 2 of these 5 families, a thrombotic event had occurred in 2 affected patients. In the first family, the male proband with Klinefelter´s syndrome, had a history of recurrent pulmonary emboli with only marginally elevated IgA-aCL. The affected mother of the proband had a history of deep venous thrombosis and had an elevated level anti OX-LDL. Her identical twinsister, with SLE complicated by lymphoma, had elevated levels of IgG-aCL, IgA-aCL, anti-prothrombin, and anti ß2-GPI, but no history of thrombosis.

In the second family, the male proband had a history of nephrotic syndrome and multiple venous thromboses, but no aPL or anti OX-LDL. His mother and her cousin also had SLE; the former was negative for all assays and did not have thrombosis, and the latter had anti OX-LDL with a history of deep venous thrombosis.

In the remaining 40 multiplex SLE families with two affected members, there was only one family in which both affected patients (sisters) had a history of thrombosis. One of these patients was positive for all assays except IgM-aCL. Serum from the other patient was not available. All healthy first-degree relatives (mother, father, and one healthy sister) were negative for all assays.

Table 8. The prevalence of antibodies to cardiolipin, prothrombin, β2-glycoprotein I (β2-GPI)and oxidized low-density lipoprotein (oX-LDL) in 292 SLE patients. The sensitivity, specificity and positive (PPV) and negative (NPV) predictive values of the tests for a history of thrombosis.

Test	Positive	Thrombosis	Sensitivity	Specificity	PPV	NPV
Any aCL isotype	102 (35%)	20/40	50 %	67 %	20 %	89 %
IgG	49 (17%)	15/40	38 %	87 %	31 %	90 %
IgA	76 (26%)	14/40	35 %	75 %	18 %	88 %
IgM	27 (9%)	5/40	13 %	91	19 %	87 %
Antiprothrombin	58 (20%)	14/40	35 %	83 %	24 %	89%
Anti β2-GPI	46 (16%)	14/40	35 %	87 %	30 %	89 %
Anti ox-LDL	68 (23%)	4/40	35 %	79 %	21 %	88 %
Any aCL isotype + antiprothrombin	123(42%)	20/40	50 %	59 %	16 %	88 %
Any aCL isotype + anti β2-GPI	118 (40%)	23/40	58 %	62 %	20 %	93 %
Any aCL isotype + anti ox-LDL	132 (45%)	25/40	63 %	58 %	19 %	93 %
IgG-aCL + anti β2-GPI	70 (24%)	19/40	48 %	80 %	27 %	91 %
IgG-aCL + antiprothrombin	84 (29%)	18/40	45 %	74 %	21 %	89 %
IgG-aCL + anti ox-LDL	94 (32%)	23/40	58 %	72 %	24 %	91 %
Anti β2-GPI + anti ox-LDL	91 (31%)	21/40	53 %	72 %	23 %	91 %
Anti β2-GPI + antiprothrombin	84 (29%)	18/40	45 %	74 %	21 %	89 %

Sensitivity is the rate of true positives (number of patients with thrombosis and a positive test result divided by the number of patients with thrombosis), specificity is the rate of true negatives (number of patients without thrombosis and a negative test result divided by the number of patients without thrombosis), PPV (positive predictive value) is the number of true positives from all positives, NPV (negative predictive value) is the number of true negatives from all negatives. aCL= anticardiolipin, IgG = immunoglobulin G, ox-LDL = oxidized low-density lipoprotein

Table 9. Relationship between individual antibody tests and thrombosis in 292 patients with SLE

	Venous thrombosis			Arterial thrombosis			Any thrombosis		
Test	Positive	Negative	OR(95% CI)	Positive	Negative	OR(95% CI)	Positive	Negative	OR(95% CI)
IgG-aCl	8/49(16%)	17/243(7%)	2.6(1.1-6.4)	8/49(20%)	8/243(3%)	5.7(2.0-16.1)	15/49(31%)	25/243(10%)	3.9(1.8-8.0)
IgA-aCl	10/67(15%)	15/225(7%)	2.5(1.1-5.8)	5/67(7%)	11/225(5%)	1.6(0.5-4.7)	14/67(21%)	26/225(12%)	2.0(1.0-4.1)
IgM-aCl	4/27(15%)	21/265(8%)	2.0(0.6-6.4)	2/27(7%)	14/265(5%)	1.4(0.3-6.7)	5/27(19%)	35/265(13%)	1.5(0.5-4.2)
Anti β2-GPI	9/46(20%)	16/246(7%)	3.5(1.4-8.5)	6/46(13%)	10/246(4%)	3.5(1.2-10.3)	14/46(30%)	26/246(11%)	3.7(1.8-7.8)
Anti-prothrombin	8/58(14%)	17/234(7%)	2.0(0.8-5.0)	7/58(12%)	9/234(4%)	3.4(1.2-9.6)	14/58(24%)	26/234(11%)	2.6(1.2-5.3)
Anti-ox-LDL	11/68(16%)	14/224(6%)	2.9(1.3-6.7)	3/68(4%)	13/211(6%)	0.7(0.2-2.7)	14/68(21%)	26/224(12%)	2.0(1.0-4.0)

OD = odds ratio, CI = confidence interval, other abbreviations as indicated in Table 8

3. Identifying new susceptibility loci for SLE in multiplex families using linkage mapping (Study III)

The primary genome wide scan was performed with 400 highly polymorphic microsatellite markers (MD-10). Information was retrieved from 388 markers with an average marker interval of 9.73 cM. Due to the pedigree structures (i.e. missing parents), we were able to capture 50 to 60% of the maximum inheritance information throughout the genome.

The genome scan data contained six gaps of 20 to 26 cM. The highest NPL score was on chromosome 6q (NPL 2.35, p = 0.01) at marker D6S441, and the two next best regions on chromosomes 3q (D3S1278) and 5p (D5S418) with NPL scores of 1.78 (p = 0.03) and 1.74 (p = 0.04). In order to establish the empirical thresholds for suggestive and significant linkage (i.e., NPL scores reached at random once per one or 20 genome scans (Lander and Kruglyak, 1995)) in our data set, a simulation with 200 iterations were performed. The best observed NPL-score under the null hypothesis of no linkage revealed that NPL = 1.7 is reached once per genome scan (suggestive linkage), and the global p-value of 0.05 corresponds to NPL = 3 (significant linkage). Hence the 3 loci obtained in our primarily genome wide scan exceeded the threshold for suggestive linkage, yet none of them was significant.

To verify the highest NPL scores in regions with suggestive linkage (NPL > 1.7) on chromosomes 3q, 5p and 6q, and to cover gaps exceeding 20 cM on chromosomes 6p (i.e. HLA-region), 8 and 14q (with corresponding NPL >1), we added 29 markers in these regions. As a result, in 2 regions with suggestive linkage, on chromosome 5p and chromosome 6q, the NPL scores increased with the added markers (Table 10). The overall NPL scores, including all genotyped markers in the genome-wide scan, are shown in Figure 5. The largest change in NPL scores was seen in the HLA region, where the NPL score increased from 1.2 to 2.1. The NPL scores for 14q increased from 1.5 to 2.2.

Table 10. The linkage results in genome wide scan before and after addition of extra markers.

Chromosome content	NPL- scores, primary genome wide scan	NPL- scores after addition of markers
3q	**1.78** (D3S1278, *p*= 0.03)	1.55 (D3S1278, *p*= 0.06)
5p	**1.74** (D5S418, *p*= 0.04)	**2.03** (D5S418, *p*= 0.02)
HLA-region	1.22 (D6S276, *p*= 0.11)	**2.17** (D6S273, *p*= 0.02)
6q25-q27	**2.35** (D6S441, *p*= 0.01)	**2.47** (D6S960, *p*= 0.008)
8	0.62 (D8S260, *p*= 0.26)	0.48 (D8S260, *p*= 0.31)
14q21-q23	1.51 (D14S288, *p*= 0.07)	**2.2** (D14S587, *p*= 0.02)

