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ASSOCIATION OF UBE2L3 GENE POLYMORPHISM AND INTERLEUKIN 10 GENE POLYMORPHISM WITH SYSTEMIC LUPUS ERYTHEMATOSUS AMONG WOMEN POPULATION IN CHINA

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ABSTRACT

Objective: To investigate the association of UBE2L3 gene polymorphism and Interleukin 10 gene polymorphism with systemic lupus erythematous among woman population in china. **Methods:** A case—control study of 111 female SLE patients and 120 healthy individuals were designed. Genomic DNA of subjects were extracted and amplified by polymerase chain reaction (PCR). second generation/next generation sequencing technology was performed to determine the single nucleotide polymorphism(SNP) in UBE2L3(rs5754217) and IL10(rs3024505). **Results:** The loci of UBE2L3 rs5754217 show the frequency of genotype GT, GG, TT as follows (52.2% and 44.2%), (18.9% and 30%) and (28.8% and 25.8%) respectively in SLE case group and control group. The frequency of G and T allele as follows (45% and 55%) and (52.1% and 49.7%) respectively in SLE case group and control group. The P values, odds ratio and 95%CI of genotype GT (P=0.10, OR=1.38,95%CI= (0.824 – 2.322)) and genotype TT (P=0.30, OR=1.16,95%CI= (0.651 – 2.075)) are statically not significant(P>0.05). The P values, odds ratio and 95%CI of genotype GG (P=0.02, OR=0.54, 95%CI= (0.294 – 1.006)) are statically significant result (p<0.05). this result indicates that may be there is 0.54 times higher risk for SLE by genotype GG. The P values, odds ratio and 95%CI of allele G (P= 0.06, OR= 0.75, 95%CI = (0.522 – 1.087)) and allele T (P=0.06, OR=1.32, 95%CI = (0.919 – 1.912)) are statically not significant. Loci of IL10rs3024505 show the frequency of genotype CT, CC as follows (7.2% and 11.7%) and (92.8% and 88.3%) respectively in SLE case group and control group. The frequency of C and T allele as follows (96.4 and 94.2%) and (3.6% and 5.8%) respectively in SLE case group and control group. The P values, odds ratio and 95%CI of genotype CT (P=0.12, OR=0.58,95%CI= (0.236 – 1.460)) and genotype CC (P=0.12, OR=1.70,95%CI= (0.690– 4.224)) are statically not significant(P>0.05). The P values, odds ratio and 95%CI of allele C (P= 0.13, OR=1.65, 95%CI = (0.684 – 4.013)) and allele T (P=0.13, OR=0.60,95%CI = (0.249 – 1.461)) are statically not significant. **Conclusion:** The results suggest that Genotype "GG" of loci rs5754217 (UBE2L3) show a positive correlation with SLE case group as compared to control group. This indicates that the genotype "GG" of rs5754217 may increase the risk of SLE in Chinese women population.

KEYWORDS: systemic lupus erythematosus (SLE); ubiquitination proteasome pathway; UBE2L3; Interleukins; IL 10; single nucleotide polymorphisms (SNPs).

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of autoantibodies to the components of the cell nucleus, complement system activation, immune complex deposition and multi-organ damage with broad spectrum of clinical presentations such as inflammation, vasculitis and vasculopathy.[2,3,4] etiology of SLE related to both genetic and environmental components with strong predominance to female sex as compared to male sex with female to male ratio of 9:1, indicating the role of hormones in etiology of SLE. development of SLE in sibling is 30 time higher as compared to individuals without an affected sibling.[2] Higher rate of concordance for SLE in monozygotic twins as compared to dizygotic twins.[4,5] Even though the exact etiology of SLE is unknown.[4] Ubiquitin is a part of the Ubiquitin Proteasome Pathway (process is known as ubiquitination), that is responsible for the degradation of more than 80% of normal and abnormal intracellular proteins by covalent ligation to ubiquitin, with the help of enzymes (E1,E2,E3) that link the chains of the polypeptide co-factors and mark them for degradation, transport or assembly into complexes.[2,3,5,40] UBE2L3 (Also known as UBCH7) is a member of E2 ubiquitin-conjugating enzyme family of Ubiquitin Proteasome Pathway and UBE2L3 is located at chromosome 22q11.21.[6,30] Recent studies show that UBE2L3 is critical for LUBAC function and is the preferred E2 for LUBAC (linear ubiquitin chain assembly complex) in vivo. The amount of UBE2L3 exerts the rate-limiting control over LUBAC-mediated NF-kB activation.[55]

