Clin Exp Immunol 2004; **137**:1–7 doi:10.1111/j.1365-2249.2004.02525.x

REVIEW

The candidate gene approach: have murine models informed the study of human SLE?

D. S. CUNNINGHAME GRAHAM & T. J. VYSE *Rheumatology Section, Imperial College Faculty of Medicine, Hammersmith Hospital, London, UK*

(Accepted for publication 30 April 2004)

SUMMARY

Genome wide linkage studies in human SLE have identified seven highly significant loci linked to SLE, and more than 20 other loci showing suggestive linkage to disease. However, pin-pointing the susceptibility alleles in candidate genes within these linkage regions is challenging, due the genetic heterogeneity, racial differences and environmental influences on disease aetiology. Utilization of murine models of spontaneous lupus nephritis provide a complementary approach, which may then identify candidate genes for analysis in human cases. This review highlights the utility of cross-species approach to identify and characterize the effect of given candidate genes in lupus. The examples described in this review demonstrate the importance of bringing together both genetic and functional information in human and mouse studies.

Keywords human SLE murine model candidate gene

INTRODUCTION

Systemic Erythematosus Lupus (SLE) is a complex multisystem autoimmune disease of unknown aetiology, characterized by the production of autoantibodies against a diverse set of nuclear and cell surface autoantigens. End organ damage results from the formation of immune complexes. The overall disease prevalence 1·5–250 in 100 000; it is less common in European than in Asian and Afro-Caribbean populations, and is approximately 10-fold more common in women of childbearing age than men [1,2].

SLE is known to have a genetic basis, but with an environmental contribution. Familial clustering indicated that SLE has a significant genetic component with a sibling risk ratio ($\lambda_s = 20$) [3], which is the same range as other autoimmune diseases, for Type 1 Diabetes $\lambda_s = 16$ and for Rheumatoid Arthritis $\lambda_s = 8$. Twin studies showed that the concordance for monozygotic twins is 10 fold more than for dizygotic twins [4]. Six genome-wide linkage studies have been performed to discover loci important in the lupus. Seven loci having highly significant linkage (LOD score = 3·3) to SLE were replicated in multiple studies. These were 1q23–24, 1q41–42; 2q37, 4p15–16, 6p11–22, 16q12–13 and 17p13 [5–10]. More than 20other loci showing suggestive linkage to SLE (LOD = 1·9) were also identified, indicating that they may also contain susceptibility loci, but of weaker effect. Linkage studies in complex traits with heterogeneous phenotypes, such as SLE, may yield false-positive results. Furthermore, linkage studies have less power than association studies to identify aetiologic alleles.

The complexity of SLE in human disease has been highlighted by the fact that no single locus was identified in every genome-wide linkage study. Whilst this may reflect the differences in study design, analysis, methodology and racial mix of the study cohorts, it is largely due to the genetic heterogeneity of the out-bred human population, with a small contribution from each locus to the overall disease susceptibility. Different study cohorts were used partially because of the difficulty in recruiting sufficient families to identify significant linkages in the analysis.

Murine models of lupus allow us to step back from the genetic heterogeneity seen in the human population. It is possible to breed large numbers of genetically homogenous inbred mice under controlled environmental conditions to facilitate large-scale genome wide linkage analyses. Furthermore, the flexibility and manipulatability of murine models allow the generation of congenic mouse strains to investigate the effect of given loci in different nonlupus prone backgrounds, or the development of knock-outs and transgenics to look at the effect of the absence or over expression of immunologically relevant genes.

Given the overall similarity of the human and mouse immune systems, it is anticipated that the linkage studies and functional genetics from knock-outs and transgenics accumulated in the mouse models can be fed back into the human disease studies. This should provide additional stimulus for a real break-through in the understanding of the human disease process and in the development of alternative therapeutic strategies.

Correspondence: Dr Deborah Cunninghame-Graham, Rheumatology Section, Imperial College Faculty of Medicine, Hammersmith Hospital, London, W12 ONN, UK.

E-mail: deborah.cunninghame-graham@imperial.ac.uk

Table 1. Susceptibility loci important in human SLE and murine models of lupus

Gene	Human location	Linkage study	Mouse location (Chromosome: cM)	Mouse linkage region	Mouse linkage reference
CRP/SAP	1q23·2	[7,8]	1: 94·2	*Sle1*/*Nba2*	[15]/[14]
DNASE1	16q13·3	[5,6]	16: 1·7	–	–
C1Q	1p36	[10]	4: 66·1	~*Nba1*	[33]
FCγRIIA/IIA	1q23	[8,10]	1: 92·3	*Nba2*	[14]
PDCD1	2q37·3	[67]	1 D	*Bxs3*	[80]