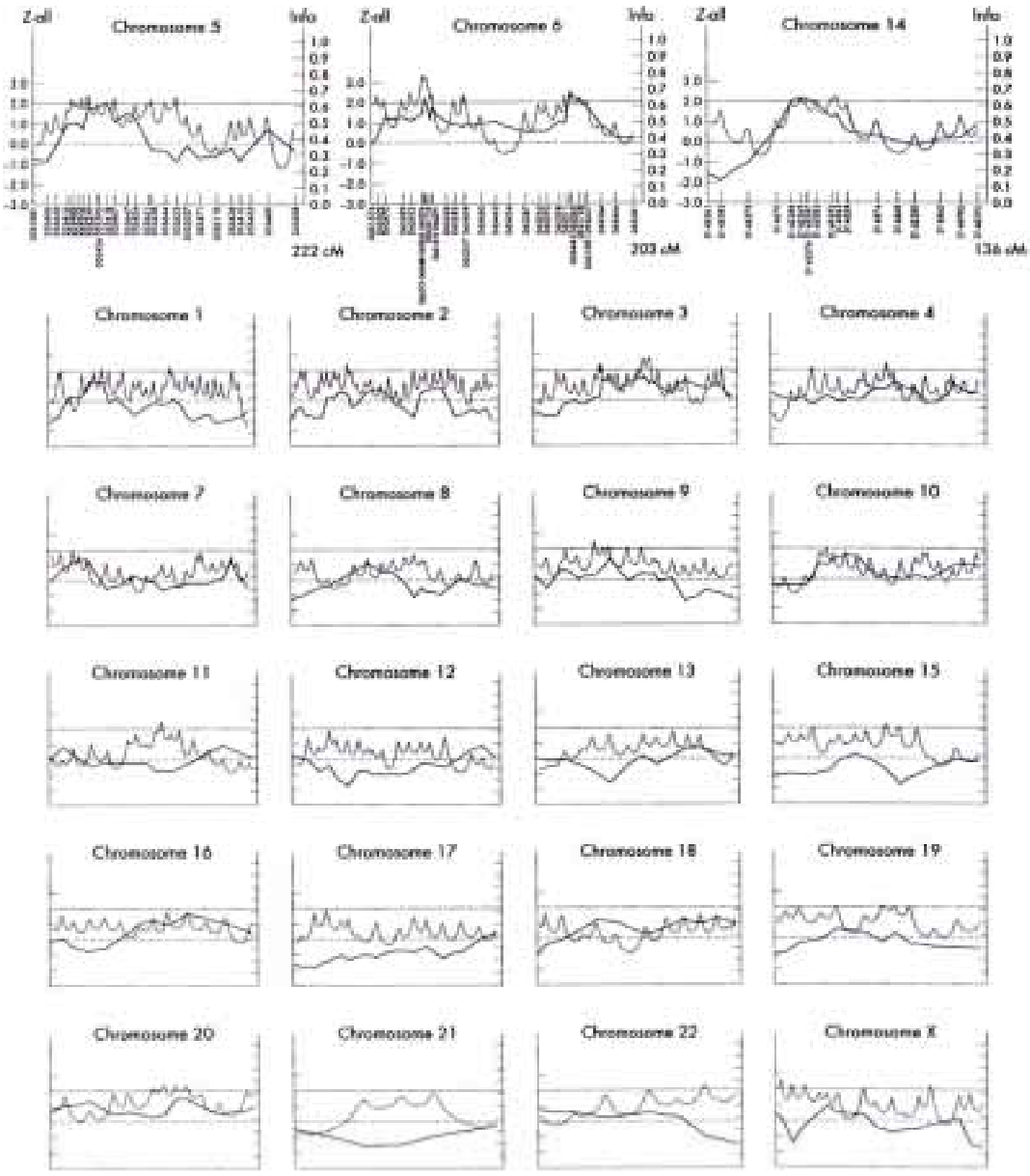


Fig 5. Nonparametric multipoint linkage analyses in 35 multiply affected SLE families. All chromosomes are shown; those with peaks exceeding NPL=2.0 are shown in large panels (top row). NPL scores are plotted with solid line, scale to the left, and information content with a dotted line, scale to the right. 417 markers were genotyped, and the average intermarker distance was 10 cM.

4. Haplotype associations on chromosomes 6q and 14q (IV)

To verify our linkage results and to narrow down the susceptibility region for SLE in Finnish families, we genotyped more markers along 10 cM streches on either side of the best NPL-scores on chromosomes 6q and 14q. On chromosome 6q, there were 25 markers across a 22 cM region, from D6S308 to D6S1035. On chromosome 14q, there were 32 additional markers, covering a region of 24 cM between markers D14S70 and D14S63. In spite of a few gaps of 2 to 4 cM within these regions, the average marker interval remained 1 cM or less. The NPL-scores after finemapping are shown in Figure 6.

Including data from 32 additional markers, the information content for chromosome 14q increased from 50 to 60% in the genome-wide scan to 72%. The highest NPL-score of 2.22 (p = 0.015) was observed at marker D14S1055, which lies 5 cM centromeric to the previous linkage peak at D14S587.

The haplotype association was studied and analyzed using TDT and HPM. Eighty-nine trios from 65 families were analyzed. Two distinct regions with excess allele sharing were identified. A significant association of a 2 cM long haplotype was seen in 10 chromosomes at 53.2-55.2 cM (markers D14S978-D14S589-D14S562, alleles 3-2-8). The pattern was not seen in the control chromosomes (the odds ratio could not be computed; $X^2 = 9.95$, p = 0.00598 for marker D14S562). The next region of allele sharing was a few cM centromeric to the previous one, at 50.1-50.2 between markers D14S1009-D14S748 (alleles 3-1). This haplotype was shared by 15 chromosomes of SLE patients, and was also present in 2 control chromosomes, but did not, however, reach significance ($X^2 = 9.99$, empirical $p = 0.14$, odds ratio = 13, 95% CI 2.9-59). For these 2 shared haplotypes, only 5 meiosis were informative for TDT analyses. The 3-2-8 was transmitted 4 times, and not-transmitted once ($p = 0.18$). The 3-1 haplotype was transmitted 6 times, and not-transmitted twice ($p = 0.157$). The TDT remained thus inconclusive.

There were three individuals who posessed both haplotypes 3-1 and 3-2-8 on the same chromosomes, suggesting a common origin. The longest shared haplotype between two unrelated SLE patients covered 8 cM, from D14S288-D14S281 (Fig. 7).

Data from 25 mapped markers across a 22 cM region on chromosome 6q were analyzed. The information content was 74%. The highest observed NPL score was 2.27 (p = 0.013), slightly lower than that observed in the genome scan (NPL= 2.47). The peak marker D6S1708 (at 157.8 cM) was 6 cM telomeric from the previous peak at D6S960 (at 151.4 cM). The NPL scores and the corresponding information content are shown in Figure 6.

Trios used for the HPM and TDT analyses were the same as for 14q analysis. There was some allele sharing between markers D6SGATA184A08 and D6S1637 (alleles 1 and 3) at 147-148 cM, 10 cM away from the linkage peak. Haplotype 1-3 was seen in 27 patient chromosomes and in 9 control chromosomes ($X^2 = 8.02$, empirical p = 0.0719 for marker D6S1637, odds ratio = 5.8, 95% CI 2.6-13). The empirical association was not significant. Haplotype 1-3 was transmitted 15 times, and not transmitted 5 times ($X^2 = 5.00$, p = 0.025).

Of the patients with excessive haplotype sharing, 5 out of the 10 chromosomes (5/9 families) with a 3-2-8-haplotype on chromocome 14q originated from Savo. Furthermore, both the 3-1 and 3-2-8- haplotypes, spanning 8 cM on chromosome 14q, were found in 2 families from neighbouring parishes in Savo.

There were 3 families (2 originating from Savo) in which both haplotypes on chromosome 14q and the haplotype on chromosome 6 were present, and 5 families (3 originating from Savo) had 6 patients, in which 2 haplotypes from both chromosome 14q and 6q were present. Excluding close relationships between the families, such haplotype sharing is highly unexpected if not associated with the selection criterion (i.e., SLE), and the clustering of the associated haplotypes in eastern Finland may suggest a founder effect, possibly involving two loci on chromosomes 14q and 6q.

D14S288 (47.57)	D14S47 (48.00)	D14S1027(49.00)	D14S259 (50.00)	D14S1009(50.10)	D14S748 (50.20)	D14S1055(50.30)	D14S255 (50.40)	D124S269(51.79)	D14S984 (52.50)	D14S978 (53.20)	D14S589 (54.20)	D14S562 (55.20)	D14S281 (55.40)
2	0	6	2	0	2	0	3	0	8	3	2	8	0
2	1	5	4	1	1	3	4	4	15	3	2	8	2
2	0	0	7	0	1	0	5	2	8	3	2	8	2
2	1	2	2	2	3	5	3	3	1	3	2	8	5
11	1	3	3	2	3	1	5	1	1	3	2	8	1
5	1	5	5	0	2	3	3	1	1	3	2	8	5
4	1	1	2	2	3	1	3	0	0	3	2	8	5
1	0	4	2	3	1	4	3	1	8	3	2	8	5
11	1	4	2	3	1	1	3	0	1	3	2	8	2
11	1	4	2	3	1	1	3	1	1	3	2	8	2

Fig. 7. Detailed analysis of independent SLE-associated haplotypes for marker loci on 14q from a set of patients of 35 multiplex and 31 singleton (Savo) Finnish families. The haplotype 3-2-8 is present in 10 patients and no controls and is transmitted 4 times and not transmitted once. The haplotype 3-1, present in 15 patients and 2 controls, is transmitted 6 times and not transmitted 2 times.

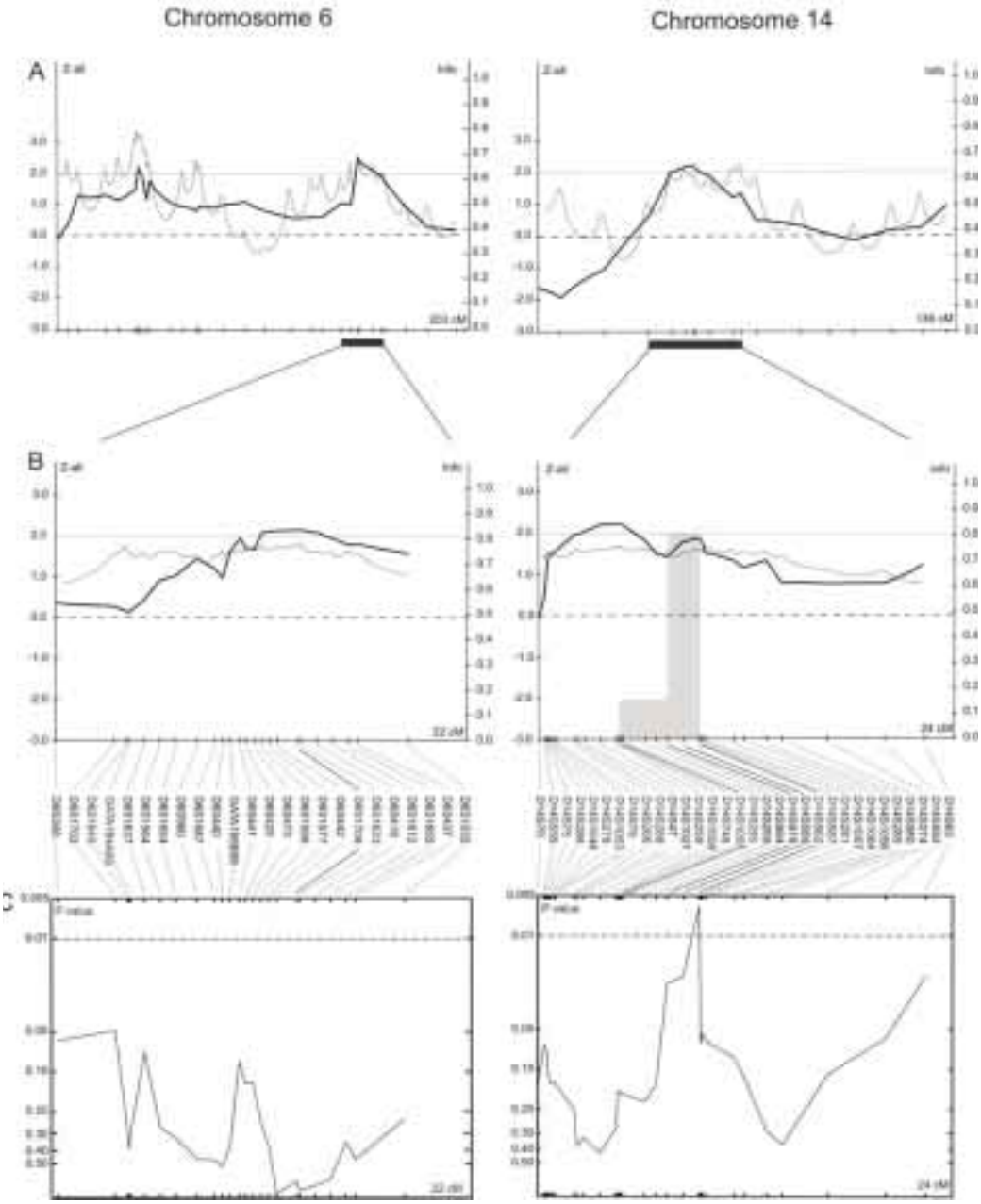


Figure 6. A. Linkage maps of chromosomes 6 and 14 from 35 Finnish families multiply affected by SLE, full chromosome views (Koskenmies et al. 2003). The distances between markers in linkage regions (black bars) are on average 3.5 cM.
B. Combined linkage results from 35 multiplex and 31 singleton (Savo) families after addition of fine-mapping markers, detailed maps. The intermarker distances are $\leq$1cM. Gray bars show the shared associating haplotypes in 10 patients by HPM analysis. Detailed haplotypes are shown in Figure 7.
C. Association analyses using HPM. The dotted line depicts the empirical significance level of 0.01. The y-axis shows the p-value, expressed in logarithmic scale. The x-axis shows the genotyped region in cM (same scale as panel B).

