

Genetic Factors Involved in the Pathogenesis of Systemic Lupus Erythematosus

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Abstract

Systemic Lupus Erythematosus (SLE) is a typical systemic autoimmune disease characterized by the production of auto antibodies reactive to nuclear and cellular components such as double-stranded DNA, RNA-proteins and phospholipids. The deposition of immune complex to tissues results in inflammatory reactions leading to tissue damage and organ failures. In recent years, enormous efforts have been undertaken to clarify the genetic susceptibility to SLE. To date, nearly 30 SLE-susceptibility loci have been mapped on the human chromosomes. Mouse models of SLE have also been studied and have contributed to the understanding of SLE pathogenesis. In this review, we summarize the recent knowledge of the SLE susceptibility loci, and we describe new insights into the epistatic interaction of closely linked polymorphic genes that is likely to play a pivotal role in the impaired homeostasis of the immune system in SLE.

Keywords: Systemic Lupus Erythematosus; Single Nucleotide Polymorphism; Genetic Susceptibility

Introduction

Clinical aspects of SLE: Systemic Lupus Erythematosus (SLE) is among the most serious immunological disorders characterized by the production of auto antibodies reactive to nucleic acids as well as to nuclear and cellular components. Deposition of immune complex on varieties of tissues leads to systemic inflammation and multiple organ failures [1]. The prevalence of SLE is reported to be between 40 and 400 cases per 100,000 individuals. Nearly 90% of SLE patients are women [2].

The immune abnormalities of SLE: The accelerated autoimmune features in SLE are represented by the class-switch and affinity maturation of auto antibodies, the mechanism of which is normally operative on the antibody productions against non-self pathogens [3]. In patients with overt SLE activity, peripheral CD4⁺ T cells show a low capacity to produce Interleukin (IL)-2 on stimulation, and a high frequency of cells positive for early activation markers such as HLA-DR, -DP and CD69 is observed [4].

Pathogenesis of SLE: The cell surface expression of CD69 on lymphocytes is under the control of type-I Interferon (IFN) [5].

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It is widely recognized that in the peripheral blood cells of SLE patients, the transcripts of genes that are induced by type-I IFN are elevated [6]. This type of gene expression profile, termed the "interferon signature," is attributed to the function of natural immunity-sensing nucleic acids [7]. Nevertheless, the role of natural immunity in the cause-effect relationship of the pathogenesis of SLE remains elusive. SLE is a multifactorial disease in which the accumulation of the allelic effects of multiple genes forms the basis of disease susceptibility [8]. Environmental factors such as viral infections, exposure to chemicals, and UV irradiation are also implicated in the onset of SLE [9].

History of SLE Genetics

Over the last few decades, there have been enormous efforts to clarify the genetic susceptibility to SLE. The history of SLE genetics can be classified into three generations of studies. The distinction between generations was made based on progress in molecular genetics methods. Before polymorphic DNA markers were available, first-generation SLE genetics was the study of the recurrence risks in siblings [10], concordance rates in monozygotic twins [11], and case-control studies focusing on potential polymorphic candidate genes involved in the immune system. Associations of SLE with HLA class II haplotypes (HLA-DR3, -DR2 and -DR4) [12, 13] and polymorphisms of the complement component genes (C1q, C2 and C4) [14-16] and Fcγ receptor genes were reported in early studies [17-19].

The second-generation SLE genetics studies began in the early 1990s. The major methodological advance that enabled second-generation SLE genetics was the new availability of polymorphic DNA markers with short nucleotide repeats (microsatellite DNA markers) that are scattered randomly throughout the entire genome. Each locus of microsatellite DNA usually has multiple alleles with different repeat numbers, and each allelic form of microsatellite DNA has been a highly informative tool in genetic linkage analyses.

Since the late 1990s, several groups of researchers have reported elaborate genome-wide linkage studies in familial SLE

from different ethnic groups. Figure 1 summarizes the results of these studies. Among multiple regions with potential linkage to SLE, the following seven regions met the threshold of genome-wide linkage studies: 1q22-24 [20], 1q41 [20, 21], 2q37 [22], 4p13-16 [22, 23], 6p21-11 [24, 25], 12q24 [26] and 16q13 [25]. The results of each independent linkage study differ markedly with respect to major genomic intervals linked to SLE. Nevertheless, the linkages with genomic intervals on chromosome 1 attracted attention because the observations were consistent in separate genome scans, and the region was syntenic to the mouse chromosome region with autoimmune susceptibility.