SUSCEPTIBILITY GENES FROM THE INNATE IMMUNE SYSTEM – THE WASTE DISPOSAL HYPOTHESIS

CRP/SAP

One of the functions of the acute phase response, mediated by the induction of two pentraxin genes, C-Reactive protein (*CRP*) and Serum Amyloid P component (*SAP*), gene nomenclature *ACPS*, appears to be the clearance of apoptotic material. Following inflammation or injury, *CRP*, which is the major acute phase protein in humans, binds to the products of apoptosis, including components of cellular matrix and nuclear antigens, to promote opsonization of all these particles either indirectly by activation of the classical pathway of the complement system or directly by binding to the Fcγ receptors on apoptotic cells [11]. However, in SLE patients, there is often little or no acute phase response [12]. This scenario is mirrored in lupus-prone NZB/W F1 mice, which show reduced induction of the murine acute phase pentraxin, *Sap*, in the disease state [13].

The human *SAP* and *CRP* genes, are located in 1q23.2, in the linkage region identified by Moser *et al*. [8], and close to that identified by Shai *et al*. [7]. The mouse homologues are located in the *Sle1*/*Nba2* linkage region on distal mouse chromosome 1 (Table 1) [14,15].

An *Acps* knock-out mouse developed spontaneous autoimmunity and glomerulonephritis. It was hypothesized that the absence of *Sap* probably preventing the clearance of chromatin. This failure in 'waste-disposal' promotes the production of autoantibodies against chromatin and DNA, with subsequent deposition in the kidney [16]. Additionally, expression of a human *CRP* transgene in the NZW/B F1 has been shown to delay the onset of lupus [17].

More recently, human association studies have shown that a single SNP, CRP4, is associated with SLE and also with the production of ANA [18]. The human gene produces two mRNA species, which differ in the length of the 3'UTR (*http://www.ncbi.nlm.nih.gov/*). CRP4 is located in the 3′UTR of the longer CRP mRNA molecules and may therefore play a role in transcript stability. Furthermore, basal levels of CRP were affected by the rare A allele of CRP4, being lowest in A/A homozygotes of CRP4. Further experiments are required to determine whether genetic variation at the CRP locus impacts on the magnitude of the acute phase CRP response, which is preserved in response to certain inciting stimuli in SLE.

DNASEI

Like the pentraxins, *DNASEI* is also involved in the removal of potential autoantigens. Interest in *DNASEI* as a susceptibility gene for SLE began with the observation that there was reduced *DNASEI* activity in the serum of SLE patients [19]. The enzyme is the major nuclease present in the serum, urine and excreta, probably being the enzyme responsible for the degradation of nuclear antigens carried by the serum, produced by apoptosis of cells at sites of tissue damage or inflammation elsewhere in the body.

More recently B6, *Dnase*$^{-/-}$ knock-out mice showed increased production of autoantibodies against nuclear antigens, especially ssDNA and nucleosomes. There may be a dose-dependent effect on gene expression, since there was only 50% DNAse I expression levels in *Dnase*$^{+/-}$ mice compared to that seen in wild-type mice. Furthermore, 17% of the *Dnase*$^{+/-}$ animals showed symptoms of glomerulonephritis, as compared to 23% of *Dnase*$^{-/-}$ mice [20].

Given the reduced enzyme activity and production of pathogenic ANA in lupus, it was suggested that polymorphisms at the *DNASEI* locus might contribute to the development of SLE. The human gene is located at 16q13.3, in an interval linked to SLE [5,6]. Furthermore, an A/T polymorphism in exon 2 was detected in two young Japanese SLE patients, who had low serum *DNASEI* activity and high levels of IgG antinucleosome antibodies [21]. However, this missense polymorphism was not found in 182 UK patients [22], 39 unrelated Tunisian SLE cases or 91 normal controls[23] 18 American SLE patients [24] or 108 Spanish SLE patients and 100 healthy controls [25].

It is interesting to note that although there are three different isoforms of the enzyme, produced by six known polymorphisms, there are no significant differences in enzyme activity between these isoforms [26]. To fully elucidate the genetic contribution of *DNASEI* to the aetiology of SLE, identification of further SNPs across each isoform of the enzyme will be necessary, prior to detailed association studies in different racial groups. Additionally, it will be interesting to determine whether the different isoforms of *DNASEI* have an individual genetic contribution to SLE and the causality of the low serum levels of *DNASEI*.

C1Q

C1q initiates the classical pathway of the complement system. A role for C1q in the genetic predisposition to SLE originated from observations that homozygous hereditary C1q deficiency is a significant risk factor, albeit an extremely rare, in the development of SLE. Almost 90% of people identified as being C1q deficient, go on to develop SLE, with a low age of onset [27–31]. However, the molecular basis for this complete C1q deficiency is heterogeneous [29] Additionally, acquired low levels of serum C1q, are also predictive of proliferative lupus nephritis [32].