activation of NF-κB leads to activation of T cells and B cells in SLE. Abnormal NF-κB signaling lead to the secretions of auto reactive T cells, which have a critical role in SLE and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases.[56] Recent genetic study of wang et al and genome-wide association studies (GWAS) shows that the genetic variants of *UBE2L3* is strongly associated with systemic lupus erythematosus (SLE) in individuals of European, Asian, African-American ancestry and weaker association evidence was also observed in the Hispanic and Gullah populations.[6,29,30] hence the association between UBE2L2 Gene and SLE depends on geographical variations, sample size etc.

Cytokines are small secreted proteins (molecular mass 5 – 20 kDa) released by cells. cytokines have a specific effect on signaling between cells.[57,58] Interleukin is one type of cytokine, which is made up of one type of leukocyte and acting on other leukocytes but the Production of interleukins are now known not to be confined only to leukocytes. interleukins are divided in to 37 types and nomenclature of interleukin family based on sequence homology, receptor chain similarity and functional property.[57,59] IL-10 is an anti-inflammatory factor that is important for the regulation of immune response. which is produced mainly by T cells, monocytes, B cells, NK cells, macrophages, Dendritic Cells and Mast cells. IL10 involves in leukocyte infiltration, inflammation, and skin disorders.[75-79] The gene for IL10 mainly localized to chromosome number 1 at 1q31-32 region, a genomic region associated with susceptibility to SLE.[62] IL-10 inhibits T cell function by suppressing the expression of pro -inflammatory cytokines such as TNF, IL-1, IL-6, IL-8, and IL-12.[63,64] It also inhibits antigen presenting cells by down regulating major histocompatibility complex class II (MHC-II).[65] IL-10 promotes B-cell-mediated functions, survival, proliferation, differentiation, and antibody production.[66] Hence, increased production of IL-10 could thus explain B cell hyperactivity and autoantibody production, two main features of the immune dysregulation in SLE. Recent studies show that cultured peripheral blood mononuclear cell (PBMC) from SLE patients spontaneously produce high levels of IL-10, also first degree relatives of SLE patients produce high levels of IL-10 compared with unrelated controls. IL10 production by monocytes, B cells in healthy members of multi-case families with SLE was significantly higher as compared to unrelated controls. Recent study of Andrew w gibson about "the role of IL 10 in autoimmune pathology" shows that several studies among Caucasians, Chinese, African-Americans and Mexican population found the association between IL 10 gene with SLE, on the other hand, several studies among Caucasians, Chinese population did not found the association between IL10 gene and SLE.[60] hence the association between IL 10 Gene and SLE depends on geographical variations, sample size etc.

The above data's shows that there is geographical variation in both genes (UBE2L3 and IL10) for the susceptibility of SLE. Hence we conduct this study to investigate the role of UBE2L3 (rs5754217) and IL10 (rs3024505) in SLE and provide further reference for future research.

In this study, we used rs3024505(IL10) and rs5754217(UBE2L3) Single nucleotide polymorphism (SNPs) determined by second generation/ next generation gene sequencing technology to know the association between SLE with UBE2L3 gene and IL10 gene. it's the most commonly used illumina sequencing technology and the cost is lower, the accuracy rate is higher and speed is faster.[1]

MATERIALS AND METHODS

Research objects

111 cases of female SLE patients were collected from the outpatient and inpatient departments of zhongnan hospital, Wuhan University and People's Hospital, wuhan university in between May 2015 – May 2016 and the average age of patient was (37.02±14.76) years old. All patients met the revised 1997 American College of Rheumatology criteria for the diagnosis of SLE. 120 normal subjects were selected as control group, they examined and screened for excluding SLE, Sjogren's syndrome, rheumatoid Arthritis, dermatomyositis and other autoimmune diseases and the average age was (42.2±15.75) years old. The subjects in this study were not related to each other. There was no significant difference in age distribution between two groups ($P > 0.05$). Informed consent was obtained from all subjects at each site, and the study was approved by ethics committee of zhongnan hospital, Wuhan university. 5ml of peripheral venous blood sample were collected for DNA extraction and genotyping.