Third-generation SLE genetics, which use extensive Genome-Wide Association Scans (GWASs), began in the late 2000s. GWAS studies are based on the achievements of the human genome project and those of the subsequent HapMap Project, the international collaborative effort to develop a haplotype map of the human genome by using Single Nucleotide Polymorphisms (SNPs). The rapid progress of DNA typing by microarray technology also accelerated this research.

To date, there have been four independent GWASs. Figure 1 also summarizes the SNP loci that fulfilled the criterion of significance levels ($p < 5 \times 10^{-8}$) in these GWAS studies [27]. In 2008, the International Consortium for Systemic Lupus Erythematosus (SLEGEN) reported GWAS studies using approx. 320,000 SNPs in 720 European SLE patients and 2,337 controls. Significant associations were observed for the HLA region, 7q32 (IRF5), 16p11.2 (ITGAM), 11p15.5 (KIAA1542), 3p14.3 (PXX) and 1q25.1 [28]. The consortium also confirmed the association at FCGR2A, PIPN22 and STAT4 regions that were previously reported to be associated with SLE. Graham et al. [29] reported GWAS studies using 310,000 SNPs in 431 SLE patients and 2,155 controls. Aside from the HLA and IRF5 regions previously reported, they observed a significant association for TNFAIP3 locus on chromosome 6. In 2009, Gateva et al. [30] reported studies using 2,466 selected SNPs that were previously suggested to have potential linkage with SLE, in 1,963 patients and 4,329 controls. They observed new

loci: TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10. Han et al. (2009) [31] reported GWAS studies in a Chinese Han population (1,047 cases and 1,205 controls). They observed new susceptibility loci (ETS1, IKZF1, RASGRP3, SLC15A4,

TNIP1, 7q11.23, 10q11.22, 11q23.3 and 16p11.2), and also confirmed seven previously reported loci (BLK, IRF5, STAT4, TNFAIP3, TNFSF4, 6q21 and 22q11.21).

