Molecular Genetics of Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease that affects multiple organs. The hallmark of SLE is the production of autoantibodies directed against various components of the cell nucleus such as anti-nuclear antibodies and antibodies to double stranded DNA (ds-DNA). These autoantibodies cause end-organ damage via inflammatory response to immune complex, which results in various clinical manifestations such as glomerulonephritis, arthritis, serositis, vasculitis and neurological disorders. The usual onset occurs between 15 and 45 years of age. SLE is more prevalent in females (female:male ratio ~ 8:1). The prevalence of SLE in the general population is approximately 1 in 2000 but it varies among ethnic groups. SLE is more common in Africans and Asians, than in Caucasians. In Thailand, there was no official report on the incidence or prevalence of SLE. However, SLE has been reported in Hong Kong, with the estimated point prevalence around 58.8 per 100,000 population (rates for men and women are 11.7 cases per 100,000 persons and 104 cases per 100,000 persons, respectively).[1] In addition, the outcome of SLE may also differ among racial/ethnic groups. Mok CC and coworker reported that Chinese patients with SLE in Hong Kong have more serious organ manifestations than Caucasians, especially renal disease. The frequencies of renal disease were 50% in Chinese patients and 31-39% in Caucasian patients. Renal manifestation has been reported in Thai SLE patients with higher frequency than Chinese patients, with the reported frequency of 78%.[2] Furthermore, another manifestation such as neuropsychiatry was also found with higher frequency in Thai patients compared to Chinese patients. Therefore, from these data, it can help to indicate that Asian patients with SLE, particularly Thais have more severity in the progression of disease.

Genetic Factors

There is clearly a genetic component to SLE susceptibility shown by familial clustering and twin's studies. The degree of familial clustering was measured by comparing the risk of a sibling with the risk in the population as a whole (λs), which varies between 20 and 40. In other words, the siblings of SLE patient are 20-40 times more likely to develop SLE than those who do not have an affected sibling. The evidence in twins study is the higher concordance rates in monozygotic twins ranging between 24% and 58%, while the concordance rate is only 2-5% in dizygotic twins. Such 10-fold difference suggests that multiple genes shared between each pair of twins considerably influence the susceptibility to SLE.

Two basic strategies have been employed in human SLE genetic studies: linkage and association analyses. In addition, a useful alternative strategy is defining the genetics of SLE in mouse models.

1. Linkage Analysis

The linkage study is used to seek for co-segregation of a particular genetic marker with disease in families of affected individuals. The markers themselves are not usually functional but are linked to disease causing variants according to possibilities that proximal consequences on chromosome will be inherited together

TABLE 1. Established linkages (LOD ≥ 3.3) with Systemic Lupus Erythematosus.

Chromosome	Location	Markers	LOD	Collection
1	1q22-23	FcγRIIA	4.30	OMRF1B
1	1q41-42	D1s2860- D1s213	3.30	UCLA
2	2q37	D2s125	4.24	UPP 1
4	4p16	D4S2366	3.62	OMRF1B
6	6p21-p11	D6S426	4.19	UMN1 & 2
16	16q13	D16s415	3.85	UMN1 & 2
17	17p13	D17s974- D17s1298	3.64	OMRF1C

UCLA = A study conducted at University of California, Los Angeles
UMN = A study conducted at University of Minnesota
OMRF = A study conducted at Oklahoma Medical Research Foundation
USC = A study conducted at University of Southern California
UPP = A study conducted at University of Uppsala

TABLE 2. Candidate genes associated with SLE.