(1) specimen collection: 5mL of peripheral venous blood were collected, specimen stored in the refrigerator at -20℃ for 3 days for DNA extraction. DNA extracted by using blood genomic DNA extraction kit (TIANGEN Biotech Co. Ltd, DP318 and BioTek Co. Ltd, DP6101) and preserved at -20℃.

(2) PCR amplification of target gene: The primers used for PCR as follows: rs3024505 upstream primer 5' –AGCCCCGTGACTTAGAGG- 3'and Downstream primer 3' –TCCCACTTCCTTCCTATGG- 5'; rs5754217 upstream primer 5' -TTCATGCTGCTGCTAAGGT- 3' and downstream primer 3' -TCAGGGAAGGAACGAATAC- 5' Synthesized by Wuhan DABOND Biotechnology Co. Ltd.

(3) PCR amplification conditions: 94℃ pre-denaturation for 3 min, 94℃ denaturation for 3 sec, 56℃ annealing for 30 sec, 72℃ extension for 30 sec, 35 cycles, last extension of 72℃ for 5min.

(4) Agarose gel electrophoresis: The amplified PCR product was electrophoresed on 2% agarose gel and the amplified results were verified, the PCR products were sequenced by Wuhan Dabond Biotechnology Co. Ltd. The results of sequencing are shown in Fig 1 – 2.

(5) Statistical analyses: The genotype and allele frequencies were statistically analyzed by direct counting method based on the results of the second-generation sequencing, Hardy-Weinberg (H-W) equilibrium test and Comparison of genotype and allele frequency differences between the two groups, done by Using software SPSS 16.0 for $\chi 2$ test. Odds Ratio (OR values) and 95% confidence intervals (95%CI) were used to evaluate differences in genotype frequencies and allele distribution. P <0.05 was statistically significant.

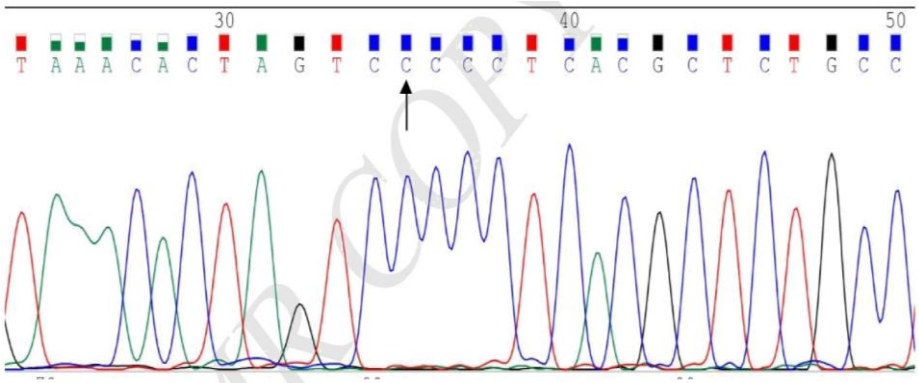

Figure 1: Result of sequenced PCR product of loci rs3024505 (contain C/T allele).

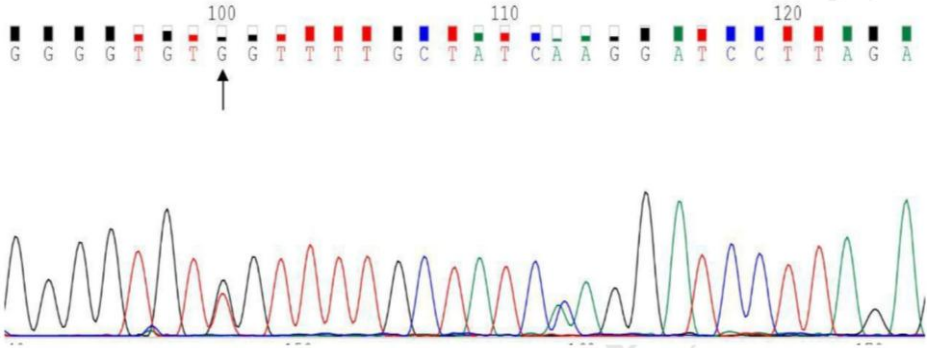

Figure 2: Result of sequenced PCR product of loci rs5754217 (contain G/T allele).