Figure 1: Kodera et al

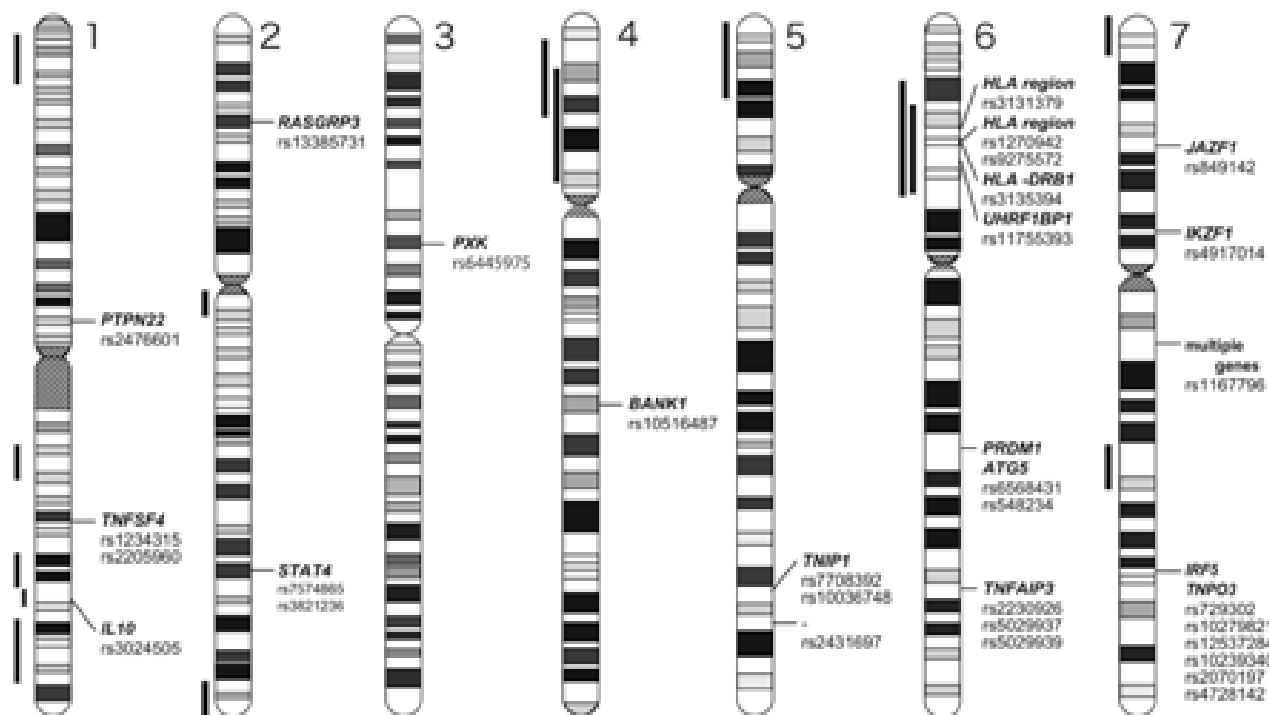


Figure 1: Results of genome-wide mapping of SLE-susceptibility loci on human chromosomes. The regions identified by genetic linkage studies (peak LOD scores larger than 3.0) are shown with solid black lines. SNP loci identified by GWAS studies are designated by their identification numbers together with the names of potential candidate genes.

Polygenic Involvement in SLE Susceptibility

The results of the recent extensive GWAS studies unveiled the extreme complexity of the genetic susceptibility to SLE. Nearly 30 SNP loci have been demonstrated to show an association with SLE with nominal p -values $< 5 \times 10^{-8}$. However, the relative risks observed for all of these susceptibility SNP loci were only low or moderate (odds ratio 1.2–2.4). It is evident that multiple allelic effects of susceptibility genes (of which

polymorphisms are commonly observed in human populations) are responsible for the basis of the genetic susceptibility to SLE. It is likely that there are subsets of SLE with respect to the combination of susceptibility genes involved, and that ethnic groups differ in the compositions of these SLE subsets. Therefore, studies using mouse SLE models should provide advantages for elucidating the critical path of SLE pathogenesis.

Studies Using SLE Model Mice

Studies using mouse SLE models have the following advantages over human research. First, SLE in each model strain of mice may represent a single SLE subset with respect to the combination of susceptibility genes. Secondly, component phenotypes (endophenotypes) underlying the loss of self-tolerance can be studied by the method of quantitative genetics

in mouse models. Thirdly, advanced methods of reverse genetics, i.e., gene-targeting technology can be applied to test the involvement of candidate genes. Several SLE models have provided insights into the cellular and molecular bases of the susceptibility to autoimmunity.

The New Zealand Black (NZB) strain of mice has been studied as an autoimmune strain that shows the spontaneous production of an anti-erythrocyte autoantibody and hemolytic anemia [32]. The F1 hybrid of this strain and the phenotypically normal New Zealand White (NZW) strain show very accelerated autoimmune phenotypes, such as the production of anti-double-stranded DNA autoantibody and immune-complex mediated glomerulonephritis. The SLE phenotypes of (NZB x NZW) F1 mice are strictly dependent on the heterozygosity of H-2^d/H-2^z [33].

Mixed haplotype MHC-class II molecule, the expression of which is ectopic on the activated antigen-presenting cells, has been implicated in the development of accelerated autoimmune phenotypes of (NZB x NZW) F1 mice. Recombinant inbred strains derived from (NZB x NZW) F1 mice, NZM/Aeg lines were also studied [34]. The MRL-Fas^{lpr} strain is a naturally occurring Fas-deficient strain [35]. Defective Fas gene plays a pivotal role in the spontaneous development of SLE-related phenotypes. Nevertheless, the autoimmune features of this strain are under the control of polygenes [36].

The BXSB strain is a recombinant inbred strain derived from a cross between the C57BL/6 and SB/Le strains. Male BXSB mice spontaneously develop the SLE phenotype, and the accelerated autoimmune phenotype of these mice is linked to Yaa (Y-linked autoimmune acceleration) locus on the Y chromosome [37]. The Yaa mutation was identified as a translocation from the telomeric end of the X chromosome containing the gene encoding for TLR7 gene [38]. Yaa mutation on the normal C57BL/6 genetic background is useful to accelerate the expression of other SLE susceptibility genes that are introduced by the advanced method of reverse genetics.