DISCUSSION

1. Clinical studies

In order to evaluate whether familial SLE influences disease expression, as evidence for a stronger genetic background, we compared the clinical manifestations in patients with sporadic and familial SLE. Familial and sporadic forms of same diseases can be clinically distinguished. For example, human non-polyposis colorectal cancer is an autosomal dominantly inherited colorectal cancer, which differs from the sporadic form by an earlier age of onset (<50 years), a right-sided location in the colon, and a family history of colorectal and/or other extra-intestinal cancers (Aaltonen and Peltomäki 1994; Vasen 1994; Suh et al. 2002). Also, familial adenomatous polyposis, another dominantly inherited colorectal cancer, affect individuals at a younger age, and is often associated with extra-intestinal manifestations including cutaneous cysts, osteomas, desmoids, and cancers (Giardiello et al. 1997). In both of these hereditary colorectal cancers several gene mutations have been already identified (Aaltonen et al. 1993; Aaltonen and Peltomaki 1994; Giardiello et al. 1997; Suh et al. 20029). Additionally, a familial form of Alzheimer´s disease, affecting approximately 10% of patients, has been identified. Patients having these mutations are young at age at onset, and have affected first-degree relatives (Haass and Baumeister 1998; St. George-Hyslop 2000).

Studies comparing the clinical manifestations in patients with sporadic and familial SLE are few. In 1976, Arnett and Schulman compared the phenotypes of first-degree relatives with SLE with those of patients with sporadic and familial SLE. They found a clear concordance for disease expression between affected first-degree relatives (parent/offspring and monozygotic twins) within families, but also found that different families were concordant for different phenotypes. These findings support both the genetic influence in development of the disease and the genetically heterogeneous nature of SLE. As there were no differences in clinical manifestations of patinents with sporadic and familial SLE, Arnett and Shulman concluded that familial SLE is unlikely to be a different disease entity from non-familial SLE.

In the present study, we compared clinical manifestations and laboratory findings of 113 patients from 53 multiplex families with those of 113 sporadic cases, mached by age, sex and duration of disease. We did not look at the concordance of disease expression between family members in multiplex families. Our multiplex families included second degree relatives, and even more distantly related pedigree members, because the patient collection was originally focused on genetic studies, and hence all available affected pedigree members were collected, and also clinically characterized. We found no differencies in clinical manifestations between sporadic and familial SLE patients. Our results coincide with those found in previous studies (Arnett and Schulman 1976; Gourley et al. 1996; Michel et al. 2001).

Although the clinical expression of SLE varies widely, the four most prevalent clinical manifestations in our patients were the presence of ANA, arthritis, photosensitivity, and malar/butterfly rash. This is in accordance with other clinical studies of SLE (Gudmundsson and Steinsson 1990; Gourley et al. 1996; Michel et al. 2001) regardless of ethnicity. The frequency of nephritis in our patients was lower (27-29%) than previously reported (Arnett and Shulman 1976; Tan et al. 1982; Cervera et al. 1993). Whether this is due to ethnic diversity or methodological differences is unclear. In a nationwide Icelandic study (Gudmundson and Steinsson 1990), and in an Irish study of familial SLE (Gourley et al.1996), the frequencies of renal manifestations were close to our findings (20% and 15%,

respectively). To study susceptibility genes in SLE, one should concentrate on those patients with rare or more severe symptoms. This, however, requires a large number of families, and in a small country such as Finland, this may be difficult to accomplish for a rare disease like SLE. Some successfull linkage studies on stratifying patients based on neurological manifestations, as an example, have been obtained worldwide (Nath et al. 2002).

In a study by Michel et al. (2001) autoimmune diseases other than SLE were evaluated in relatives of affected SLE patients. More than 20% of first-degree relatives of a patient, and as many as 11% of lupus propands, were found to have another distinct SLE associated autoimmune disease. Similar results have been reported earlier (Arnett 1997). The frequency of other autoimmune diseases in sporadic patients or their relatives was not reported. It has been speculated (Michel et al. 2001) that because autoimmune diseases may share some common susceptibility genes, families with multiple cases of various autoimmune disorders should be considered in genetic studies. In our genome-wide scan study, there were only 2 patients and 3 first-degree relatives, who were reported to have another autoimmune disease, other than SLE. However, with a total number of 169 genotyped individuals, these 5 cases could not have any influence on the linkage results.

The main motivation for Study II was to investigate one SLE subphenotype, antiphospholipid syndrome (APS), as a possible tool for further genetic studies. First-degree relatives of patients with SLE or APS have a higher prevalence of anticardiolipin antibodies (aCL), suggesting a genetic predisposition to the development of these antibodies (Mackworth-Young et al 1987; Goldberg et al. 1995). The strongest evidence for a familial form of APS has been the identification of several kindreds with an increased frequency of antiphospholipid antibodies and the associated clinical manifestations, including thrombosis and recurrent abortions (Mackie et al. 1987; Matthey et al. 1989; Ford et al. 1990). In our study, there was no difference between multiplex and sporadic families in the occurrence of thrombosis. Additionally, antiphospholipid antibodies were not more frequent in the relatives of patients in the 3 multiplex families, where thrombosis had occurred at least in two pedigree members with SLE. However, the number of families identified was very small, and hence no definitive conclusions of the existence of familial ASP can be drawn from our study. Moreover, the recruitment of patients was basically targeted toward genetic studies. This means that only those individuals important for linkage were recruited, and especially in a large kindred, the status of aPLs of many pedigree members were not evaluated, suggesting that a putative clustering of aPLs in our families may have been underestimated. Finally, aPLs are a heterogeneous group of antibodies associated with many clinical manifestations (such as infections and malignancies). This means that diseases other than APS in SLE patients and their relatives have to be taken into account when seeking possible clustering of antiphospholipid antibodies in a family. In our study, the thrombotic events, at least in some cases, were probably due to factors other than those associated with these antibodies, suggesting that the etiopathogenesis of thrombosis in patients with familial SLE is multifactorial.

2. Genetic studies

This is the first nationwide study mapping SLE susceptibility genes in the Finnish population. More than 80% of hospital patients with SLE in Finland population were recruited. From the identified families, 53 were multiply affected by SLE and 35 of these families, informative for linkage, was included in our primary genome-wide scan. In spite of the suggested

homogeneous population structure in Finland, we could not find any single major locus contributing to the disease in our sample set. Three loci, however, exceeded the linkage threshold of 1.7, corresponding to the definition of suggestive linkage genome-wide (Lander and Kruglyak 1995). With addition of markers after the primary scan, we were able to increase the information content genome wide, but could not reach the threshold for significant linkage for any locus. Ideally, one would like to verify the results in an independent data set, but this is not possible because we had already sampled almost all available SLE patients in Finland. These results can, however, be used to guide further association mapping with very high-density marker maps (Elston et al. 1996).

Suggestive linkage was found in two previously reported regions on chromosomes 6p (near HLA region) and 14q21-q23 (Gaffney et al. 1998), and in two new locations. Linkage to chromosome 5p has not been described previously. Linkage on chromosome 6q25-q27 has some overlapping results with one previous SLE study (Moser et al. 1998), however, for a different map position. Linkage to the HLA region is in accordance with previous studies. The association between SLE and the HLA complex was reported already in 1970´s (Grumet et al. 1971), and since then, studies in patients of European descent have consistently shown an associations, especially between SLE and DR2 and DR3 (Harley et al. 1998; Tan and Arnett 1998). More recently, Gaffney et al. (1998) observed strong linkage to the HLA-region in their genome- wide scan, with supporting results from studies by Shai et al. (1999) and Lindqvist et al. (2000).

Linkage to 14q21-q23 has been suggested in three previous genome wide scans. The best NPL score (2.81, $p < 0.0016$) was at marker D14S276 (Gaffney et al. 1998), which lies only 1 cM telomeric from the linkage peak in our genome wide scan. High LODs were also found for D14S258 (LOD = 2.02, $p = 0.02$) (Shai et al. 1999), and D14S592 (LOD = 1.5) (Lindqvist et al. 2000). These data indicates locus heterogeneity between the different datasets, and even though none of these studies yielded significant linkage, they support evidence for SLE susceptibility on chromosome 14q.

The relevance of linkages to 5p and 6q were more difficult to evaluate. Linkage to chromosome 5p has not been demonstrated previously, while linkage to 6q, about 20 cM telomeric to our linkage peak, has been implicated in one previous SLE study (Moser et al. 1998). In studies with other autoimmune diseases, however, linkages to the same general region have been identified. A significant lod score of 4.5 (between markers D6S476 and D6S473) was observed on 6q (IDDM5) for type I diabetes (Davies et al. 1996; Todd 1997). In patients with rheumatoid arthritis there is evidence for linkage disequilibrium on chromosome 6q, at markers D6S446, D6S311 and D6S440 (Myerscough et al. 2000). Our linkage peak at D6S960 (NPL = 2.47, $p = 0.08$) is 2 cM away from theirs peak at D6S440. Linkage to chromosome 6q in different autoimmune diseases suggests that chromosome 6q25-27 may harbour a gene influencing predisposition to autoimmunity.