The *C1QA*, *-B* and *-C* genes in humans are located at 1p36, which has been identified as a suggestive linkage region for SLE by Gray-McGuire *et al*. [10] (Table 1). The C1q genes in mice are found on mouse chromosome 4 at 66·1 cM, close to the *Nba1* linkage region mapped in the NZB/W F1 model of lupus nephritis [33]. A *C1qa*$^{-/-}$ knock-out mouse, on a hybrid 129 × C57/BL6 background, produced high levels of ANA and antihistone autoantibodies. 25% of these animals developed glomerulonephritis, with a relative increased frequency of glomerular apoptotic bodies [34]. Since C1q is produced directly from cells of the monocyte-macrophage lineage, reconstitution of normal serum levels of C1q in the *C1qa*$^{-/-}$ knock-out was seen within 4–6 weeks after transplantation with wild type bone marrow cells [35].

Although it is clear that hereditary C1q deficiency is a significant risk factor in the development of human SLE, the contribution of genetic variants with the potential to exert subtle effects on C1q expression and function is less clear-cut, and awaits further investigation. In a recent small study it was suggested that a synonymous SNP in Gly70 was associated with decreased levels of C1q in subacute cutaneous lupus erythematosus [36]. However, the molecular basis of the reduction in C1q levels was explored in NZB and NZB/W F1 mice. There were no polymorphisms detected in the two exons and single intron of each of the *C1qa*, *-b* or *-c* genes. However, a ~3·5 kb insertion in the upstream region of the *C1qa* gene was linked to low levels of C1q in young NZB and NZB/W F1 serum. The exact location of this insertion was not reported, but the *D4Mit70* microsatellite in the region of the insertion was linked to low levels of C1q and development of nephritis [37].

THE Fc RECEPTORS

A number of lines of evidence have implicated the Fcγ receptors in the development of autoimmunity. *Nba2* and *Sle1* are two key linkage intervals for lupus nephritis, on mouse distal chromosome 1 [14,38,39], these loci contain two *Fc*γ receptor genes, *Fcgr2* and *Fcgr3*, with the peak of linkage in *Nba2* being close to *Fcgr2* (Table 1) [40,41]. Although there are membrane-bound Fc receptors described for each class of antibody, IgG, IgM, IgA and IgE, it is *Fcgr2* and *Fcgr3* which are important in lupus. This family of receptors is important in the clearance of antibodies or immune complexes, a process crucial to the regulation of the immune response. The clearance effect of the *Fc*γ receptor system is a balance between activating and inhibitory receptors, with *Fcgr*3 being an activating receptor and *Fcgr*2 being an inhibitory receptor.

The human orthologues of *Fcgr2* and *Fcgr3,* FcγRII and FcγRIII are located in the 1q23–24 linkage region on human chromosome 1, identified in genome-wide linkage studies for SLE [8,10]. However, there is not a direct gene-to-gene match of the human and mouse genes, since in mice there are single genes for *Fcgr2* and *Fcgr3*, whereas in humans there is gene duplication, leading to five genes corresponding to *Fcgr2* and *Fcgr3*; encoded in the order FcγRIIA, FcγRIIIA, FcγRIIC, FcγRIIIB and FcγRIIB.

Many association studies have investigated polymorphisms in the stimulatory FcγRIIa (CD32) and FcγRIIIa (CD16) genes, both of which are low affinity binders of IgG molecules. These genes make good candidates for SLE, because they both possess SNPs causing nonsynonymous amino acid changes that are associated with disease.

In the case of FcγRIIa the nonsynonymous polymorphism converts amino acid residue 131 from histidine (H) to an arginine (R), with the R allele binding with less avidity to IgG2 than the H allele. Since FcγRIIa is the major receptor class for IgG2, this missense mutation is important because anti-dsDNA autoantibodies are predominantly of the IgG2 subclass, so the mutation may lead to delayed clearance of dsDNA-IgG2 containing immune complexes. However, the results of a series of association studies investigating the involvement of the R131 allele with lupus nephritis were inconclusive. In SLE cases from African American [42], white Dutch [43] and Hispanic populations with high level of renal disease [44], there was increased FcγRIIa R131 allele compared to the H allele. However, this was not observed in other European, Asian or African Caribbean populations both with and without renal disease [42,45–50]. In an attempt to clarify the inconsistencies of these results, meta-analysis was carried out on genotyping information from 17 studies. This analysis showed that the RR genotype was more common in the total number of SLE cases (Odds Ratio (OR) = 1·30, 95% confidence interval (CI) 1·10–1·52) and in those patients without renal disease (OR = 1·27, 95% CI 1·04–1·55), both compared to normal controls. There was a potential dose–response relationship, most clearly seen in the European Caucasian racial group, between the R allele, encoding the low affinity receptor, and the risk of SLE, but not for lupus nephritis [51].