Candidate Genes Identified

Recent GWAS studies in human populations were proven to be powerful in narrowing the region of SLE-susceptibility loci. However, the involvement of the polymorphism of each candidate gene needs to be demonstrated by the reverse genetic approach using mice. The following genes are the candidate susceptibility genes; their possible involvement in SLE pathogenesis is also summarized.

FCGR2B gene

FCGR2B gene codes for an inhibitory Fc receptor, FcγRIIB, which is expressed on myeloid cells and B cells. The FcγRIIB molecule has an ITIM motif in the cytoplasmic region of the molecule, and is thought to be responsible for the regulation of the terminal differentiation of B-lineage cells [39]. The potential involvement of FcγRIIB in SLE susceptibility was first suggested by the observation of SLE-related phenotypes in FcγRIIB-deficient mice [40]. Subsequently, based on the genetic linkage studies of autoantibody production in NZB mice, fcgr2b gene from NZB mice with deletion polymorphism in the promoter region was identified to be responsible for the autoantibody production in NZB mice [41, 42].

NZB mice are known to be defective in susceptibility to the Bovine γ Globulin (BGG)-specific high-dose tolerance induced by high doses of Deaggregate BGG (DBGG) [43]. This type of tolerance induction has long been studied as a model of peripheral immune tolerance. Defective high-dose tolerance in NZB mice was also shown to be linked to the fcgr2b locus of NZB mice. Moreover, the epistatic interaction of defective fcgr2b gene and the autoimmune-susceptible haplotype of SLAM family genes were found to be responsible for the defective immune tolerance and the SLE-related autoimmune phenotypes in these mice [44].

In humans, homozygosity of the I232T missense mutation on the coding sequence of FCGR2B gene was reported to be in significant linkage to SLE [45]. It was concluded that the human FcγRIIB molecule with 232T is unable to inhibit the

activation signals from B-cell antigen receptors because it is excluded from sphingolipid rafts on the cell surface of B cells [46]. Polymorphism in the promoter region of human FCGR2B gene was also suggested to bear SLE susceptibility [47]. The

involvement of SLAM family gene polymorphism was also suggested in humans [48]. Figure 2 shows the structures of SLE-susceptibility regions on human chromosome 1 as well as on syntenic mouse chromosome 1.