To verify our linkage results and to proceed toward genetic association study we performed fine mapping with a dense set map of markers in two identified regions on chromosomes 14q22-q23 and 6q25-q27. These regions were chosen mainly because our genome wide scan results coincided with those of previous studies of SLE (14q) and other autoimmune diseases (6q). Even though linkage to the HLA region has been observed in previous studies, we did not include it in our fine-mapping, as the HLA region (because of it´s importance in many autoimmune disese) has been and is being studied worldwide. While planning the fine

mapping project, the sequence of chromosome 5 in public sequence repositories was still very fragmented, and hence fine mapping of this region was postponed.

The fine-mapping was performed using microsatellite markers with an average marker distance of 1 cM. Families originating from a restricted Savo region in central eastern Finland were added to our data set under the hypothesis of shared ancestral mutations among the patients. This geographical region has shown founder effects for hereditary nonpolyposis colorectal cancer and chloride diarrhea, which both are monogenic diseases (Nystrom-Lahti et al. 1994; Hoglund et al. 1995).

There was excess allele sharing in both chromosomes, 6q and 14q, and clustering of associated haplotypes in Savo samples. The length of the associated haplotype was 2 cM on chromosome 14q (alleles 3-2-8) and 1 cM on chromosome 6q (alleles 1-3). The length of the associated haplotypes is roughly in accordance with the population history. The area was settled in the early 16th century, and founder effects have been noted there (Lahermo et al. 1996; de la Chapelle 1998). Despite the small number of associated families, we found significantly excessive haplotype sharing between patients (IV). Two patients sharing a long conserved haplotype (8cM) originated from neighbouring Savo parishes, but the patients´ relationship was excluded at least back to the mid-19th century. Moreover, six patients from five unrelated families shared conserved haplotypes for two loci on different chromosomes, 6q and 14q, an observation which is highly unlikely if not causally associated with the disease.

The results from our association study verified the linkage regions identified in our genome-wide scan, and refined the search regions considerably. The conserved haplotype on chromosome 14q is located next to the best linkage peak observed in our genome wide scan, and coincides with the peak of Gaffney et al. (1998).

For chromosome 6q, the conserved haplotype lies 3 cM centromeric from the observed linkage peak in our genome wide scan, verifying the linkage region. Moreover, the over-transmitted haplotype coincides with the peak for rheumatoid arthritis (Myerscough et al. 2000), suggesting that this general region is highly interesting not only for SLE susceptibility genes, but also for possible genes influencing the predisposition to autoimmunity in general.

CONCLUSIONS AND FUTURE PROSPECTS

This study was performed to search for susceptibility genes among Finnish families multiply affected by SLE. A total of 53 multiplex families were identified during an intensive recruitment phase covering more than 80% of all hospital-based SLE patients in Finland. The clinical characteristics of SLE patients in multiplex families were evaluated, and were compared with those of families with sporadic patients, but no obvious phenotype for "familial" SLE, as a tool for further genetic analyses, was found. Moreover, stratifying our patients according to one SLE subphenotype, antiphospholipid syndrome, did not reveal any evidence for familial aggregation of antiphospholipid antibodies in association with thrombosis in multiplex families. Hence, clinically, familial and sporadic SLE were shown to be the same entity, suggesting that results from further genetic studies in multiplex families can be extrapolated to all patients with SLE.

We found no single major gene contributing to the risk of the disease in Finland, despite its population structure. However, in 35 families multiply affected by SLE, we found a suggestive linkage to four loci, providing further evidence for SLE loci at two of these, on chromosome 6p and 14q. Linkage to chromosome 5p has not been described previously, whereas linkage to chromosome 6q was reported in one previous SLE study. The same genomic region on 6q has also been identified in studies with type I diabetes and rheumatoid arthritis, and hence can harbour a gene influencing the predisposition for autoimmunity.

The association study was performed on 2 of the linkage regions, on chromosomes 6q and 14q. With 31 additional families originating from central eastern Finland, a region with a known founder effect, we observed excess sharing of a haplotype on 14q and excess transmission of a haplotype on 6q. Our finding not only verified the suggestive linkage results observed in our genome-wide scan, but also coincided with those of three independent data sets, and refined the search regions considerably. Our results are compatible with the idea of a founder effect for susceptibility genes in SLE in central eastern Finland, and suggest a path to the isolation of putative susceptibility genes.

In the near future, our aim is to fine map the linkage region on chromosome 5p in order to verify these linkage results. Moreover, to confirm the suggested identity-by-descent of the identified haplotypes on both chromosomes 14q and 6q, further fine-mapping with tightly-spaced SNP markers within and near the haplotypes will be conducted. Thereafter, positional cloning can proceed to verify new transcripts and to study their polymorphisms and possible associations with SLE in this and other sample sets. Our research group envisions that these genetic studies, incorporating careful clinical evaluation of patients, systematic linkage and association studies, and finally, future positional identification of susceptibility genes, will contribute to our understanding of mechanisms in this puzzling disease.

ACKNOWLEDGEMENTS

This study was performed during the years 1997-2004 at the Department of Medical Genetics, University of Helsinki. During these years, a number of people have contributed to this work in many ways, and without their help and encouragement this work would have never been accomplished.

I thank the former and present heads of the Department of Medical Genetics, Professors Juha Kere and Pertti Aula, acting-Professor Anna-Elina Lehesjoki, Professor Leena Palotie and acting-Professor Kristiina Aittomäki for the excellent research facilities.

My warmest gratitude goes to my supervisors Juha Kere, Elisabeth Widén and Heikki Julkunen. It has been a privilege and joy to work in Juha´s group. Juha has a great ability to support, encourage and guide during miserable times, when nothing seemed to work out and the giving up seemed the only possible thing to do. I thank Elisabeth for her expertise in disease gene mapping and her valuable advice and comments. You always had time when I needed help. Heikki is thanked for many things. For practical matters, first of all, for being the link between patients (patient collection) and this work. His contribution in Studies I and II has been essential, and his expertise in the field of rheumatology has helped me to understand this clinically very diverse, variable and difficult disease that is SLE.

Professor Kimmo Aho and Docent Maija Wessman, the official reviewers of this thesis, are thanked for their valuable advice and constructive criticism. Professor Kimmo Aho is a great personality, with whom I had the pleasure to have many interesting and instructive scientific conversations in the field of rheumatology. I thank Docent Maija Wessman for her expertise in genetics and for valuable comments. It was a pleasure having you as my reviewer.

I am deeply grateful to all SLE patients and their family members for participating in this study.

My warmest thanks go to laboratory technician Riitta Lehtinen. Thank you for patiently teaching all the laboratory techniques and helping me in every way.

I thank my collaborators in this work. Päivi Lahermo and Päivi Tikka for helping with characterizing markers and genotyping, Vesa Ollikainen for performing simulations, and Päivi Onkamo and Petteri Sevón for their essential biostatistical contribution to this work. Sirkka Ruohomäki, Päivi Hantula, Anitta Hottinen, Sirkku Ekström, Päivi Malinen and Teija Baas are thanked for excellent technical assistance and Anne Lehto for invaluable help with data management.

I thank my co-authors Timo Palosuo and Outi Vaarala, as well laboratory technicians Tuula Kalenius and Pirjo Mäki, who all contributed essentially to Study II.

I warmly thank Sinikka Lindh for her help with sample collection, and Minna Maunula and Elina Lampainen for their help in many practical matters.

During the past years I have had an opportunity to become acquainted with many young scientists, and each and every one of them is thanked for the enjoyable times together. Especially I want to thank Kati Asumalahti, Tarja Laitinen, Siv Knaappila, Ralf Krahe, Pia

Höglund, Katariina Hannula-Jouppi, Siru Mäkelä, Outi Elomaa, Minna Kujala, Hanne Lohi, Paula Kauppi, Ulla Hodgson and Anne Polvi. Hannu Turunen is also thanked for his expertise in computers and for the help with them during the past years. My special thanks go to Hannele Koillinen and Jaana Nopola-Hemmi. It has been a joy having you as friends during all these years, not only in the laboratory but also outside the University. It has been a relief sharing the highlights and disappointments that mapping complex diseases, but also of life in general, has brought along. Thank you for your friendship.

I acknowledge Jodie Painter for revising the language of my thesis.

Hugest thanks to all of my friends outside the Department. It would take too many pages to list all the good friends I am surrounded with in my life. I thank you all collectively for sharing the joys and sorrows of life, and for having you as friends. I especially want to thank Sirkku, Riikka, Eeva and Tiina. Even though our timetables in this rushed world do not allow us to get together as often as we used to as students, it is always a great pleasure and great fun, whenever we have the opportunity to do so. Thanks for those moments.

I am grateful to my parents Eila and Reijo Koskinen for their love, support and encouragement not only for and during this work but also for every matter in my life. Particularly, I want to thank them for their indispensable assistance in child-care during these years. I thank my sisters Saila and Sanna, and my brother in-law Petri for their love, friendship and helpfulness. My mother-in-law, Hilkka Koskinen, is thanked for her never-ending optimism, kindness and help, and Outi and Jukka Koskinen for their friendship.

Finally, my deepest of all gratitude goes to my family: my husband Jari-Pekka, who has patiently shared the up and downs of life during these years. Thank for your love and affection, and for having two wonderful children, Linnea and Elias, together. You three are the most precious things in my life.