RESULT

(1) Hardy-Weinberg equilibrium test was performed on both experimental group and control group. The distribution of locus in both experimental and control groups were consistent with Hardy-Weinberg equilibrium test (P > 0.05), which Indicates that the samples selected in this experiment have a good representation.

(2) The genotype and allele frequency of both rs3024505 and rs5754217 are shown in table 1. The loci of UBE2L3 rs5754217 shows the frequency of genotype GT, GG, TT as follows (52.2% and 44.2%), (18.9% and 30%) and (28.8% and 25.8%) respectively in SLE case group and control group. The frequency of G and T allele as follows (45% and 55%) and (52.1% and 49.7%) respectively in SLE case group and control group. The P values, odds ratio and 95%CI of genotype GT (P=0.10, OR=1.38, 95%CI= (0.824 – 2.322)) and genotype TT (P=0.30, OR=1.16, 95%CI= (0.651 – 2.075)) are statically not significant (P>0.05). The P values, odds ratio and 95%CI of genotype GG (P=0.02, OR=0.54, 95%CI= (0.294 – 1.006)) are statically significant result (p<0.05). this result indicates that may be there is 0.54 times higher risk for SLE by genotype GG. The P values, odds ratio and 95%CI of allele G (P= 0.06, OR= 0.75, 95%CI = (0.522 – 1.087)) and allele T (P=0.06, OR=1.32, 95%CI = (0.919 – 1.912)) are statically not significant.

Loci of IL10 rs3024505 shows the frequency of genotype CT, CC as follows (7.2% and 11.7%) and (92.8% and 88.3%) respectively in SLE case group and control group. The frequency of C and T allele as follows (96.4 and 94.2%) and (3.6% and 5.8%) respectively in SLE case group and control group. The P values, odds ratio and 95%CI of genotype CT (P=0.12, OR=0.58, 95%CI= (0.236 – 1.460)) and genotype CC (P=0.12, OR=1.70, 95%CI= (0.690 – 4.224)) are statically not significant (P>0.05). The P values, odds ratio and 95%CI of allele C (P= 0.13, OR=1.65, 95%CI = (0.684 – 4.013)) and allele T (P=0.13, OR=0.60, 95%CI = (0.249 – 1.461)) are statically not significant

Table 1: UBE2L3 gene polymorphism and IL10 gene polymorphism in SLE case group and control group.

Loci	Genotype/Allele	SLE Cases (%)	Control group (%)	P value	OR(95% CI)
rs57541	GT	58(52.2)	53(44.2)	0.10(>0.05)	1.38(0.824 – 2.322)
	GG	21(18.9)	36(30)	0.02(<0.05)	0.54(0.294 – 1.006)
	TT	32(28.8)	31(25.8)	0.30(>0.05)	1.16(0.651 – 2.075)
	G	100(45.0)	125(55.0)	0.06(>0.05)	0.754(0.522 – 1.087)
	T	122(52.1)	115(47.9)	0.06(>0.05)	1.32(0.919 – 1.912)
rs3024505	CT	8(7.2)	14(11.7)	0.12(>0.05)	0.58(0.236 – 1.460)
	CC	103(92.8)	106(88.3)	0.12(>0.05)	1.70(0.690 – 4.224)
	C	214(96.4)	226(94.2)	0.13(>0.05)	1.65(0.684 – 4.013)
	T	8(3.6)	14(5.8)	0.13(>0.05)	0.60(0.249 – 1.461)