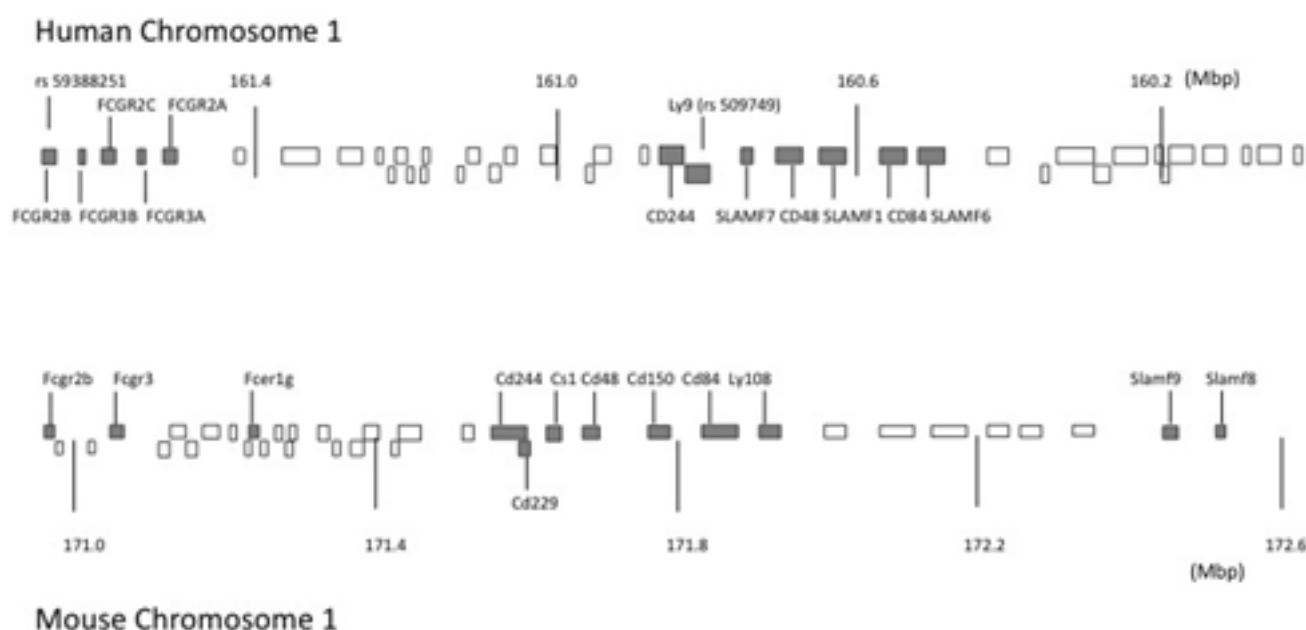


Figure 2: Structures of the SLE-susceptibility region on human telomeric chromosome 1, and that on syntenic mouse chromosome 1. Reported susceptibility SNP loci are shown by their identification numbers.

MHC Region

Early studies demonstrated that the risk of the development of SLE is associated with HLA class II alleles HLA-DR3 and -DR2 in European populations [12, 13]. The structure and polymorphism of the MHC haplotype differ among ethnic groups, and non-European populations have shown associations with other alleles such as HLA-DR4 [13]. In an early genetic linkage analysis of familial SLE, the lack of consistency in the linkage to HLA loci was a puzzling problem [25]. The involvement of MHC-class II alleles was consistently observed in recent GWAS studies. Associations with HLA-DRB1 alleles were replicated in GWAS studies in different ethnic groups.

Because of the strong linkage disequilibrium in HLA-class II regions, the involvement of HLA-DQA1 and HLA-DQB1 alleles as well as the class I and class III regions has not yet been excluded [49].

TNFIP3

TNFIP3 (Tumor Necrosis Factor alpha Inducible Protein 3 gene) codes for an ubiquitin-modifying enzyme, A20, which plays a pivotal role in the regulation of NF- κ B activity [50]. Based on the findings of GWASs and the subsequent fine mapping and resequencing, Adrianto et al. (2011) [51] identified a risk haplotype with a deletion polymorphism in the intronic region of TNFIP3 gene in SLE patients of European and Korean ancestry, and because of its location in the region of possible involvement in the regulatory element, this deletion polymorphism was implicated in the defective regulation of TNFIP3 gene. Compared with the normal gene, TNFIP3 gene

with this deletion polymorphism showed reduced transcription and A20 protein expression in cell lines obtained from SLE patients. A20-deficient mice were reported to develop systemic organ inflammation and SLE-related phenotypes [52, 53].

Conclusion

The emerging new trend in SLE genetics is comprised of the identification of susceptibility genes, the characterization of their risk polymorphisms, and the demonstration of the involvement of each susceptibility allele by reverse genetics methods using mice. Next-generation sequencing technology has already been applied to the studies of risk haplotypes of genomic regions. Advanced technology for gene targeting in mice — especially the availability of tissue-specific gene knock-out or knock-down methods using the Cre/loxP system—has already proven to be useful to elucidate the molecular mechanisms underlying the involvement of susceptibility genes [54]. The recent rapid development of RNA-guided genome-editing technology will accelerate the reverse genetics [55].

SLE has been one of the most problematic and devastating systemic autoimmune diseases. The genetic susceptibility to SLE was once thought to be too complex to be studied, but international collaborative efforts are now revealing multiple clues for understanding the genetic susceptibility to SLE. The ultimate goal is the complete elucidation of the critical pathway of the abnormal autoimmune reaction, and the continuing progress toward this goal allows for optimism.