In FcγRIIIA, the most frequently studied SNP also leads to nonsynonymous amino acid change; from phenylalanine (F) to valine (V) change at residue 158. The F158 allele binds IgG1 and IgG3 with lower affinity than the V158 allele. This is important since evidence in both human SLE and in murine models of lupus nephritis suggests that IgG2 and IgG3 may have a pathogenic role in kidney disease [52,53].

Consequently, alleles of FcγRIIA reducing the clearance of IgG3 antibodies may increase the extent of renal disease. As with FcγRIIA, the results from association studies were not conclusive, with increased levels of the low binding F158 allele in reported in Hispanic [44] Caucasian [48], Korean [50] and an ethnically diverse population [54], but not in African American [55] or Caucasian SLE patients [47].

Association of F158 to lupus nephritis was found in a Caucasian, Korean and a multiethnic population [50] but not in Germans or in Hispanics, Asian/Pacific Islanders and African Americans [56]. A recent meta-analysis of the F/V158 polymorphism using data from 11 studies showed association of the low-binding F158 allele with lupus nephritis {OR = 1·2, 95% CI 1·06–1·36, $P = 0·003$]. This risk was higher in FF homozygotes compared to VV homozygotes (OR = 1·47, 95% CI 1·11–1·93, $P = 0·006$). The association to SLE was uncertain [57]. Overall, the association data for the H/R131 and F/V158 polymorphisms is not strong enough to fully account for the linkage identified in 1q23–24, indicating that other genes encoded in this region are involved in the genetic susceptibility to SLE.

However, functional data, supported by murine knock-outs, indicate that both *Fcgr2* and *Fcgr3* may be involved in disease pathogenesis. The γ chain, located at 1q23, forms part of the functional FcγRIII and FcγRI molecules, hence a γ chain knock-out did not express either FcγRIII or FcγRI. Phenotypically, these mice were able to generate and deposit immune complexes, but did not develop the inflammation and nephritis mediated by *Fcgr3* [58]. Additionally, NZB/W nice possessing a disrupted *Fcgr3b* gene did not develop glomerulonephritis, despite having

increased levels of antidsDNA autoantibodies with renal deposition [59]. In the *Fcgr2* knock-out mouse there were increases in both B cell responses and in levels of immunoglobulins. These data are important since *Fcgr2b* is only expressed on B cells, where it inhibits B cell receptor-mediated activation of B cells. However, other pathways are also involved in the regulation of antibody levels, since these mice do not show uncontrolled immunoglobulin production [60]. *Fcgr2b* knock-out mice do not show significant renal disease [61]. More recently, the Fcγ RIIB deficiency in combination with the Fas mutation has been shown to be important in the development of autoimmune disease, since the B6.Fas$^{lpr/lpr}$-Fcγ RIIB deficient mice develop systemic autoimmunity, but the B6.Fas$^{lpr/lpr}$ do not [62].

Investigation of a molecular mechanism for the involvement of *Fcgr2* in the pathogenesis of autoimmune disease revealed single nucleotide polymorphisms (SNPs) in the promoter of *Fcgr2b*. These SNPs, identified in spontaneous mouse models of autoimmunity (NZB, BXSB, MRL and NOD), led to down regulation of the levels of *Fcgr2b* in germinal centre B cells, which produce high affinity IgG anti-DNA autoantibodies [63–65].

Although there is good circumstantial evidence to support a role for murine *Fcγ* receptors in lupus, there two major problems in the interpretation of the data. Murine knock-outs of genes in distal chromosome 1, including the *Fcγ* receptors, have been developed using the B6/129 hybrid system. However, in the fragment of DNA used for the homologous recombination, there are other genes, adjacent to the disrupted gene which have been implicated in autoimmunity. There may be epistatic interactions between unidentified 129 or B6 genes in the background of the knock-out which interact with the targeted gene to contribute to the autoimmune phenotype [66]. In addition, as mentioned earlier, there are structural differences in the locus containing the Fcγ receptors in mouse and human. However, given these caveats, the association data in humans, the functional genetic studies in mice, the balance between activation and inhibition of the Fcγ receptor pathways in both systems, together with the divergence in antibody specificity for the Fc receptors, it may be that combinations of the alleles at multiple Fcγ receptor genes make a bigger contribution to the development of SLE than the individual genes alone.

OTHER GENES

PDCD1

Human linkage studies have also identified a locus, *SLEB2*, at 2q37.3, which contains the *PDCD1* gene [67]. *PDCD1* is an inhibitory transmembrane receptor which down-regulates lymphocyte proliferation and cytokine secretion (mediated by the T cell receptor) [68,69]. The gene is a member of the immunoglobulin family and is induced on peripheral T and B lymphocytes [68,69].