DISCUSSION

Systemic lupus erythematous

Systemic lupus erythematous (SLE) is a prototypic autoimmune disease characterized by the production of autoantibodies to the components of the cell nucleus, complement system activation, immune complex deposition and multi-organ damage. Major organs involved in SLE are skin, kidneys, lungs, heart and the central nervous system with broad spectrum of clinical presentations.[2,3,4] etiology of SLE related to both genetic components (HLA-DR,PTPN22, STAT4, IRF5, BLK, OX40L, FCGR2A, BANK1,SPP1, IRAK1, TNFAIP3, C2, C4, CIq, PXK) and environmental components (ultraviolet light and infectious or endogenous viruses or viral-like elements (especially Epstein–Barr virus)) with strong predominance to female sex as compared to male sex with female to male ratio of 9:1. Child bearing age group of females have higher risk to get SLE, indicating the role of hormones in etiology of SLE and certain drugs also cause SLE known as DIL(drug induced lupus), particularly those drugs that are metabolized by acetylation such as procainamide and hydralazine, but the pathogenesis of DIL is unknown. development of SLE in sibling is 30 time higher as compared to individuals without an affected sibling.[2] Familial aggregation and a higher rate of concordance for SLE in monozygotic twins (25% - 50%) than in dizygotic twins (5%).[5,4] There are marked disparities in SLE incidence and prevalence around world, SLE prevalence varies depends on ethnic and geographical variations.[6] The prevalence of SLE ranges from 31 to 70 cases per 100,000 persons among Chinese populations[7] and from 7 to 71 cases per 100,000 persons in European populations and lupus nephritis is more prevalent in Chinese populations than in European populations.[6] in pathogenesis of SLE, Immune responses against endogenous nuclear antigens are characteristic. Autoantigens released by apoptotic cells are presented by dendritic cells to T cells, leading to the activation of T cell. Activated T cells help B cells to produce antibodies to these self-constituents by secreting cytokines such as interleukin 10 (IL10) and IL23 and by cell surface molecules such as CD40L and CTLA-4. In addition to this antigen-driven T cell-dependent production of autoantibodies, recent data support T cell-independent mechanisms of B cell stimulation via combined B cell antigen receptor (BCR) and toll-like receptors (TLR) signaling. The pathogenesis of SLE involves a multitude of cells and molecules that participate in apoptosis, innate and adaptive immune responses. There have been significant improvements in long term survival, but patients with SLE still have higher risks of premature mortality compared to the general population. Factors contributing to mortality in SLE includes major organ involvement, especially nephropathy, thrombosis, accelerated atherosclerosis, and an increased risk of cancer.[2,8]

Ubiquitin Proteasome Pathway

Irwin Rose along with Avram Hershko, Aaron Ciechanover was awarded Nobel Prize in Chemistry in 2004 for the discovery of ubiquitin - mediated protein degradation.[9,10,11,12] During the past two decades, the Ubiquitin Proteasome Pathway (UPP) has taken the center stage in our understanding of the control of protein turnover. Ubiquitin is small protein (76 -amino-acid residue and molecular mass of 8.5 kDa) found only in eukaryotes. Ubiquitin is a part of the UPP, that is responsible for the degradation of more than 80% of normal and abnormal intracellular proteins by covalent ligation to ubiquitin with the help of enzymes (E1, E2, E3) that link the chains of the polypeptide co-factors and mark them for degradation.[2,3,5] This tagging process leads to their recognition by 26S proteasome (molecular mass of 700 kDa), a very large multi-catalytic protease complex, that degrades ubiquitinated proteins Into small peptides known as proteolytic action. These Proteasomal degradation removes denatured, misfolded, damaged or improperly translated proteins from cells. The products resulting from this degradation has different sequences, lengths and biological functions.[4,13] Ubiquitination also involved in non-proteolytic action, such as receptor internalization and down regulation; assembly of multi-protein complexes; intracellular trafficking; inflammatory signaling; autophagy; DNA repair.[12,14,15,16] Ubiquitin covalently coupled to lysine residues on target proteins by a cascade of enzymatic reactions carried out by activating (E1), conjugating (E2) and ligating (E3) enzymes. These three types of enzymes act sequentially. Ubiquitin is first activated by E1 and is then transferred into an E2 conjugating enzyme. Subsequently, E3 Ubiquitin ligases interact at the same time with Ubiquitin - loaded E2 and the substrate protein, mediate isopeptide bond formation between the C terminus of Ubiquitin and substrate lysine.[17] These actions leads to formation of Ubiquitin chains.it can be either monoubiquitin or