References

1. Arnett FC. 1997. The genetic basis of lupus erythematosus. In: Dubois J (ed) *Lupus erythematosus*. pp.77-117. Williams & Wilkins, Baltimore, MD.
2. Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, et al. (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum*, 58: 15-25.
3. Hirose S, Wakiya M, Kawano-Nishi Y, Yi J, Sanokawa R, et al. (1993) Somatic diversification and affinity maturation of IgM and IgG anti-DNA antibodies in murine lupus. *Eur J Immunol*, 23: 2813-2820.
4. Daca A, Czuszyńska Z, Smolenska Z, Zdrojewski Z, Witkowski JM, Bryl E (2011) Two systemic lupus erythematosus (SLE) global disease activity indexes--the SLE Disease Activity Index and the Systemic Lupus Activity Measure--demonstrate different correlations with activation of peripheral blood CD4+ T cells. *Hum Immunol*, 72: 1160-1167.
5. Shiow LR, Rosen DB, Brdickova N, Xu Y, An J, et al. (2006) CD69 acts downstream of interferon- α/β to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature*, 440: 540-544.
6. Becker AM, Dao KH, Han BK, Kornu R, Lakhanpal S, et al. (2013) SLE peripheral blood B cell, T cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. *PLoS One*, 8: e67003.
7. Bizzaro N, Villalta D, Giavarina D, Tozzoli R (2012) Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis. *Autoimmun Rev*, 12: 97-106.
8. Hirose S, Jiang Y, Hamano Y, Shirai T (2000) Genetic aspects of inherent B-cell abnormalities associated with SLE and B-cell malignancy: lessons from New Zealand mouse models. *Int Rev Immunol*, 19: 389-421.
9. Miller FW, Cooper GS. 2007. Environmental aspect of lupus. In: Wallace, D J & Hahn, B H (eds), *Dubois' Lupus Erythematosus*, Seventh ed. pp.21-33. Lippincott Williams & Wilkins, Philadelphia, PA.
10. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, et al. (2005) Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort.

- Arthritis Rheum, 52: 1138-1147.
11. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, et al. (1992) A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum*, 35: 311-318.
12. Tsao BP, Wu H. 2007. The Genetics of Human Lupus. In: Wallace, D J & Hahn, B H (eds), *Dubois' Lupus Erythematosus*, Seventh ed. pp.54-81. Lippincott Williams & Wilkins, Philadelphia, PA.
13. Galeazzi M, Sebastiani GD, Morozzi G, Carcassi C, Ferrara GB, et al. (2002) HLA class II DNA typing in a large series of European patients with systemic lupus erythematosus: correlations with clinical and autoantibody subsets. *Medicine (Baltimore)*, 81: 169-178.
14. Bowness P, Davies KA, Norsworthy PJ, Athanassiou P, Taylor-Wiedeman J, et al. (1994) Hereditary C1q deficiency and systemic lupus erythematosus. *QJM*, 87: 455-464.
15. Davies KA, Erlendsson K, Beynon HL, Peters AM, Steinsson K, Valdimarsson H, Walport MJ (1993) Splenic uptake of immune complexes in man is complement-dependent. *J Immunol*, 151: 3866-3873.
16. Ehrenfeld M, Urowitz MB, Platts ME (1984) Selective C4 deficiency, systemic lupus erythematosus, and Whipple's disease. *Ann Rheum Dis*, 43: 91-94.
17. Duits AJ, Bootsma H, Derksen RH, Spronk PE, Kater L, et al. (1995) Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum*, 38: 1832-1836.
18. Salmon JE, Millard S, Schachter LA, Arnett FC, Ginzler EM, et al. (1996) Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J Clin Invest*, 97: 1348-1354.
19. Enenkel B, Jung D, Frey J (1991) Molecular basis of IgG Fc receptor III defect in a patient with systemic lupus erythematosus. *Eur J Immunol*, 21: 659-663.
20. Moser KL, Neas BR, Salmon JE, Yu H, Gray-Mcguire C, et al. (1998) 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: 14869-14874.
21. Tsao BP, Cantor RM, Grossman JM, Shen N, Teophilov NT, et al. (1999) PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. *J Clin Invest*, 103: 1135-1140.
22. Lindqvist AK, Steinsson K, Johanneson B, Kristjansdottir H, Arnasson A, et al. (2000) A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. *J Autoimmun*, 14: 169-178.
23. Gray-Mcguire C, Moser KL, Gaffney PM, Kelly J, Yu H, et al. (2000) Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16-15.2. *Am J Hum Genet*, 67: 1460-1469.
24. Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, et al. (1998) A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci U S A*, 95: 14875-14879.
25. Gaffney PM, Ortmann WA, Selby SA, Shark KB, Ockenden TC, et al. (2000) 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: 547-556.
26. Nath SK, Quintero-Del-Rio AI, Kilpatrick J, Feo L, Ballesteros M, Harley JB (2004) Linkage at 12q24 with systemic lupus erythematosus (SLE) is established and confirmed in Hispanic and European American families. *Am J Hum Genet*, 74: 73-82.
27. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT (2011) Basic statistical analysis in genetic case-control studies. *Nat Protoc*, 6: 121-133.
28. International Consortium for Systemic Lupus