Gene	Location	Associated allele	Statistical significance[a]	References
FcGR2A	1q23	R131	1.30 (1.1-1.52), p=0.0016	Karassa FB et al., 2002
			2.6 (1.3-5.2), p=0.002	Lee HS et al., 2003
			2.01 (1.28-3.14), p=001	Hirankarn N et al., 2006
FcGR3A	1q23	F176	$\chi2$=9.87, p<0.01	Wu J et al., 1997
			1.2 (1.06-1.36), p=0.006	Karassa FB et al., 2003
CRP	1q23	CRP4A	$\chi2$=3.81, p=0.05	Russell AI et al., 2004
		CRP (GT)16	$\chi2$=3.82, p=0.05	Russell AI et al., 2004
FASL	1q23	−844C	p=0.024	Wu J et al., 2003
		-844 CC	OR=1.53, p=0.014	Chen JY et al., 2005
IL10	1q31-32	-1082A, -819T and -592A haplotype	OR=1.9, p=0.02	Rood MJ et al., 1999
			$\chi2$=5.85, p<0.05	Khoa PD et al., 2005
		-1082G allele	P<0.05	Gibson AW et al., 2005
		-2763AA genotypes	1.47(1.02−2.12),p=0.03	Hirankarn N et al., 2006
		-1082A, -819C and -592C haplotype		
PTPN22	1p13	1858T allele (W620)	1.49(1.28−1.75), p <0.00001	Lee YH et al., 2007
PDCD-1	2q37	PD-1.3A	2.6 (1.6−4.4), p=0.00001	Prokunina L et al., 2002
			2.73 (1.35-5.56), p=0.0019	Velázquez-Cruz R et al., 2007
STAT4	2q32	rs7574865 (T allele)	1.55(1.34-1.79), p=1.87x10^{-9}	Remmers EF et al., 2007
CTLA-4	2q33	+49G	1.72(1.2-2.4), p=0.003	Ahmed S et al., 2001
			1.23 (1.08−1.41), p=0.002	Lee YH et al., 2005
HLADR2, DR3	6p21	DR2/DR3	$\chi2$=35.0/76.0, p<0.0005	Hartung K et al., 1992
			1.75(1.4-2.19), p=0.000001 /2.02(1.44-2.83), p=0.0004	Castaño-Rodríguez N,2008
TNFa	6p21	-308A	3.7 (2.24−6.11), p<0.05	van der Linden MW et al., 2001
		-238A	OR=3.62, p=0.02	Zúñiga J et al., 2004
		-308A	2.3 (1.4-3.9), p=0.001	Parks CG et al, 2004
		-308A	2.6 (1.77−3.83), p < 0.0001	Correa PA et al., 2005
		-238A	0.04 (0-0.28), p<0.0001	Correa PA et al., 2005
		-863A allele and -863A,-308G,238G haplotype	1.85 (1.21-2.83), p=0.009	Hirankarn N et al., 2007
TNFb	6p21	TNFB*2	RR=3.4, p<0.0001	Kim TG et al., 1996
			p<0.05	Takeuchi F et al., 2005
C4	6p21	AQ0	p<10^{-6}	Hartung K et al., 1992
IRF5	7q32	Intron -3835T (rs2004640)	p=4.4 x 10^{-16}	Graham RR et al., 2006
		TInCA haplotype from rs2004640, In/Del exon6, rs2070197, rs10954213	$\chi2$=24.457, p=5.72 x 10^{-8}	Kozyrev SV et al., 2007
MBL	10q11.2-q21	codon 54 B allele	1.406 (1.221-1.608), p=0.001	Lee YH et al., 2005
FAS	10q24	297C/416G	RR=5, p= 0.01	Horiuchi T et al., 1999
		-670A	p=0.004	Kanemitsu S et al., 2002
Bcl-2	18q21	Multiple alleles	$\chi2$=34.95, p=0.0001	Mehrian R et al., 1998
TYK2	19p13.2	Exon (V/F) 13430	p= 5.60x10^{-5}	Sigurdsson S et al., 2005
		Exon (I/S) 19107	p=1.50x10^{-4}	

[a] Odd ratio (OR) with 95% confidence interval, relative risk (RR) if available or x2 with p value.

at meiosis. The consistent linkage of a marker with disease in several affected families therefore identifies a susceptibility region in the genome. However, linkage signals detected in complex diseases may result from the combined effect of a cluster of genes of modest effects. False positive results can occur due to multiple testing since numerous numbers of genetic markers are assayed in a genome scan. Only loci with a highly significant threshold that can be confirmed by independent studies are believed to be true positive results. There are seven loci that reached the threshold for significant linkage to SLE (Table 1) and all loci (excepting for 17p13) have been confirmed in at least one independent cohort for linkage to SLE.[3,4]