Aged *Pdcd1* knock-out B6 mice develop spontaneous lupus-like disease with glomerulonephritis and arthritis. Using a T cell receptor transgenic system, it was shown that the regulatory effect of PDCD1 operated the level of the T cell receptor, in maintaining peripheral tolerance [70]. Interestingly, although *Pdcd1* knock-out mice develop lupus-like illness, there are increased levels of PDCD-1, compared to controls, in several autoimmune diseases, including SLE [71]. However, the reasons behind for this apparent contradiction are unclear.

A polymorphism in intron 4 (PD1·3), allele A, was associated with SLE ($P = 0{\cdot}00001$), in Europeans [72] and in European Americans [73]. The A allele of this SNP, which is located in an intronic enhancer sequence, disrupts the binding site for the RUNX1 transcription factor. It was proposed that the polymorphism resulted in the inappropriate regulation of PDCD1, leading to a breakdown in tolerance and to lymphocyte hyperactivity. Recently a significant association with renal disease was reported by the same group in Swedes, but not in European Americans [73]. However, a SNP in exon 6, in amino acid residue C268, was not associated with SLE in Chinese patients, but was associated with Rheumatoid Arthritis [74].

CONCLUSIONS

This review summarizes several examples in which mouse genetics have provided a powerful tool in the analysis of candidate genes for SLE. This can be seen in the murine linkage studies, which have identified linkage regions containing the *CRP/SAP*, *Fcgr2* and *Fcgr3* genes, with orthologous genes also found in regions linked to human SLE. However, divergence between the human and mouse genomes may mean that for given candidate genes there may not be direct orthologues between the two species. Nevertheless, this lack of orthology may not be a serious problem for the cross species approach to the genetic analysis of lupus. This can be seen most clearly in the case of the Fcγ receptors and pentraxin genes, where it was still possible to gain valuable information from murine candidates, despite the absence of direct human orthologues.

Functional data has also been informative, with mouse knock-outs implicating several genes as good candidates for SLE, with a diversity seen in the autoimmune phenotypes. This highlights the complexity of the disease pathology for lupus. However, using data from mouse models of lupus will not give us complete picture of the factors involved in human SLE. This is because the predominant disease phenotype in mice is of glomerulonephritis, whereas in human SLE which involves multiple organ systems, there are a much wider set of clinical manifestations, including serositis, skin involvement and disorders of the central nervous system. This limitation of the disease phenotypes in mice can be seen by looking at the reports of several murine knock-outs. The *ACPS* knock-out mice developed glomerulonephritis. *DNASE1* knock-out mice had increased levels of autoantibodies against nuclear antigens in addition to glomerulonephritis, however, glomerulonephritis was less prevalent in the C1q knock-out animals, since only 25% of the animals had renal disease. Furthermore, the autoantibodies produced were predominantly against ANA and histones. The γ chain knock-out did not develop the inflammation and severe nephritis characteristic of some other knock-outs, but did have proteinuria and glomerular deposition of anti-DNA autoantibodies and IgG1, -2a and -2b immune complexes [58]. The *Fcgr3b* knock-out also did not develop glomerulonephritis, but had renal deposition of antidsDNA autoantibodies [59] and the Fcgr2b knock-out, despite having elevated immunoglobulin levels, also did not develop renal disease [60,61]. *Pdcd1* knock-out mice develop multiple immunoglobulins with glomerulonephritis and deposition of IgG3 autoantibodies [70]. However, as mentioned previously, there can also be problems in the utilization of murine knock-outs. In the case of C1q, a 129/sv genomic library was used to isolate the C1q gene. If the segment of DNA around this 129/Sv C1q gene, which is included in the DNA fragment used for homologous recombination also contains susceptibility loci, it may be difficult to interpret whether the phenotype is due to the knocked out gene or these other suscep-

tibility genes. In the future, it will be possible to use RNAi approaches to specifically knock out a single gene, thereby avoiding the 'carry over' of flanking DNA sequence. Nevertheless, it is likely that the variants contributing to a complex disease trait do not completely abrogate the function of their gene product.

In mice, the identification of loci important in disease aetiology, may be hampered by the limited sequence heterogeneity seen in inbred mice. However, this can be overcome by mapping loci in different lupus-prone strains and in the context of different genetic backgrounds to increase sequence diversity [75]. Furthermore, this may counteract the problems associated with the extreme genetic heterogeneity in human samples, which may act to reduce the chances of finding a strong genetic effect. It should be noted that the extent of haplotype diversity is not infinite in the human population, with a variation in both the size and the constituent variants of the discrete haplotype blocks found throughout the genome [76–79].

In summary, this shows that integrating data from murine models of lupus may facilitate the identification of susceptibility genes for SLE in human populations, because they are a good experimental system in which to test out functional hypotheses for the aetiology of SLE and potential therapeutic strategies. The examples given in this paper serve to demonstrate that this cross-species approach can be an important tool in unravelling the complexity of the human disease.