- Erythematosus G, Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, et al. (2008) Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXX, KIAA1542 and other loci. *Nat Genet*, 40: 204-210.
29. Graham RR, Cotsapas C, Davies L, Hackett R, Lessard CJ, et al. (2008) Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet*, 40: 1059-1061.
30. Gateva V, Sandling JK, Hom G, Taylor KE, Chung SA, et al. (2009) A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet*, 41: 1228-1233.
31. Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, et al. (2009) Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet*, 41: 1234-1237.
32. Howie JB, Helyer BJ (1968) The immunology and pathology of NZB mice. *Adv Immunol*, 9: 215-266.
33. Hirose S, Jiang Y, Nishimura H, Shirai T (2006) Significance of MHC class II haplotypes and IgG Fc receptors in SLE. *Springer Semin Immunopathol*, 28: 163-174.
34. Morel L, Wakeland EK (2000) Lessons from the NZM2410 model and related strains. *Int Rev Immunol*, 19: 423-446.
35. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*, 356: 314-317.
36. Nose M, Nishihara M, Fujii H (2000) Genetic basis of the complex pathological manifestations of collagen disease: lessons from MRL/lpr and related mouse models. *Int Rev Immunol*, 19: 473-498.
37. Izui S, Ibnou-Zekri N, Fossati-Jimack L, Iwamoto M (2000) Lessons from BXSB and related mouse models. *Int Rev Immunol*, 19: 447-472.
38. Santiago-Raber ML, Kikuchi S, Borel P, Uematsu S, Akira S, Kotzin BL, Izui S (2008) Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. *J Immunol*, 181: 1556-1562.
39. Nimmerjahn F, Ravetch JV (2006) Fcgamma receptors: old friends and new family members. *Immunity*, 24: 19-28.
40. Bolland S, Ravetch JV (2000) Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity*, 13: 277-285.
41. Jiang Y, Hirose S, Abe M, Sanokawa-Akakura R, Ohtsuji M, et al. (2000) Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. *Immunogenetics*, 51: 429-435.
42. Jiang Y, Hirose S, Sanokawa-Akakura R, Abe M, Mi X, et al. (1999) 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*, 11: 1685-1691.
43. Staples PJ, Talal N (1969) Relative inability to induce tolerance in adult NZB and NZB-NZW F1 mice. *J Exp Med*, 129: 123-139.
44. Fujii T, Hou R, Sato-Hayashizaki A, Obata M, Ohtsuji M, et al. (2011) Susceptibility loci for the defective foreign protein-induced tolerance in New Zealand Black mice: implication of epistatic effects of Fcgr2b and Slam family genes. *Eur J Immunol*, 41: 2333-2340.
45. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, et al. (2002) Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum*, 46: 1242-1254.
46. Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR,

- Macary PA, et al. (2005) Loss of function of a lupus-associated FcγRIIb polymorphism through exclusion from lipid rafts. *Nat Med*, 11: 1056-1058.
47. Su K, Wu J, Edberg JC, Li X, Ferguson P, et al. (2004) A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. *J Immunol*, 172: 7186-7191.
48. Cunninghame Graham DS, Vyse TJ, Fortin PR, Montpetit A, Cai YC, et al. (2008) Association of LY9 in UK and Canadian SLE families. *Genes Immun*, 9: 93-102.
49. Morris DL, Taylor KE, Fernando MM, Nititham J, Alarcon-Riquelme ME, et al. (2012) Unraveling multiple MHC gene associations with systemic lupus erythematosus: model choice indicates a role for HLA alleles and non-HLA genes in Europeans. *Am J Hum Genet*, 91: 778-793.
50. Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, et al. (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol*, 5: 1052-1060.
51. Adrianto I, Wen F, Templeton A, Wiley G, King JB, et al. (2011) Association of a functional variant downstream of TNFAIP3 with systemic lupus erythematosus. *Nat Genet*, 43: 253-258.
52. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A (2000) Failure to regulate TNF-induced NF-κB and cell death responses in A20-deficient mice. *Science*, 289: 2350-2354.
53. Tavares RM, Turer EE, Liu CL, Advincula R, Scapini P, et al. (2010) The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. *Immunity*, 33: 181-191.
54. Nagy A (2000) Cre recombinase: the universal reagent for genome tailoring. *Genesis*, 26: 99-109.
55. Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, Jaenisch R (2013) One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell*, 154: 1370-1379.