polyubiquitin chains of variable length with any of the seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 or Lys63) or the amino-terminal Met (Met1). which can be further degraded into small amino acids with the help of Deubiquitinating enzymes (DUB) and used for the synthesis of new proteins.[18,19,20] It is believed that polyubiquitin chains linked through lysine at position 48 of ubiquitin (Lys 48) target protein substrates for degradation by the proteasome, whereas polyubiquitin chains of alternative linkages (such as Lys 63) carry out signaling functions independent of proteolysis. In addition, some proteins such as the histones H2A and H2B are modified by a single ubiquitin that has a non-degradative regulatory function.[12,14,15,16] monoubiquitin or polyubiquitin chains serve as three dimensional signals, that are recognized by different types of ubiquitin-binding domain (UBD). The human proteome contains two ubiquitin E1 enzymes, about 50 E2 enzymes, 600 E3 enzymes, 90 DUB enzymes and 20 types of UBD, indicating the central role of ubiquitin in cell regulation. E3 enzymes are categorized into two classes, one containing a HECT (Homologous to the E6-AP C-Terminus) domain and the other containing a RING or RING-like (for example, U-box or PHD) domain. Both HECT- and RING-domain E3 enzymes have key roles in regulating immune responses.[21]

The pathological states associated with ubiquitin system in relation to human disease can be classified into two groups: (a) those that result from loss of function leads to mutation in a ubiquitin system enzyme or target substrate that result in stabilization of the later, and (b) those that result from gain of function leads to abnormal or accelerated degradation of the protein target. deregulation of ubiquitination has broad consequences. It may lead to aberrant activation or deactivation of pathways, improper or insufficient assembly of protein complexes, accumulation of misfolded proteins or mis-localization of proteins from their functional cellular compartment. Each of these changes can be detrimental to cell functioning leads to many disorders such as malignancies, neurodegenerative diseases and possible systemic autoimmunity.[12,14,15,16,22] Better understanding of Ubiquitin Proteasome Pathway and identification of the components involved in the degradation of key regulatory proteins has led to the development of mechanism-based therapeutics in various diseases.[23-25] A growing body of evidence suggests that targeting theUbiquitin Proteasome Pathway is an attractive approach to treat inflammatory and autoimmune diseases.[26-28]

UBE2L3

UBE2L3 (Also known as UBCH7) is a member of E2 ubiquitin-conjugating enzyme family of Ubiquitin Proteasome Pathway and UBE2L3 is located at chromosome 22q11.21. Recent genetic study of wang et al and genome-wide association studies(GWAS) shows that the genetic variants of *UBE2L3* is strongly associated with systemic lupus erythematosus (SLE) in individuals of European, Asian, African-American ancestry and weaker association evidence was also observed in the Hispanic and Gullah populations.[6,29,30] hence the association between UBE2L2 Gene and SLE depends on geographical variations, sample size etc.UBE2L3 also associated with multiple autoimmune diseases, Crohn's disease (CD), celiac disease (CeD) and rheumatoid arthritis (RA), psoriasis (PS), inflammatory bowel disease (IBD), Hashimoto's thyroiditis(in Han Chinese population),[30,37] its cellular functions remained unknown, Because of E2 enzymes appeared to be substitutable in ubiquitination assays. E2 has been demonstrated to participate in the ubiquitination of p53,[42] c-Fos and the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) precursor p105 *in vitro*.[38,39] Recent studies have further revealed that *UBE2L3* is involved in cell proliferation.[40] Additionally, UBE2L3 interacts with Triad3A (RNF216), which can regulate the degradation of Toll-like receptors (TLR).[43] In SLE, signaling through the endosomal TLR is thought to be an important pathway for the generation of interferon-α (IFN-α).[44] E2 enzyme has function with only selected E3 ligases in vivo and have a critical role in determining ubiquitin (Ub) chain type,[41] Because E2 enzymes act as ubiquitin shuttles, the kinetics transfer of ubiquitin from E2 to substrate in the case of RING E3 ligases, or HECT enzymes of E3, might limit the speed of polyubiquitin chain formation.[18] But UBE2L3 is incapable of conjugating ubiquitin into free lysine and directly into the target substrate and necessary for standard RING E3 ligases.[45] therefore UBE2L3 is restricted to HECT-like E3s, dual RING E3 ligases with a RBR motif (RING-in-between-RING) and seven of the nine HECT E3 ligases.[46]