This study was financially supported by the Sigrid Jusélius Foundation, Helsinki University Central Hospital research funds, and the Academy of Finland, and the Stockmann foundation

Helsinki, February 2004

ELECTRONIC DATABASE INFORMATION

The Marshfield genetic maps http://research.marshfieldclinic.org/genetics/ (for selecting markers)

National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov (for verifying the marker order for fine-mapping)

UCSC Genome Bioinformatics Site http://genome.ucsc.edu (for verifying the marker order in fine-mapping)

REFERENCES

Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR. *Science* 260: 812-6, 1993

Aaltonen LA, Peltomaki P. Genes involved in hereditary nonpolyposis colorectal carcinoma. *Anticancer Res* 14: 1657-60, 1994

Abu-Shakra M, Lee P. Mortality in systemic sclerosis: a comparison with the general population. *J Rheumatol* 22: 2100-2, 1995

Alarcon-Segovia D, Deleze M, Oria CV, Sanchez-Guerrero J, Gomez-Pacheco L, Cabiedes J, Fernandez L, Ponce de Leon S. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine (Baltimore)* 68: 353-65, 1989

Arnett FC, Shulman LE. Studies in familial systemic lupus erythematosus. *Medicine* (Baltimore). 55: 313-22, 1976

Arnett FC. The genetics of human lupus erythematosus. In: *Duboi´s lupus erythematosus*. Wallace DJ, Hahn BH (eds). Baltimore: Willimas and Wilkins: 1997, 77-117

Arnett FC, Reveille JD. Genetics of systemic lupus erythematosus. *Rheum Dis Clin North Am* 18: 865-92, 1992

Asherson RA, Khamashta MA, Ordi-Ros J, Derksen RH, Machin SJ, Barquinero J, Outt HH, Harris EN, Vilardell-Torres M, Hughes GR. The "primary" antiphospholipid syndrome: major clinical and serological features. *Medicine (Baltimore)* 68: 366-74, 1989

Asumalahti K, Veal C, Laitinen T, Suomela S, Allen M, Elomaa O, Moser M, de Cid R, Ripatti S, Vorechovsky I, Marcusson JA, Nakagawa H, Lazaro C, Estivill X, Capon F, Novelli G, Saarialho-Kere U, Barker J, Trembath R, Kere J; Psoriasis Consortium. Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus. *Hum Mol Genet* 11: 589-97, 2002

Becker KG, Simon RM, Bailey-Wilson JE, Freidlin B, Biddison WE, McFarland HF, Trent JM. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. *Proc Natl Acad Sci U S A* 95:9979-84, 1998

Bell DA, Maddison PJ. Serologic subsets in systemic lupus erythematosus: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum* 23: 1268-73, 1980

Botto M, Theodoridis E, Thompson EM, Beynon HL, Briggs D, Isenberg DA, Walport MJ, Davies KA. Fc gamma RIIa polymorphism in systemic lupus erythematosus (SLE): no association with disease. *Clin Exp Immunol* 104: 264-8, 1996

Bowness P, Davies KA, Norsworthy PJ, Athanassiou P, Taylor-Wiedeman J, Borysiewicz LK, Meyer PA, Walport MJ. Hereditary C1q deficiency and systemic lupus erythematosus. *QJM* 87: 455-64, 1994
Branch DW. Thoughts on the mechanism of pregnancy loss associated with the antiphospholipid syndrome. *Lupus* 3: 275-80, 1994

Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, Domenech I, Aydintug AO, Jedryka-Goral A, de Ramon E, Galeazzi M, Haga HJ, Mathieu A, Houssiau F, Ingelmo M, Hughes G. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine (Baltimore)* 72:113-24, 1993

Cervera R, Asherson RA. Clinical and epidemiological aspects in the antiphospholipid syndrome. *Immunobiology* 207: 5-11, 2003

Chakravarti A. Population genetics--making sense out of sequence. *Nat Genet* 21(Suppl 1): 56-60, 1999

de la Chapelle A, Wright FA. Linkage disequilibrium mapping in isolated populations: the example of Finland revisited. *Proc Natl Acad Sci U S A* 95: 12416-23, 1998

Cohen PL, Eisenberg RA. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu Rev Immunol* 9: 243-69, 1991

Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. *Genome Res* 8: 1229-31, 1998

Cordell HJ. Epistasis: what it means, what it doesn´t mean, and statistical methods to fetect it in humans. *Hum Mol Genet* 11: 2463-2468, 2002

Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. *Nat Genet* 29: 229-32, 2001

Davies JL, Cucca F, Goy JV, Atta AA, Merriman ME, Wilson A, Barnett AH, Bain SC, Todd JA. Saturation multipoint mapping of chromosome 6q in type 1 diabetes. *Hum Mol Genet* 5: 1071-4, 1996

Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, Walker A, Mack TM. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 35:311-8, 1992

Dijstelbloem HM, Bijl M, Fijnheer R, Scheepers RH, Oost WW, Jansen MD, Sluiter WJ, Limburg PC, Derksen RH, van de Winkel JG, Kallenberg CG. Fcgamma receptor polymorphisms in systemic lupus erythematosus: association with disease and in vivo clearance of immune complexes. *Arthritis Rheum* 43: 2793-800, 2000

Drake CG, Babcock SK, Palmer E, Kotzin BL. Genetic analysis of the NZB contribution to lupus-like autoimmune disease in (NZB x NZW)F1 mice. *Proc Natl Acad Sci U S A* 91: 4062-6, 1994

Drake CG, Rozzo SJ, Vyse TJ, Palmer E, Kotzin BL. Genetic contributions to lupus-like disease in (NZB x NZW)F1 mice. *Immunol Rev* 144:51-74, 1995

Drake CG, Rozzo SJ, Hirschfeld HF, Smarnworawong NP, Palmer E, Kotzin BL. Analysis of the New Zealand Black contribution to lupus-like renal disease. Multiple genes that operate in a threshold manner. *J Immunol* 154: 2441-7, 1995

Duits AJ, Bootsma H, Derksen RH, Spronk PE, Kater L, Kallenberg CG, Capel PJ, Westerdaal NA, Spierenburg GT, Gmelig-Meyling FH, van de Winkel JG. Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum* 38: 1832-6, 1995

Emlen W. Immune complexes in systemic lupus erythematosus. In: Wallace DJ, Hahn BH (eds). *Duboi´s lupus erythematosus*. Lea & Febiger, Philadelphia, 1993, 100-7

Elston RC, Guo X, Williams LV. Two-stage global search designs for linkage analysis using pairs of affected relatives. Genet Epidemiol 13: 535-58, 1996

Fessel WJ. Systemic lupus erythematosus in the community. Incidence, prevalence, outcome, and first symptoms; the high prevalence in black women. *Arch Intern Med* 134: 1027-35, 1974

Fielder AH, Walport MJ, Batchelor JR, Rynes RI, Black CM, Dodi IA, Hughes GR. Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. *BMJ* 286: 425-8, 1983

Ford PM, Brunet D, Lillicrap DP, Ford SE. Premature stroke in a family with lupus anticoagulant and antiphospholipid antibodies. *Stroke* 21: 66-71, 1990

Formica MK, Palmer JR, Rosenberg L, McAlindon TE. Smoking, alcohol consumption, and risk of systemic lupus erythematosus in Black Women´s Healt Study. *J Rheumatol* 30: 1222-6, 2003

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D.The structure of haplotype blocks in the human genome. *Science* 296: 2225-9, 2002

Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, Malmgren ML, Rohlf KE, Ockenden TC, Messner RP, King RA, Rich SS, Behrens TW. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci U S A* 95: 14875-9, 1998

Gaffney PM, Ortmann WA, Selby SA, Shark KB, Ockenden TC, Rohlf KE, Walgrave NL, Boyum WP, Malmgren ML, Miller ME, Kearns GM, Messner RP, King RA, Rich SS, Behrens TW. Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am J Hum Genet* 66: 547-56, 2000

Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesman PJ, Barbui T, Zwaal RF, Bevers EM. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 335: 1544-7, 1990

Galli M, Ruggeri L, Barbui T. Differential effects of anti-beta2-glycoprotein I and antiprothrombin antibodies on the anticoagulant activity of activated protein C. *Blood* 91: 1999-2004, 1998

Galli M, Barbui T. Antiprothrombin antibodies: detection and clinical significance in the antiphospholipid syndrome. *Blood* 93: 2149-57, 1999

Giardiello FM, Petersen GM, Piantadosi S, Gruber SB, Traboulsi EL, Offerhaus GJ, muro K, Krush AJ, Booker SV, Luce MC, Laken SJ, Kinzler KW, Vogelstein B, Hamilton SR. APC gene mutations and extraintestinal phenotype of familial adenomatous polyposis. *Gut* 40: 521-5, 1997

Gilliam JN, Sontheimer RD. Skin manifestations of SLE. *Clin Rheum Dis* 8: 207-18, 1982

Gnaussy NO, Sibbitt W Jr, Bankhurst AD, Qualls CR. Cigarette smoking and disease activity in systemic lupus erythematosus. *J Rheumatol* 30: 1215-21, 2003

Goldberg SN, Conti-Kelly AM, Greco TP. A family study of anticardiolipin antibodies and associated clinical conditions. *Am J Med* 99: 473-9, 1995

Gourley IS, Cunnane G, Bresnihan B, FitzGerald O, Bell AL. A clinical and serological comparison of familial and non-familial systemic lupus erythematosus in Ireland. Lupus 5: 288-93, 1996

Gourley IS, Pattersson CC, Bell AL: The prevalence of systemic lupus erythematosus in Northern Ireland. *Lupus* 6: 399-403, 1997

Graham RR, Langefeld CD, Gaffney PM, Ortmann WA, Selby SA, Baechler EC, Shark KB, Ockenden TC, Rohlf KE, Moser KL, Brown WM, Gabriel SE, Messner RP, King RA, Horak P, Elder JT, Stuart PE, Rich SS, Behrens TW. Genetic linkage and transmission disequilibrium of marker haplotypes at chromosome 1q41 in human systemic lupus erythematosus. *Arthritis Res* 3: 299-305, 2001

Graham RR, Ortmann WA, Langefeld CD, Jawaheer D, Selby SA, Rodine PR, Baechler EC, Rohlf KE, Shark KB, Espe KJ, Green LE, Nair RP, Stuart PE, Elder JT, King RA, Moser KL, Gaffney PM, Bugawan TL, Erlich HA, Rich SS, Gregersen PK, Behrens TW. Visualizing human leukocyte antigen class II risk haplotypes in human systemic lupus erythematosus. *Am J Hum Genet* 71: 543-53, 2002

Granados J, Vargas-Alarcon G, Drenkard C, Andrade F, Melin-Aldana H, Alcocer-Varela J, Alarcon-Segovia D. Relationship of anticardiolipin antibodies and antiphospholipid syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE). *Lupus* 6: 57-62, 1997

Gray-McGuire C, Moser KL, Gaffney PM, Kelly J, Yu H, Olson JM, Jedrey CM, Jacobs KB, Kimberly RP, Neas BR, Rich SS, Behrens TW, Harley JB. Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16-15.2. *Am J Hum Genet* 67: 1460-9, 2000

Grennan DM, Parfitt A, Manolios N, Huang Q, Hyland V, Dunckley H, Doran T, Gatenby P, Badcock C. Family and twin studies in systemic lupus erythematosus. *Dis Markers* 13: 93-8, 1997

Gribenberg-Gahmberg M, Konttinen Y. Systeemiset sidekudostaudit. In: Leirisalo-Repo M, Hämäläinen M, Moilanen E (eds). *Reumataudit.* Duodecim, Helsinki, 2002, 222-8

Grumet FC, Coukell A, Bodmer JG, Bodmer WF, McDevitt HO. Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. A possible genetic predisposition to disease. *N Engl J Med* 285: 193-6, 1971

Gudmundsson S, Steinsson K. Systemic lupus erythematosus in Iceland 1975 through 1984. A nationwide epidemiological study in an unselected population. *J Rheumatol* 17: 1162-7, 1990

Guo SW, Thompson ET. Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. *Biometrics* 48: 361-72, 1992

Gutierrez MA, Garcia ME, Rodriguez JA, Rivero S, Jacobelli S. Hypothalamic-pituitary-adrenal axis function and prolactin secretion in systemic lupus erythematosus. *Lupus* 7: 404-8, 1998

Haass C, Baumeister R. What do we learn from a few familial Alzheimer´s disease case? *J Neural Transm* 54(Suppl.1) 137-45, 1998

Hahn BH. An overview of the pathogenesis of systemic lupus erythematosus. In: Wallace DJ, Hahn BH (eds). *Duboi´s lupus erythematosus*. Lea & Febiger, Philadelphia, 1993, 67-70.