2. Association Studies

A classical association study (or candidate gene study) is the assessment of alleles or haplotypes, or any DNA polymorphisms within a gene of interest in the difference of frequencies between affected patients and appropriate controls. A polymorphism observed with greater than expected frequency in affected individuals either suggests that the polymorphism being analyzed is the actual disease causing allele, or one located very closely to the responsible gene. Several candidate genes within confirmed susceptibility loci from Table 1 have been studied and found to be associated with SLE (Table 2). In addition, there are other SLE associated genes that were not confirmed in the SLE-linked region (Table 2).[3,4] Mostly, they are the genes involving in apoptosis such as *FAS, FASL, BCL2* and *PDCD-1*; the genes that play an important role in the regulation of the immune system including *HLA, CTLA-4, TNF, IL-10, STAT4* and *PTPN22*; the genes that are implicated

TABLE 3. SLE susceptibility genes identified from genome wide association studies.

Gene	Locus	SNP	P value	OR	Potential function
PTPN22[5]	1p13.2	rs2476601	0.0000052	1.53	Inhibition of lymphocyte activation
FCGR2A[5]	1q23	rs1801274	6.78E-07	0.74	Clearance of immune complexes
STAT4[6]	2q32.3	rs7574865	9E-14		Modulation of the production of cytokines in T cells and natural killer cells; activation of response of macrophages to interferon-α
PXK[5]	3p14.3	rs6445975	7.1E-09	1.25	Unknown effect of serine–threonine kinase
BANK1[7]	4q24	rs10516487	0.0064		B-cell adaptor protein
HLA[5,6]	6p21.32-33	rs2187668	3E-21		Presentation of antigen
		rs1270942	1.71E-51	2.35	
		rs3131379	1.71E-52	2.36	
IRF5[5,6]	7q32.1	rs10488631	2E-11		Production of interferon-α
		rs729302	2.00E-10	0.78	
		rs10279821	6.50E-09	0.8	
		rs12537284	3.61E-19	1.54	
c8orf13-BLK[6]	8p23.1	rs2736340	0.0000004	1.37	unknown (c8orf13)-Activation of B cells (BLK)
		rs13277113	0.00000008	1.39	
KIAA1542[5]	11p15.5	rs4963128	3.00E-10	0.78	Linkage disequilibrium with IRF7; production of type I interferon
ITGAM-ITGAX[6]	16p11.2	rs9937837	0.0000007	1.28	Adhesion of leukocytes to endothelial cells
		rs11574637	0.0000005	1.3	
ITGAM[5]	16p11.2	rs9888739	1.61E-23	1.62	Adhesion of leukocytes to endothelial cells
		rs1143678	8.50E-14	1.4	
		rs4548893	2.36E-12	1.34	
		rs1143679	1.70E-17	1.78	

in clearance of the immune complex such as *FcG Receptor, C4, MBL* and *CRP*. Since the breakthrough discovery of the central role of type I interferon in SLE pathogenesis from expression microarray, candidate genes within type I interferon (IFN) pathway have been proven to be significantly associated with SLE risk such as *TYK2* and *IRF5*. Nevertheless, many of the genes associated with SLE have not been confirmed and remain controversial.

Even if genome-wide linkage analysis using microsatellite markers has been successful in the identification of numerous complex disease loci, high-density single-nucleotide polymorphism (SNP) maps can provide greater information. In addition, most studies in a candidate gene approach using a few single nucleotide polymorphisms (SNPs) do not cover the whole gene or relevant haplotype block. With the development of genotyping platforms that permit analysis of hundreds of thousands of SNPs, it is now possible to apply this principle of indirect association to the whole genome rather than just candidate genes or candidate linkage regions. Recently, three genome wide association studies (GWAS) in Caucasian patients with SLE from international collaborations have been reported.[5-7] Results from these studies confirm known candidate genes including *HLA, FCGR, PTPN22, STAT4* and *IRF5*. Interestingly, several new candidate genes have been discovered as summarized in Table 3. For instance, the discovery of *BLK* and *BANK1* genes emphasize the crucial role of the B cell in the pathogenesis of SLE. Another novel

TABLE 4. Loci confirmed in interval-congenic mice.