REFERENCES

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

2 Rus V, Hochberg MC. The Epidemiology of Systemic Lupus Erythematosus. In: Wallace DJ, Hahn BH, eds. Dubois' Lupus Erythematosus. Baltimore: Lippincott, Williams & Wilkins 2002:65–83.

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

4 Deapen D, Escalante A, Wienrib L *et al.* A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 1992; **35**:311–8.

5 Gaffney PM, Kearns GM, Shark KB *et al.* A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. Proc Natl Acad Sci USA 1998; **95**:14875–9.

6 Gaffney PM, Ortmann WA, Selby SA *et al.* 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 2000; **66**:547–56.

7 Shai R, Quismorio FP Jr, Li L *et al.* Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. Hum Mol Genet 1999; **8**:639–44.

8 Moser KL, Neas BR, Salmon JE *et al.* Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. Proc Natl Acad Sci USA 1998; **95**:14869–74.

9 Johanneson B, Steinsson K, Lindqvist AK *et al.* A comparison of genome-scans performed in multicase families with systemic lupus erythematosus from different population groups. J Autoimmun 1999; **13**:137–41.

10 Gray-McGuire C, Moser KL, Gaffney PM *et al.* Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16–15. 2 Am J Hum Genet 2000; **67**:1460–9.

11 Mold C, Baca R, Du Clos TW. Serum Amyloid P Component and C-Reactive Protein Opsonize Apoptotic Cells for Phagocytosis through Fc[gamma] Receptors. J Autoimmunity 2002; **19**:147–54.

12 Pepys MB, Lanham JG, De Beer FC. C-reactive protein in SLE. Clin Rheum Dis 1982; **8**:91–103.

13 Rordorf C, Schnebli HP, Baltz ML, Tennent GA, Pepys MB. The acute-phase response in (NZB X NZW) F1 and MRL/1 MICE. J Exp Med 1982; **156**:1268–73.

14 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 1997; **158**:5566–74.

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

16 Bickerstaff MC, Botto M, Hutchinson WL *et al.* Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat Med 1999; **5**:694–7.

17 Szalai AJ, Weaver CT, McCrory MA *et al.* Delayed lupus onset in (NZB x NZW) F1 mice expressing a human C-reactive protein transgene. Arthritis Rheum 2003; **48**:1602–11.

18 Russell AI, Cunninghame Graham DS, Shepherd C *et al.* Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Human Mol Genet 2004; **13**:137–47.

19 Chitrabamrung S, Rubin RL, Tan EM. Serum deoxyribonuclease I and clinical activity in systemic lupus erythematosus. Rheumatol Int 1981; **1**:55–60.

20 Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, Moroy T. Features of systemic lupus erythematosus in Dnase1-deficient mice. Nat Genet 2000; **25**:177–81.

21 Yasutomo K, Horiuchi T, Kagami S *et al.* Mutation of DNASE1 in people with systemic lupus erythematosus. Nat Genet 2001; **28**:313–4.

22 Simmonds MJ, Heward JM, Kelly MA *et al.* A nonsense mutation in exon 2 of the DNase I gene is not present in UK subjects with systemic lupus erythematosus and Graves' disease: Comment on the article by Rood *et al.* Arthritis Rheum 2002; **46**:3109–10.

23 Chakraborty P, Kacem HH, Makni-Karray K, Jarraya F, Hachicha J, Ayadi H. The A/T mutation in exon 2 of the DNASE1 gene is not present in Tunisian patients with systemic lupus erythematosus or in healthy subjects. Arthritis Rheum 2003; **48**:3297–8.

24 Tew MB, Johnson RW, Reveille JD, Tan FK. A molecular analysis of the low serum deoxyribonuclease activity in lupus patients. Arthritis Rheum 2001; **44**:2446–7.

25 Balada E, Ordi-Ros J, Hernanz S *et al.* DNASE I mutation and systemic lupus erythematosus in a Spanish population: comment on the article by Tew *et al.* Athritis Rheum 2002; **46**:1974–6.

26 Nadano D, Yasuda T, Kishi K. Measurement of deoxyribonuclease I activity in human tissues and body fluids by a single radial enzyme-diffusion method. Clin Chem 1993; **39**:448–52.

27 Bowness P, Davies KA, Norsworthy PJ *et al.* Hereditary C1q deficiency and systemic lupus erythematosus. Quart J Med 1994; **87**:455–64.

28 Mark J, Walport. Complement and systemic lupus erythematosus. Arthritis Res 2002; **4** (Suppl. 3):S279–S293.

29 Petry F. Molecular basis of hereditary C1q deficiency. Immunobiology 1998; **199**:286–94.

30 Tsao BP. The Genetics of Human Lupus. In: Wallace DJ, Hahn BH, eds. Dubois' Lupus Erythematosus. Baltimore: Lippincott, Williams & Wilkins 2002:97–119.