Linear ubiquitination, which involves sequential bonding of a ubiquitin moiety into the Met-1 (M1) residue of ubiquitin[47] and is mediated by E3 complex LUBAC (linear ubiquitin chain assembly complex). LUBAC composed of HOIL-1, HOIP, and Sharpin.[48,49,50,51] LUBAC has pivotal role in inflammation, through crucial regulation of NF-κB and it forms linear (M1) ubiquitin chains on NEMO(NF-kB Essential Modulator) to activate the IKK(inhibitor of kappa B Kinase) complex. Deficiency of HOIL-1 or Sharpin inhibits phosphorylation and degradation of the NF-kB sequestration protein IkBa, leading to impaired activation of NF-kB. HOIL-1-deficient mice have defective NF-kB responses.[48,52] and rare human loss-of-function mutations in HOIL-1 led to defective TNF signaling and abnormal IL-1 response.[53] Sharpin deficiency diminishes NF-kB activation, while increasing pro-inflammatory TNF-induced cell death, led to chronic proliferative dermatitis in Sharpin-deficient mice.[50] Thus LUBAC has been shown to be critical for NF-kB activation downstream of the TNF receptor 1 (TNFR1) and CD40 with plasma blast and plasma cell expansion in SLE. Presence of HOIP in B cells was necessary for CD40 signaling.[52,54] HOIL-1 and HOIP are both RBR

E3 ligases. Recent studies show that UBE2L3 is critical for LUBAC function and is the preferred E2 for LUBAC in vivo. The amount of UBE2L3 exerts rate-limiting control over LUBAC-mediated NF-kB activation, and together UBE2L3 and LUBAC play an important role in late-phase of NF-kB activation[55] and activation of NF-κB leads to activation of T cells and B cells in SLE.NF-κB signaling needs for the proper maturation and development of lymphocytes and dendritic cells. Abnormal NF-κB signaling lead to the secretions of auto reactive T cells, which have a critical role in SLE and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases.[56] Hence the regulation on UBE2L3 can provides novel strategies for the prevention and treatment of SLE and other disease related to UBE2L3.

Cytokines and Interleukins

Cytokines are small secreted proteins (molecular mass 5 – 20 kDa) released by cells. cytokines have a specific effect on signaling between cells. cytokine effects includes embryonic, changes in cognitive functions and progression of the degenerative processes of aging development, disease pathogenesis, non-specific response to infection, specific response to antigen and cytokines are part of stem cell differentiation, allograft rejection, vaccine efficacy.[57,58] There are many types of Cytokines (chemokines, interferons, interleukins, lymphokines, tumour necrosis factor….etc) based on the cells present in cytokines and their interaction with other cells. Interleukins are discovered in 1977, since the discovery of interleukins, there are around 200,000 articles published in relation with interleukins. interleukins are made up of one type of leukocyte and acting on other leukocytes but the Production of interleukins is now known not to be confined only to leukocytes. interleukins are secreted proteins, which binds to their specific receptors and play their roles in signaling among leukocytes. interleukins are divided in to 37 types and nomenclature of interleukin family based on sequence homology, receptor chain similarity and functional property.[57,59]