Harbige LS. Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutr Health* 10: 285-312, 1996

Harley JB, Moser KL, Gaffney PM, Behrens TW. The genetics of human systemic lupus erythematosus. *Curr Opin Immunol* l10: 690-6, 1998

Haywood ME, Hogarth MB, Slingsby JH, Rose SJ, Allen PJ, Thompson EM, Maibaum MA, Chandler P, Davies KA, Simpson E, Walport MJ, Morley BJ. Identification of intervals on chromosomes 1, 3, and 13 linked to the development of lupus in BXSB mice. *Arthritis Rheum* 43: 349-55, 2000

Hellan M, Kuhnel E, Speiser W, Lechner K, Eichinger S. Familial lupus anticoagulant: a case report and review of the literature. *Blood Coagul Fibrinolysis* 9: 195-200, 1998

Helve T. Prevalence and mortality rates of systemic lupus erythematosus and causes of death in SLE patients in Finland. *Scand J Rheumatol* 14: 43-6, 1985

Hochberg MC. Prevalence of systemic lupus erythematosus in England and Wales, 1981-2. *Ann Rheum Dis* 46: 664-6, 1987

Hochberg MC. The application of genetic epidemiology to systemic lupus erythematosus. *J Rheumatol* 14: 867-9, 1987

Hochberg MC . The epidemiology of systemic lupus erythematosus. In: Wallace DJ, Hahn BH (eds). *Duboi´s lupus erythematosus*. Lea & Febiger, Philadelphia, 1993, 49-57

Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 40: 1725, 1997

Hogarth MB, Slingsby JH, Allen PJ, Thompson EM, Chandler P, Davies KA, Simpson E, Morley BJ, Walport MJ. Multiple lupus susceptibility loci map to chromosome 1 in BXSB mice. *J Immunol* 161: 2753-61, 1998

Hoglund P, Sistonen P, Norio R, Holmberg C, Dimberg A, Gustavson KH, de la Chapelle A, Kere J. Fine mapping of the congenital chloride diarrhea gene by linkage disequilibrium. *Am J Hum Genet* 57: 95-102, 1995

Horowitz DA, Stohl W, Gray JD. T-lymphocytes, natural killer cells, cytokine and immune regulation. In Duboi´s lupus erythematosus. Edited by Wallace DR, Hahn BH. Baltimore: Williams & Wilkins; 1997: 199-209

Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajarvi R, Juvonen H, Kokko-Sahin ML, Vaisanen L, Mannila H, Lonnqvist J, Peltonen L. A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. *Am J Hum Genet* 65: 1114-24, 1999

Hughes GR. The antiphospholipid syndrome: ten years on. *Lancet* 342: 341-4, 1993

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411: 599-603, 2001

Izui S, Iwamoto M, Fossati L, Merino R, Takahashi S, Ibnou-Zekri N. The Yaa gene model of systemic lupus erythematosus. *Immunol Rev* 144:137-56, 1995

James JA, Kaufman KM, Farris AD, Taylor-Albert E, Lehman TJ, Harley JB An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J Clin Invest* 100: 3019-3026, 1997

Jara LJ, Medina G, Vera-Lastra O, Shoenfeld Y. Atherosclerosis and antiphospholipid syndrome. *Clin Rev Allergy Immunol* 25: 79-88, 2003

Jorde LB. Linkage disequilibrium as a gene-mapping tool. Am J Hum Genet 56: 11-4, 1995

Kameda S, Naito S, Tanaka K, Kajiyama K, Kunihiro K, Nishiguori S, Jimi S, Yanase T. HLA antigens of patients with systemic lupus erythematosus in Japan. *Tissue Antigens* 20: 221-2, 1982

Kaslow RA, Masi AT. Age, sex, and race effects on mortality from systemic lupus erythematosus in the United States. *Arthritis Rheum* 21: 473-9, 1978

Kelley VE, Winkelstein A. Age- and sex-related glomerulonephritis in New Zealand white mice. *Clin Immunol Immunopathol* 16: 142-50, 1980

Kelly JA, Moser KL, Harley JB. The genetics of systemic lupus erythematosus: putting the pieces together. *Genes Immun* 3 (Suppl 1): 71-85, 2002

Kelly JA, Thompson K, Kilpatrick J, Lam T, Nath SK, Gray-McGuire C, Reid J, Namjou B, Aston CE, Bruner GR, Scofield RH, Harley JB. Evidence for a susceptibility gene (SLEH1) on chromosome 11q14 for systemic lupus erythematosus (SLE) families with hemolytic anemia. *Proc Natl Acad Sci U S A* 99: 11766-71, 2002

Kere J. Human population genetics: lessons from Finland. *Annu Rev Genomics Hum Genet* 2: 103-28, 2001

Keswani SC, Chauhan N. Antiphospholipid syndrome. *J R Soc Med* 95: 336-42, 2002

Kong A, Cox NJ. Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61: 1179-88, 1997

Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. *Nat Genet* 31: 241 – 247, 2002.

Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, Theofilopoulos AN. Lupus susceptibility loci in New Zealand mice. *Proc Natl Acad Sci U S A* 91: 10168-72, 1994

Kruglyak L, Lander ES. High-resolution genetic mapping of complex traits. *Am J Hum Genet* 56: 1212-23, 1995

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58: 1347-63, 1996

Kruglyak L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nat Genet* 22: 139-44, 1999

Kuokkanen S, Gschwend M, Rioux JD, Daly MJ, Terwilliger JD, Tienari PJ, Wikstrom J, Palo J, Stein LD, Hudson TJ, Lander ES, Peltonen L. Genomewide scan of multiple sclerosis in Finnish multiplex families. *Am J Hum Genet* 61: 1379-87, 1997

Lahermo P, Sajantila A, Sistonen P, Lukka M, Aula P, Peltonen L, Savontaus ML. The genetic relationship between the Finns and the Finnish Saami (Lapps): analysis of nuclear DNA and mtDNA. *Am J Hum Genet* 58: 1309-22, 1996

Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petays T, Green T, Cargill M, Haahtela T, Lander ES, Laitinen LA, Hudson TJ, Kere J. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 28: 87-91, 2001

Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 265: 2037-48, 1994

Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11: 241-7, 1995

Lander ES. The new genomics: global views of biology. *Science* 274: 536-9, 1996

von Landenberg P, Matthias T, Zaech J, Schultz M, Lorber M, Blank M, Shoenfeld Y. Antiprothrombin antibodies are associated with pregnancy loss in patients with the antiphospholipid syndrome. *Am J Reprod Immunol* 49: 51-6, 2003

Lawrence JS, Martins CL, Drake GL. A family survey of lupus erythematosus. 1. Heritability . *J Rheumatol* 14: 913-21, 1987

Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 41: 778-99, 1998

Leech NJ, Kitabchi AE, Gaur LK, Hagopian WA, Hansen J, Burghen GA, Palmer JP, Nepom GT. Genetic and immunological markers of insulin dependent diabetes in Black Americans. *Autoimmunity* 22: 27-32, 1995

Lechner O, Dietrich H, Oliveira dos Santos A, Wiegers GJ, Schwarz S, Harbutz M, Herold M, Wick G. Altered cicardian rhytms of the stress hormone and melatonin response in lupus prone MRL/MP-fas (lpr) mice. *J Autoimmun* 14: 325-33, 2000

Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med* 346: 752-63, 2002

Lindqvist AK, Alarcon-Riquelme ME. The genetics of systemic lupus erythematosus. *Scand J Immunol* 50: 562-71, 1999

Lindqvist AK, Steinsson K, Johanneson B, Kristjansdottir H, Arnasson A, Grondal G, Jonasson I, Magnusson V, Sturfelt G, Truedsson L, Svenungsson E, Lundberg I, Terwilliger JD, Gyllensten UB, Alarcon-Riquelme ME. A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. *J Autoimmun* 14: 169-78, 2000

Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med* 112: 682-98, 1990

Mackworth-Young C, Chan J, Harris N, Walport M, Bernstein R, Batchelor R, Hughes G, Gharavi A. High incidence of anticardiolipin antibodies in relatives of patients with systemic lupus erythematosus. *J Rheumatol* 14: 723-6, 1987

Mackie IJ, Colaco CB, Machin SJ. Familial lupus anticoagulants. *Br J Haematol* 67: 359-63, 1987

Malide D, Londono I, Russo P, Bendayan M. Ultrastructural localization of DNA in immune deposits of human lupus nephritis *Am J Pathol* 143: 304-11, 1993

Manger K, Repp R, Spriewald BM, Rascu A, Geiger A, Wassmuth R, Westerdaal NA, Wentz B, Manger B, Kalden JR, van de Winkel JG. Fcgamma receptor IIa polymorphism in Caucasian patients with systemic lupus erythematosus: association with clinical symptoms. *Arthritis Rheum* 41: 1181-9, 1998

Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 145: 408-15, 1997

Matthey F, Walshe K, Mackie IJ, Machin SJ. Familial occurrence of the antiphospholipid syndrome. *J Clin Pathol* 42: 495-7, 1989