Locus	Chromosome	Susceptible strain	Major autoimmune trait	Reference
Sle1	1	NZM2410 (NZW)	Antinucleosome Ab	Morel L et al., 2001; Boackle SA et al., 2001; Wandstrat AE et al., 2004
FCgnz1	1	NZM2328 (NZW)	Antichromatin Ab, Glomerulonephritis (GN)	Waters ST et al, 2004
-	1	129	Antinuclear Ab, GN	Bygrave AE et al., 2004
Nba2	1	NZB	B cell hyperactivity, autoAb	Atencio S et al., 2004; Wither JE et al., 2003
BxS1-4[a]	1	BXSB	Anti-dsDNA Ab, GN (4 overlapping intervals)	Haywood ME et al., 2004; Haywood ME et al., 2006
Sle2	4	NZM2410 (NZW, NZB)	B cell hyperactivity, incr. B1 cells	Xu Z et al., 2005
Adnz1	4	NZM2328	Glomerulonephritis	Waters ST et al., 2004
Lbw2	4	NZB	B cell hyperactivity	Haraldsson MK et al., 2005
Sle3/Sle5	7	NZM2420 (NZW)	T cell hyperactivity from hyperstimulatory antigen-presenting cells	Sobel ES et al., 2002; Zhu J et al., 2005
Nba5	7	NZB	Anti-gp70 Ab, GN	Kikuchi S et al., 2005
Sles1	17	NZW	Suppression of lupus-like disease	Subramanian S et al., 2005

[a] Four different congenic strains with chromosome 1 intervals containing one or more of the four Bxs loci

gene namely, *ITGAM* was also identified which is an adhesion molecule that regulates leukocyte adhesion to endothelial cells and may contribute to vasculitis in patients with SLE. Nevertheless, these are the studies restricted to only Caucasian patients, the exploration of SLE susceptibility genes in other populations are still required.

3. Genetic studies in mouse models

The studies in mouse models have been used to further understanding of the lupus. There are two mouse models of the lupus comprising of synthetic and spontaneous mouse models. The synthetic ones consist of both transgenic and knock out mouse models of various apoptosis genes implicated in SLE pathogenesis. For example, *Fas, FasL* and *Bcl2* were silenced or overexpressed, resulting in the deregulation of apoptosis. In addition to synthetic mouse models, there are classic spontaneous models, including the (NZB × NZW)F1 (or NZB/W) mouse and the congenic recombinant NZM2410 strain derived from this cross, the MRL/*lpr* mouse, and the BXSB/*yaa* mouse. These strains were subjected to linkage analysis in order to pinpoint the chromosomal regions linked with SLE susceptibility. So far, more than 50 loci have been localized. Some confirmed loci on chromosomes 1, 4, 7 and 17 have been shown in Table 4.[8] Results from mouse models are useful in identifying novel genes responsible for human SLE.

Other Genetic Studies in SLE

SLE is a complex disease which is mostly caused by multiple genes interacting with various environmental factors. A great effort has been put in to identify multiple susceptibility genes as reviewed above. However, it should be mentioned that a rare subset of SLE patients are caused by a single gene mutation. For example, a complete deficiency of early classical complement component e.g., *C1q, C1r, C1s, C2,* or *C4* as well as defective complement receptor function lead to defects in immune complex clearance and 75% of these genetic-defect patients develop SLE symptoms.[9] Similar to the lupus mouse model, a rare subset of SLE patients with mutation in Fas or FasL have been identified.[10] Interestingly, mutation in *DNASE1* and *TREX1* (another type of DNase) have been identified in SLE patients.[11,12] This fact underscores the importance role of DNase in clearing DNA from released nuclear material. Recently, another form of genetic polymorphism known as copy number variation (CNV) has been shown to also play a role in SLE pathogenesis. For example, a low copy number of C4 was shown to be associated with a low level of C4 protein in the serum of SLE patients and contributes as one genetic risk factor for SLE. Although this CNV in C4 is not the only factor that causes a disease like complete complement deficiency, since a low copy number of C4 can be found in healthy controls as well.[13] Low FcgammaRIIIb CNV associated with low protein expression has just recently been reported to play a part in SLE pathogenesis via the defective clearance of immune complexes.[14] More and more data have suggested that epigenetics especially DNA methylation in CD4+ T cells plays a role in SLE pathogenesis as well.[15]

Molecular genetic studies both in human and animal models have identified important genes that contribute to SLE pathogenesis. This knowledge has greatly improved our understanding of this complex disease and lead to a new hope of identifying a more effective therapy by targeting various key proteins especially the ones in IFN type I or B cell signaling pathway.

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