31 Peter H, Schur and Lloyd Klickstein. Complement and Systemic Lupus Erythematosus. In: Wallace DJ, Hahn BH, eds. Dubois' Lupus Erythematosus. Baltimore: Lippincott, Williams & Wilkins 2002:247–57.

32 Gunnarsson I, Sundelin B, Heimburger M *et al.* Repeated renal biopsy in proliferative lupus nephritis – predictive role of serum C1q and albuminuria. J Rheumatol 2002; **29**:693–9.

33 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 USA 1994; **91**:4062–6.

34 Botto M, Dell'Agnola C, Bygrave AE *et al.* Homozygous C1q

deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nat Genet 1998; **19**:56–9.
35 Petry F, Botto M, Holtappels R, Walport MJ, Loos M. Reconstitution of the Complement Function in C1q-Deficient (C1qa–/–) Mice with Wild-Type Bone Marrow Cells. J Immunol 2001; **167**:4033–7.
36 Racila DM, Sontheimer CJ, Sheffield A, Wisnieski JJ, Racila E, Sontheimer RD. Homozygous single nucleotide polymorphism of the complement C1QA gene is associated with decreased levels of C1q in patients with subacute cutaneous lupus erythematosus. Lupus 2003; **12**:124–32.
37 Miura-Shimura Y, Nakamura K, Ohtsuji M *et al.* C1q Regulatory Region Polymorphism Down-Regulating Murine C1q Protein Levels with Linkage to Lupus Nephritis. J Immunol 2002; **169**:1334–9.
38 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 1995; **154**:2441–7.
39 Kono DH, Burlingame RW, Owens DG *et al.* Lupus susceptibility loci in New Zealand mice. Proc Natl Acad Sci USA 1994; **91**:10168–72.
40 Vyse TJ, Morel L, Tanner FJ, Wakeland EK, Kotzin BL. Backcross analysis of genes linked to autoantibody production in New Zealand White mice. J Immunol 1996; **157**:2719–27.
41 Vyse TJ, Todd JA, Kotzin BL. Non-MHC Genetic Contributions to Autoimmune Disease. In: Rose NR, Mackay IR, eds. The autoimmune diseases. San Diego: Teackmic Press, 1998:85–118.
42 Salmon JE, Millard S, Schachter LA *et al.* Fcgamma RIIA Alleles Are Heritable Risk Factors for Lupus Nephritis in African Americans. J Clin Invest 1996; **97**:1348–54.
43 Duits AJ, Bootsma H, Derksen RH *et al.* Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. Arthritis Rheum 1995; **38**:1832–6.
44 Zuniga R, Ng S, Peterson MG *et al.* Low-binding alleles of Fcgamma receptor types IIA and IIIA are inherited independently and are associated with systemic lupus erythematosus in Hispanic patients. Arthritis Rheum 2001; **44**:361–7.
45 Botto M, Theodoridis E, Thompson EM *et al.* Fc gamma RIIa polymorphism in systemic lupus erythematosus (SLE): no association with disease. Clin Exp Immunol 1996; **104**:264–8.
46 D'Alfonso S, Rampi M, Bocchio D, Colombo G, Scorza-Smeraldi R, Momigliano-Richardi P. Systemic lupus erythematosus candidate genes in the Italian population: evidence for a significant association with interleukin-10. Arthritis Rheum 2000; **43**:120–8.
47 Dijstelbloem HM, Bijl M, Fijnheer R *et al.* Fcgamma receptor polymorphisms in systemic lupus erythematosus: association with disease and in vivo clearance of immune complexes. Arthritis Rheum 2000; **43**:2793–800.
48 Koene HR, Kleijer M, Swaak AJ *et al.* The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. Arthritis Rheum 1998; **41**:1813–8.
49 Manger K, Repp R, Spriewald BM *et al.* Fcgamma receptor IIa polymorphism in Caucasian patients with systemic lupus erythematosus: association with clinical symptoms. Arthritis Rheum 1998; **41**:1181–9.
50 Salmon JE, Ng S, Yoo DH, Kim TH, Song GG. Altered distribution of Fcgamma receptor IIIA alleles in a cohort of Korean patients with lupus nephritis. Arthritis Rheum 1999; **42**:818–9.
51 Karassa FB, Trikalinos TA, Ioannidis JP. FcgammaRIIa-SLE Meta-Analysis Investigators. Role of the Fcgamma receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. Arthritis Rheum 2002; **46**:1536–71.
52 Zuniga R, Markowitz GS, Arkachaisri T, Imperatore EA, D'Agati VD, Salmon JE. Identification of IgG subclasses and C-reactive protein in lupus nephritis: The relationship between the composition of immune deposits and FC receptor type IIA alleles. Arthritis Rheum 2003; **48**:460–70.