Interleukin 10

IL-10 is an anti-inflammatory factor that is important for the regulation of immune response. which is produced mainly by T cells, monocytes, B cells, NK cells, macrophages, Dendritic Cells and Mast cells. IL10 involves in leukocyte infiltration, inflammation, and skin disorders.~IL-10 is secreted as a homodimer that consists of 2 subunits (178 amino acids with a molecular weight of 18 kda). The receptor complex for IL-10 is made up of 2 IL-10R1 and 2 IL-10R2 chains.[75-79] The gene for IL10 mainly localized to chromosome number 1 at 1q31-32 region. Studies shows that cluster of genes for IL*19*, *IL20* and *IL24* along with IL10 also located in 1q31-32 region, a genomic region associated with susceptibility to SLE. several genome scans have also identified the regions 1q21-23, 1q42-44 contain susceptibility loci for SLE and other autoimmune diseases. Recent genome-wide association studies(GWAS) in European ancestry show that single nucleotide polymorphism (SNP) in IL10 at loci rs3024505 have been associated with increased risk for SLE, Crohn's disease (CD) and ulcerative colitis (UC) and decreased risk for type 1 diabetes mellitus.[60,61] The diseases caused by IL10 can be further subdivided into two types: (a) diseases with IL-10 overproduction, such as melanoma, systemic lupus erythematosus, systemic sclerosis, lymphomas, etc. and (b) diseases with IL-10 deficiency such as Crohn's disease, ulcerative colitis, psoriasis, rheumatoid arthritis, etc.[62] IL-10 inhibits T cell function by suppressing the expression of pro-inflammatory cytokines such as TNF, IL-1, IL-6, IL-8, and IL-12.[63,64] It also inhibits antigen presenting cells by down regulating major histocompatibility complex class II (MHC-II) and B7 expression.[65] IL -10 promotes B-cell-mediated functions, survival, proliferation, differentiation, and antibody production.[66] Hence, increased production of IL-10 could thus explain B cell hyperactivity and autoantibody production, two main features of the immune dysregulation in SLE. Recent studies show that cultured peripheral blood mononuclear cell (PBMC) from SLE patients spontaneously produce high levels of IL-10, also first degree relatives of SLE patients produce high levels of IL-10 compared with unrelated controls. IL10 production by monocytes, B cells in healthy members of multi-case families with SLE was significantly higher as compared to unrelated controls. These studies indicate the role of IL10 in pathogenesis or disease susceptibility or severity for SLE. other study shows that high level of IL10 results in earlier disease onset or exacerbation of disease rather than protection. Genetic polymorphisms that regulate IL-10 production represent genetic risk factors for autoimmune diseases, but the underlying molecular mechanism remain unknown or less characterized.[60,61,67,73] several studies suggest that CD4+ICOS+FoxP3+T cells also have the capability to produce greater amount of IL-10 among the circulating CD4+T cell pool and also significant positive correlation between the frequency of CD4+ICOS+ FoxP3+T cells and the serum level of IL-10 in SLE patients.[62,74] there are geographical variations in association of IL10 with SLE, but most of the data's and studies suggest that IL10 has a positive correlation with pathogenesis of SLE. According to ishida et al study about IL10 role in murine lupus, reports shows that continuous administration of anti-IL-10 antibodies in the murine lupus model in New Zealand delayed the onset of autoimmunity and improved the survival rate from 10 to 80%,[72] it indicates the promising the findings of anti-IL-10 monoclonal antibody for the prevention and treatment of SLE patients and other diseases related to IL 10.[61] Recent study of Andrew w gibson about "the role of IL 10 in autoimmune pathology" shows that several studies among Caucasians, Chinese, African-Americans and Mexican population found the association between IL 10 gene with SLE, on the other hand, several studies among Caucasians, Chinese population did not found the

association between IL10 gene and SLE.[60] hence the association between IL 10 Gene and SLE depends on geographical variations, sample size etc.

In this study, the result shows that the frequency of "GG" genotype of loci rs5754217 (UBE2L3) were statically significant (P=0.02, OR = 0.54, 95%CI = (0.294 – 1.006). This indicates that there is positive (0.54 times) correlation between "GG" genotype of loci rs5754217 with SLE case group (women population in china) as compared to control group(women population in china). other genotype and allele of both rs5754217 (UBE2L3) and rs3024505 (IL 10) are statically not significant among women population in china.

CONCLUSION

The main conclusions of this study as follows: The objective of this study was to investigate the association of UBE2L3 gene polymorphism and Interleukin 10 gene polymorphism with systemic lupus erythematous among woman population in china. The results suggest that Genotype "GG" of loci rs5754217 (UBE2L3) show a positive correlation with SLE case group as compared to control group. This indicates that the genotype "GG" of rs5754217 may increase the risk of SLE in Chinese women population. This result provides a new way for later researches in future. Because of the small sample size and narrower geographical range, the results need to be confirmed by more studies.

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