McKay JD, Lesueur F, Jonard L, Pastore A, Williamson J, Hoffman L, Burgess J, Duffield A, Papotti M, Stark M, Sobol H, Maes B, Murat A, Kaariainen H, Bertholon-Gregoire M, Zini M, Rossing MA, Toubert ME, Bonichon F, Cavarec M, Bernard AM, Boneu A, Leprat F, Haas O, Lasset C, Schlumberger M, Canzian F, Goldgar DE, Romeo G. Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. *Am J Hum Genet* 69: 440-6, 2001

McMurray RW. Sex hormones in the pathogenesis of systemic lupus erythematosus. *Front Biosci* 6: E193-206, 2001

McMurray RW, May W. Sex hormones and systemic lupus erythematosus: review and meta-analysis. *Arthritis Rheum* 48: 2100-10, 2003

McNally T, Purdy G, Mackie IJ, Machin SJ, Isenberg DA. The use of an anti-beta 2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. *Br J Haematol* 91: 471-3, 1995

Mehrian R, Quismorio FP Jr, Strassmann G, Stimmler MM, Horwitz DA, Kitridou RC, Gauderman WJ, Morrison J, Brautbar C, Jacob CO. Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum* 41: 596-602, 1998

Meier CR, Sturkenboom MC, Cohen AS, Jick H. Postmenopausal estrogen replacement therapy and the risk of developing systemic lupus erythematosus or discoid lupus. *J Rheumatol* 25: 1515-9, 1998

Michel M, Johanet C, Meyer O, Frances C, Wittke F, Michel C, Arfi S, Tournier-Lasserve E, Piette JC; Group for Research on Auto-Immune Disorders (GRAID). Familial lupus erythematosus. Clinical and immunologic features of 125 multiplex families. *Medicine* (Baltimore) 80: 153-8, 2001

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow P, Norris F, Helvering L, Morrison P, Rosteck P, Lai M, Barrett C, Lewis C, Neuhausen S, Cannon-Albright L, Goldbar D, Wiseman R, Kmb A, Skolnick M. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266: 66-71, 1994

Mojcik CF, Klippel JH. End-stage renal disease and systemic lupus erythematosus. *Am J Med* 101: 100-7, 1996

Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. *J Clin Pathol* 56: 481-90, 2003

Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1: 219-29, 1994

Morel L, Tian XH, Croker BP, Wakeland EK. Epistatic modifiers of autoimmunity in a murine model of lupus nephritis. *Immunity* 11: 131-9, 1999

Morel L, Croker BP, Blenman KR, Mohan C, Huang G, Gilkeson G, Wakeland EK. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97: 6670-5, 2000

Moser KL, Neas BR, Salmon JE, Yu H, Gray-McGuire C, Asundi N, Bruner GR, Fox J, Kelly J, Henshall S, Bacino D, Dietz M, Hogue R, Koelsch G, Nightingale L, Shaver T, Abdou NI, Albert DA, Carson C, Petri M, Treadwell EL, James JA, Harley JB. Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc Natl Acad Sci U S A* 95: 14869-74, 1998

Myerscough A, John S, Barnett JH, Ollier WER, Worthington J. Linkage of rheumatoid arthritis to insulin-dependent diabetes mellitus loci. Evidence supporting a hypothesis for the existence of common autoimmune susceptibility loci. *Arthr Rheum* 43: 2771-5, 2000

Namjou B, Nath SK, Kilpatrick J, Kelly JA, Reid J, James JA, Harley JB. Stratification of pedigrees multiplex for systemic lupus erythematosus and for self-reported rheumatoid arthritis detects a systemic lupus erythematosus susceptibility gene (SLER1) at 5p15.3. *Arthritis Rheum* 46: 2937-45, 2002a

Namjou B, Nath SK, Kilpatrick J, Kelly JA, Reid J, Reichlin M, James JA, Harley JB. Genome scan stratified by the presence of anti-double-stranded DNA (dsDNA) autoantibody in pedigrees multiplex for systemic lupus erythematosus (SLE) establishes linkages at 19p13.2 (SLED1) and 18q21.1 (SLED2). *Genes Immun* 3(Suppl 1): 35-41, 2002b

Nath SK, Kelly JA, Namjou B, Lam T, Bruner GR, Scofield RH, Aston CE, Harley JB. Evidence for a susceptibility gene, SLEV1, on chromosome 17p13 in families with vitiligo-related systemic lupus erythematosus. *Am J Hum Genet* 69: 1401-6, 2001

Nath SK, Kelly JA, Reid J, Lam T, Gray-McGuire C, Namjou B, Aston CE, Harley JB. SLEB3 in systemic lupus erythematosus (SLE) is strongly related to SLE families ascertained through neuropsychiatric manifestations. *Hum Genet* 111: 54-8, 2002

Nyström-Lahti M, Sistonen P, Mecklin JP, Pylkkänen L, Aaltonen LA, Jarvinen H, Weissenbach J, de la Chapelle A, Peltomaki P. Close linkage to chromosome 3p and conservation of ancestral founding haplotype in hereditary nonpolyposis colorectal cancer families. *Proc Natl Acad Sci USA* 94: 6054–58, 1994

O'Connell JR, Weeks DE. PedCheck: A Program for identification of genotype. Incompatibilities in linkage analysis. *Am J Hum Genet* 63: 259-66, 1998

Oh M, Petri MA, Kim NA, Sullivan KE. Frequency of the Fc gamma RIIIA-158F allele in African American patients with systemic lupus erythematosus. *J Rheumatol* 26: 1486-9, 1999

Olson JM. A general conditional-logistic model for affected-relative-pair linkage studies. *Am J Hum Genet* 65: 1760-9, 1999

Ott J, Bhat A.Linkage analysis in heterogeneous and complex traits. *Eur Child Adolesc Psychiatry* 8 (Suppl 3): 43-6, 1999

Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, Suomalainen AJ, Syvanen AC, Lehtimaki T, Viikari JS, Laakso M, Taskinen MR, Ehnholm C, Peltonen L. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat Genet* 18: 69-73, 1998

Palosuo T, Virtamo J, Haukka J, Taylor PR, Aho K, Puurunen M, Vaarala O. High antibody levels to prothrombin imply a risk of deep venous thrombosis and pulmonary embolism in middle-aged men. *Thromb Haemost* 78: 1178-82, 1997

Patil N, Berno AJ, Hinds DA, Barrett WA, Doshi JM, Hacker CR, Kautzer CR, Lee DH, Marjoribanks C, McDonough DP, Nguyen BT, Norris MC, Sheehan JB, Shen N, Stern D, Stokowski RP, Thomas DJ, Trulson MO, Vyas KR, Frazer KA, Fodor SP, Cox DR.Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* 294: 1719-23, 2001

Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. *Hum Mol Genet* 8: 1913-23, 1999

Pericak-Vance MA. Linkage disequilibrium and allelic association. In: Haines JL, Pericak-Vance MA(eds) Approaches to gene mapping in complex human diseases. John Wiley & Sons Inc., New York 1998, 323-33

Petri M. Epidemiology of the antiphospholipid antibody syndrome. *J Autoimmun* 15: 145-51, 2000

Pramatarov KD. Drug-induced lupus erythematosus. *Clin Dermatol* 16: 367-77, 1998

Prins JB, Todd JA, Rodrigues NR, Ghosh S, Hogarth PM, Wicker LS, Gaffney E, Podolin PL, Fischer PA, Sirotina A, Peterson LB. Linkage on chromosome 3 of autoimmune diabetes and defective Fc receptor for IgG in NOD mice. *Science* 260: 695-8, 1993

Prete PE. The mechanism of action of L-canavanine in inducing autoimmune phenomena. *Arthritis Rheum* 28: 1198-200, 1985

Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, Brookes AJ, Tentler D, Kristjansdottir H, Grondal G, Bolstad AI, Svenungsson E, Lundberg I, Sturfelt G, Jonssen A, Truedsson L, Lima G, Alcocer-Varela J, Jonsson R, Gyllensten UB, Harley JB, Alarcon-Segovia D, Steinsson K, Alarcon-Riquelme ME. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 32: 666-9, 2002

Puurunen M, Vaarala O, Julkunen H, Aho K, Palosuo T. Antibodies to phospholipid-binding plasma proteins and occurence of thrombosis in patients with systemic lupus erythematosus. *Clin Immunol Immunopathol* 80: 16-22, 1996

Quintero-Del-Rio AI, Kelly JA, Kilpatrick J, James JA, Harley JB. The genetics of systemic lupus erythematosus stratified by renal disease: linkage at 10q22.3 (SLEN1), 2q34-35 (SLEN2), and 11p15.6 (SLEN3). *Genes Immun* 3 (Suppl 1): 57-62, 2002

Radeke HH, Gessner JE, Uciechowski P, Magert HJ, Schmidt RE, Resch K. Intrinsic human glomerular mesangial cells can express receptors for IgG complexes (hFc gamma RIII-A) and the associated Fc epsilon RI gamma-chain. *J Immunol* 153: 1281-92, 1994

Ratnoff WD. Inherited deficiencies of complement in rheumatic diseases. *Rheum Dis Clin North Am* 22: 75-94, 1996

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES.Linkage disequilibrium in the human genome. *Nature* 411: 199-204, 2001

Reich DE, Lander ES. On the allelic spectrum of human disease. *Trends Genet* 17: 502-10, 2001

Reich DE, Schaffner SF, Daly MJ, McVean G, Mullikin JC, Higgins JM, Richter DJ, Lander ES, Altshuler D. Human genome sequence variation and the influence of gene history, mutation and recombination. *Nat Genet* 32: 135-42, 2002

Risch N. Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40: 1-14, 1987

Risch NJ. Searching for genetic determinants in the new millennium. *Nature* 405: 847-56. 2000

Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 273: 1516-7, 1996

Robson MG, Walport MJ. Pathogenesis of systemic lupus erythematosus (SLE). *Clin Exp Allergy* 31: 678-85, 2001

Robson MG, Cook HT, Botto M, Taylor PR, Busso N, Salvi R, Pusey CD, Walport MJ, Davies KA. Accelerated nephrotoxic nephritis is exacerbated in C1q-deficient mice. *J Immunol* 166: 6820-8, 2001

Roubinian JR, Papoian R, Talal N. Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. *J Clin Invest* 59: 1066-70, 1977

Ruiz-Irastorza G, Khamashta MA, Castellino G, Hughes GR. Systemic lupus erythematosus. *Lancet* 357: 1027-32, 2001

Rus V, Hajeer A, Hochberg MC. Systemic lupus erythematosus. In: Silman AJ, Hochberg MC (eds). *Epidemiology of the Rheumatic Diseases*. Oxford University Press, 2001, 123-136

Salmon JE, Edberg JC, Kimberly RP. Fc gamma receptor III on human neutrophils. Allelic variants have functionally distinct capacities. *J Clin Invest* 85: 1287-95, 1990

Salmon JE, Edberg JC, Brogle NL, Kimberly RP. Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. *J Clin Invest* 89: 1274-81, 1992

Salmon JE. Abnormalities in immune complex clearance and receptor function. In: Wallace DJ, Hahn BH (eds). *Duboi´s lupus erythematosus*. Lea & Febiger, Philadelphia, 1993, 108-119.