53 Takahashi S, Nose M, Sasaki J, Yamamoto T, Kyogoku M. IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. J Immunol 1991; **147**:515–9.
54 Wu J, Edberg JC, Redecha PB *et al.* A Novel Polymorphism of Fcgamma RIIIa (CD16) Alters Receptor Function and Predisposes to Autoimmune Disease. J Clin Invest 1997; **100**:1059–70.
55 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 1999; **26**:1486–9.
56 Seligman VA, Suarez C, Lum R *et al.* The Fcgamma receptor IIIA-158F allele is a major risk factor for the development of lupus nephritis among Caucasians but not non-Caucasians. Arthritis Rheum 2001; **44**:618–25.
57 Karassa FB, Trikalinos TA, Ioannidis JPA. The *FcRIIIA-F158* allele is a risk factor for the development of lupus nephritis: a meta-analysis. Kidney Int 2003; **63**:1475–82.
58 Clynes R, Dumitru C, Ravetch JV. Uncoupling of Immune Complex Formation and Kidney Damage in Autoimmune Glomerulonephritis. Science 1998; **279**:1052–4.
59 Ravetch JV. Fc receptors. Curr Opin Immunol 1997; **9**:121–5.
60 Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature 1996; **379**:346–9.
61 Muhlfeld AS, Segerer S, Hudkins K *et al.* Deletion of the Fcgamma Receptor IIb in Thymic Stromal Lymphopoietin Transgenic Mice Aggravates Membranoproliferative Glomerulonephritis. Am J Pathol 2003; **163**:1127–36.
62 Yajima K, Nakamura A, Sugahara A, Takai T. FcgammaRIIB deficiency with Fas mutation is sufficient for the development of systemic autoimmune disease. Eur J Immunol 2003; **33**:1020–9.
63 Pritchard NR, Cutler AJ, Uribe S, Chadban SJ, Morley BJ, Smith KG. Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcgammaRII. Curr Biol 2000; **10**:227–30.
64 Jiang Y, Hirose S, Abe M *et al.* Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. Immunogenetics 2000; **51**:429–35.
65 Jiang Y, Hirose S, Sanokawa-Akakura R *et al.* Genetically determined aberrant down-regulation of FcgammaRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. Int Immunol 1999; **11**:1685–91.
66 Bygrave AE, Rose KL, Cortes-Hernandez J *et al.* Spontaneous Autoimmunity in 129 and C57BL/6 Mice – Implications for Autoimmunity Described in Gene-Targeted Mice. Public Library of Science 2004; **172**.
67 Lindqvist AK, Steinsson K, Johanneson B *et al.* A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. J Autoimmun 2000; **14**:169–78.
68 Agata Y, Kawasaki A, Nishimura H *et al.* Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol 1996; **8**:765–72.
69 Nishimura H, Agata Y, Kawasaki A *et al.* Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4–CD8–) thymocytes. Int Immunol 1996; **8**:773–80.
70 Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 1999; **11**:141–51.
71 Prokunina L, Alarcon-Riquelme M. The Genetic Basis of Systemic Lupus Erythematosus – knowledge of today and thoughts for tomorrow. Human Molecular Genetics 2004: ddh076.
72 Prokunina L, Castillejo-Lopez C, Oberg F *et al.* A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 2002; **32**:666–9.
73 Prokunina L, Gunnarsson I, Sturfelt G *et al.* The systemic lupus erythematosus-associated PDCD1 polymorphism PD1.3A in lupus nephritis. Arthritis Rheum 2004; **50**:327–8.

74 Lin SC, Yen JH, Tsai JJ *et al.* Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. Arthritis Rheum 2004; **50**:770–5.

75 Rigby Robert J, Rozzo SJ, Boyle JJ, Lewis M, Kotzin BL, Vyse TJ. New Loci from New Zealand Black and New Zealand White on Chromosomes 4 and 12 Contribute to Lupus-Like Disease in the Context of BALB/c. J.Immunol 2004; **172**:4609–17.

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

77 Gabriel SB, Schaffner SF, Nguyen H *et al.* The Structure of Haplotype Blocks in the Human Genome. Science 2002; **296**:2225–9.

78 Wall JD, Pritchard JK. Haplotype Blocks and Linkage Disequilibrium in the Human Genome. Nature Rev Genet 2003; **4**:587–97.

79 Zhang J, Rowe WL, Clark AG, Buetow KH. Genomewide Distribution of High-Frequency, Completely Mismatching SNP Haplotype Pairs Observed To Be Common across Human Populations. Am J Hum Genet 2003; **73**:1073–81.

80 Hogarth MB, Slingsby JH, Allen PJ *et al.* Multiple Lupus Susceptibility Loci Map to Chromosome 1 in BXSB Mice. J Immunol 1998; **161**:2753–61.