Salmon JE, Millard S, Schachter LA, Arnett FC, Ginzler EM, Gourley MF, Ramsey-Goldman R, Peterson MG, Kimberly RP. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J Clin Invest* 97: 1348-54, 1996

Samuelsson L, Enlund F, Torinsson A, Yhr M, Inerot A, Enerback C, Wahlstrom J, Swanbeck G, Martinsson T. A genome-wide search for genes predisposing to familial psoriasis by using a stratification approach. *Hum Genet* 105: 523-9, 1999

Sanchez-Guerrero J, Liang MH, Karlson EW, Hunter DJ, Colditz GA. Postmenopausal estrogen therapy and the risk for developing systemic lupus erythematosus. *Ann Intern Med* 122: 430-3, 1995

Sanchez-Guerrero J, Karlson EW, Liang MH, Hunter DJ, Speizer FE, Colditz GA. Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum* 40: 804-8, 1997

Sawalha AH, Namjou B, Nath SK, Kilpatrick J, Germundson A, Kelly JA, Hutchings D, James J, Harley J. Genetic linkage of systemic lupus erythematosus with chromosome 11q14 (SLEH1) in African-American families stratified by a nucleolar antinuclear antibody pattern. *Genes Immun* 3 (Suppl 1): 31-4, 2002

Schaid DJ. Transmission disequilibrium, family controls, and great expectations. *Am J Hum Genet* 63: 935-41, 1998

Scofield RH, Bruner GR, Kelly JA, Kilpatrick J, Bacino D, Nath SK, Harley JB. Thrombocytopenia identifies a severe familial phenotype of systemic lupus erythematosus and reveals genetic linkages at 1q22 and 11p13. *Blood* 101: 992-7, 2003

Sebastiani GD, Galeazzi M, Morozzi G, Marcolongo R. The immunogenetics of the antiphospholipid syndrome, anticardiolipin antibodies, and lupus anticoagulant. Semin Arthritis Rheum. 25: 414-20, 1996

Sestak AL, Shaver TS, Moser KL, Neas BR, Harley JB. Familial aggregation of lupus and autoimmunity in an unusual multiplex pedigree. *J Rheumatol* 26: 1495-9, 1999

Sevon P, Ollikainen V, Onkamo P, Toivonen HT, Mannila H, Kere J. Mining associations between genetic markers, phenotypes, and covariates. *Genet Epidemiol* 21(Suppl 1): 588-93, 2001

Shai R, Quismorio FP Jr, Li L, Kwon OJ, Morrison J, Wallace DJ, Neuwelt CM, Brautbar C, Gauderman WJ, Jacob CO. Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum Mol Genet* 8: 639-44, 1999

Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52: 506-16, 1993

Strasser A, Harris AW, Vaux DL, Webb E, Bath ML, Adams JM, Cory S. Abnormalities of the immune system induced by dysregulated bcl-2 expression in transgenic mice. *Curr Top Microbiol Immunol* 166: 175-81, 1990

Stockl F, Muller S, Batsford S, Schmiedeke T, Waldherr R, Andrassy K, Sugisaki Y, Nakabayashi K, Nagasawa T, Rodriguez-Iturbe B, et al. A role for histones and ubiquitin in lupus nephritis? *Clin Nephrol* 41: 10-7, 1994

St. George-Hyslop PH. Molecular genetics of Alzheimer´s disease. Biol Psychiatry 47: 183-99, 2000

Sturfelt G. The complement system in systemic lupus erythematosus. *Scand J Rheumatol* 31: 129-32, 2002

Suh JH, Lim SD, Kim JC, Hong SH, Kang GH.Comparison of clinicopathologic characteristics and genetic alterations between microsatellite instability-positive and microsatellite instability-negative sporadic colorectal carcinomas in patients younger than 40 years old. *Dis Colon Rectum* 45: 219-28, 2002

Talbott JH. Historical background of discoid and systemic lupus erythematosus. In: Wallace DJ, Hahn BH (eds). *Duboi´s Lupus erythematosus*. Lea & Febiger, Philadelphia, 1993, 3-10

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 25: 1271-77, 1982

Tan FK, Arneet FC. The genetics of lupus. *Curr Opin Rheumatol* 10: 399-408, 1998

Terwilliger JD, Ott J. Handbook of human genetic linkage. John Hopkins University Press, Baltimore, USA, 1994

Thomas RH. Hypercoagulability syndromes. *Arch Intern Med* 161: 2433-39, 2001

Todd JA. Genetics of type 1 diabetes. *Pathol Biol (Paris)* 45: 219-27, 1997

Toivonen HT, Onkamo P, Vasko K, Ollikainen V, Sevon P, Mannila H, Herr M, Kere J. Data mining applied to linkage disequilibrium mapping. *Am J Hum Genet* 67: 133-45, 2000

Triplett DA, Brandt JT, Musgrave KA, Orr CA. The relationship between lupus anticoagulants and antibodies to phospholipid. *JAMA* 259: 550-4, 1988

Tsao BP, Cantor RM, Kalunian KC, Chen CJ, Badsha H, Singh R, Wallace DJ, Kitridou RC, Chen SL, Shen N, Song YW, Isenberg DA, Yu CL, Hahn BH, Rotter JI. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J Clin Invest* 99: 725-31, 1997

Tsao BP, Cantor RM, Grossman JM, Shen N, Teophilov NT, Wallace DJ, Arnett FC, Hartung K, Goldstein R, Kalunian KC, Hahn BH, Rotter JI. PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. *J Clin Invest* 103: 1135-40, 1999

Tsao BP, Cantor RM, Grossman JM, Kim SK, Strong N, Lau CS, Chen CJ, Shen N, Ginzler EM, Goldstein R, Kalunian KC, Arnett FC, Wallace DJ, Hahn BH. Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to systemic lupus erythematosus. *Arthritis Rheum* 46: 2928-36, 2002

Tsokos GC. Systemic lupus erythematosus. A disease with a complex pathogenesis. *Lancet* 358 (Suppl 1): 65, 2001

Urowitz MB, Bookman AAM, Koehler BE, Gordon DA, Smythe HA, Ogryzlo MA. The bimodal mortality pattern of systemic lupus erythematosus. *Am J Med* 60: 221-5, 1976

Vaarala O, Alfthan G, Jauhiainen M, Leirisalo-Repo M, Aho K, Palosuo T. Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* 341: 923-5, 1993

Vaarala O, Vaara M, Palosuo T. Effective inhibition of cardiolipin-binding antibodies in gram-negative infections by bacterial lipopolysaccharide. *Scand J Immunol* 28: 607-12, 1988

Vasen HF. What is hereditary nonpolyposis colorectal cancer (HNPCC). Anticancer Res 14: 1657-60, 1994

Vila P, Hernandez MC, Lopez-Fernandez MF, Batlle J. Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. *Thromb Haemost* 72: 209-13, 1994

Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. Immunity, 15: 397-408, 2001

Walker SE, Jacobson JD. Roles of prolactin and gonadotropin-releasing hormone in rheumatic diseases. *Rheum Dis Clin North Am* 26: 713-36, 2000

Wanstrat A, Wakeland E. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nature Immunology* 9: 802-9, 2001

Witztum JL. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10: 325-35, 1990

Witztum JL. The role of oxidized LDL in atherosclerosis. *Adv Exp Med Biol* 285: 353-65, 1991

Witztum JL, Horkko S. The role of oxidized LDL in atherogenesis: immunological response and anti-phospholipid antibodies. *Ann N Y Acad Sci* 811: 88-96; discussion 96-9, 1997

Whittingham S, Mathews JD, Schanfield MS, Tait BD, Mackay IR. HLA and Gm genes in systemic lupus erythematosus. *Tissue Antigens* 21: 50-7, 1983

Vidal S, Kono DH, Theofilopoulos AN. Loci predisposing to autoimmunity in MRL-Fas lpr and C57BL/6-Faslpr mice. *J Clin Invest* 101: 696-702, 1998

Wilson WA, Gharavi AE. Genetic risk factors for aPL syndrome. *Lupus* 5:398-403, 1996

Wilson WA. Classification criteria for antiphospholipid syndrome. *Rheum Dis Clin North Am* 27: 499-505, 2001

Voss AB, Green A, Junker P. Systemic lupus erythematosus in the county of Fynen. An epidemiologic study. *Ugeskr Laeger* 161: 3837-40, 1999.

Wooster R, Stratton MR. Breast cancer susceptibility: a complex disease unravels. *Trends Genet* 11: 3-5, 1995

Wright AF, Carothers AD, Pirastu M. Population choice in mapping genes for complex diseases. *Nat Genet* 23: 397-404, 1999

Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell* 85: 311-8, 1996

Vyse TJ, Kotzin BL. Genetic basis of systemic lupus erythematosus. *Curr Opin Immunol* 8: 843-51, 1996

Vyse TJ, Rozzo SJ, Drake CG, Izui S, Kotzin BL. Control of multiple autoantibodies linked with a lupus nephritis susceptibility locus in New Zealand black mice. *J Immunol* 158: 5566-74, 1997

Vyse TJ, Kotzin BL. Genetic susceptibility to systemic lupus erythematosus. *Annu Rev Immunol* 16: 261-92, 1998

Xu J, Meyers D, Pericak-Vance M. Lod score analyses. In: Haines JL, Pericak-Vance MA(eds) Approaches to Gene Mapping in Complex Human Diseases. John Wiley & Sons Inc., New York 1998, 253-72