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Genetic Risk Factors in Lupus Nephritis and IgA Nephropathy – No Support of an Overlap

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Abstract

Background: IgA nephropathy (IgAN) and nephritis in Systemic Lupus Erythematosus (SLE) are two common forms of glomerulonephritis in which genetic findings are of importance for disease development. We have recently reported an association of IgAN with variants of *TGFB1*. In several autoimmune diseases, particularly in SLE, *IRF5*, *STAT4* genes and *TRAF1-C5* locus have been shown to be important candidate genes. The aim of this study was to compare genetic variants from the *TGFB1*, *IRF5*, *STAT4* genes and *TRAF1-C5* locus with susceptibility to IgAN and lupus nephritis in two Swedish cohorts.

Patients and Methods: We genotyped 13 single nucleotide polymorphisms (SNPs) in four genetic loci in 1252 DNA samples from patients with biopsy proven IgAN or with SLE (with and without nephritis) and healthy age- and sex-matched controls from the same population in Sweden.

Results: Genotype and allelic frequencies for SNPs from selected genes did not differ significantly between lupus nephritis patients and SLE patients without nephritis. In addition, haplotype analysis for seven selected SNPs did not reveal a difference for the SLE patient groups with and without nephritis. Moreover, none of these SPNs showed a significant difference between IgAN patients and healthy controls. *IRF5* and *STAT4* variants remained significantly different between SLE cases and healthy controls. In addition, the data did not show an association of *TRAF1-C5* polymorphism with susceptibility to SLE in this Swedish population.

Conclusion: Our data do not support an overlap in genetic susceptibility between patients with IgAN or SLE and reveal no specific importance of SLE associated SNPs for the presence of lupus nephritis.

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Introduction

Several common gene variations have recently been shown to associate with different autoimmune diseases, particularly Systemic Lupus Erythematosus (SLE). Some nucleotide polymorphisms (SNPs) have been shown to associate with single autoimmune disease, while other SNPs associate with several diseases. Interferon regulatory factor 5 *(IRF5)* polymorphism has been shown to be a risk factor for the development of SLE [1,2,3,4,5], rheumatoid arthritis (RA) [6,7,8,9], multiple sclerosis (MS) [10], Sjögren's syndrome [11] and inflammatory bowel disease [12]. Signal transducers and activator of transcription 4 *(STAT4)* and TNF receptor-associated factor 1-Complement component 5 (*TRAF1-C5*) polymorphisms have been found to associate with both SLE and RA [13,14,15,16]. Recently we reported that transforming growth factor-β1 *(TGFB1)*, an important cytokine gene, is in association with IgA nephropathy (IgAN) [17].

Immunological and biochemical similarities between SLE and IgAN demonstrate a direct link to impaired immune function in both diseases [18]. Patients with lupus nephritis and IgAN both have circulating immune complexes and display anti-C1q antibodies, which might point to certain pathogenic similarities in these glomerular disorders [18,19]. Moreover, lupus nephritis and IgAN are both chronic renal diseases that are classified in the "predominant" inflammatory group, based on morphological similarities [20,21]. We hypothesized that it may be an overlap in genetic susceptibility between lupus nephritis and IgAN and that there could be specific genetic makers associated to the development of nephritis in SLE patients.

To test this hypothesis we compared the genotype, allelic and haplotype frequencies from *IRF5*, *STAT4* and *TRAF1-C5* polymorphisms between IgAN patients and healthy controls, and SLE patients with and without nephritis, from *TGFB1* polymorphisms between SLE patients and healthy controls.

Materials and Methods

Patients and healthy subjects

Two cohorts of patients with SLE or IgAN, altogether 1252 individuals, were included in the present study. The cohort of patients with SLE, consisted of 272 SLE patients, all self-reported Caucasians from 18 to 80 years of age (mean age 45±14 years). 106 SLE patients had biopsy proven nephritis (39%) and 166 SLE patients had no clinical or laboratory signs of nephritis (61%). The control group for SLE patients consisted of 307 healthy age-matched individuals from the same population in Sweden, who were 17 to 70 years old, mean age 44±13 years.

In the IgAN cohort, there were altogether 673 DNA samples, of which 196 samples were obtained from patients with biopsy-proven IgAN, all self-reported Caucasians, and 477 samples were collected from gender- and age matched healthy controls from the same population in Sweden. Patients with Henoch-Schönlein purpura or with other forms of glomerulonephritis and individuals with self-reported non-Caucasian ancestry were excluded in our study. All patients gave written informed consent and the study was approved by the Ethics Committee of the Karolinska University Hospital, Stockholm, Sweden.

DNA extraction, selection of genetic markers, and genotyping

DNA was extracted from EDTA blood samples (5–10 ml) by the "salting out" method, as described elsewhere [22]. The SNPs were selected because they had previously been shown to be associated with SLE [5,14,15], RA [9,13,15], or with IgAN [17]. The SNPs were genotyped by fluorescent single base extension using the multiplex SNPstream system (Beckman Coulter Inc) or by TaqMan allelic discrimination assay (Applied Biosystems, Foster City, U.S.A) (Table 1). All analyzed SNPs were in Hardy-Weinberg equilibrium and the average positive rate of the genotype detection was 97.2%. DNA samples with poor performances in genotyping (<95% successful genotypes) were excluded from the statistical evaluation.

Statistical analysis

To assess genotype, allele and haplotype frequencies, Pearson Chi-square and/or Fisher's Exact Tests were performed when appropriate with PASWStatistics 18.0 Software. Haplotype analysis was carried out by HaploView [23]. Power calculation was performed for two-tail or one-tail tests when appropriate for 5% threshold of significance.

Results

IRF5, *STAT4* and *TRAF1-C5* polymorphisms did not show an association with susceptibility and/or severity of IgAN

We did not find an association with disease susceptibility for the investigated SNPs in *IRF5*, *STAT4* genes and *TRAF1-C5* locus, in both the co-dominant model and the reccessive/dominant model, comparing patients with IgAN and healthy controls. One *IRF5* SNP (rs12539741) showed a difference between genotype distribution in IgAN patients and healthy controls in males in the dominant C model ($p = 0.04$). However, this association was not significant after Bonferroni correction for multiple comparisons. Comparing the allele and haplotype frequencies, we did not observe any significant differences for the SNPs between IgAN patients and controls (Table 2).

TGFB1 polymorphisms did not show an association with susceptibility to SLE or to lupus nephritis

No significant differences in genotype distribution or allele frequencies were observed between SLE patients and healthy controls for four investigated SNPs from the TGFB1 gene. In addition, there was no significant difference between lupus nephritis and healthy controls in both the co-dominant model and the recessive/dominant model of genotype frequencies and allelic frequencies (Table 3). *TGFB1* polymorphisms in selected SNPs did not show an association with SLE or with lupus nephritis among SLE patients.

Genetic variations associated with susceptibility to SLE did not correspond to a specific association with lupus nephritis

To determine if SLE-related genetic variants associate specifically with nephritis in SLE patients, we genotyped up to nine variants from the *IRF5*, *STAT4* genes and the *TRAF1-C5* locus. We compared genotype frequencies in both the co-dominant

Table 1. Polymorphisms of *TGFB1*, *IRF5*, *STAT4* genes, *TRAF1-C5* locus in the study.

Gene	SNP	Position	Chromosome	Chromosome position	Alleles	Methods
STAT4	rs10181656	Intron 3	2q32.2-q32.3	183829287	C/G	TaqMan
IRF5	rs729302	Promoter	7q32	128356196	A/C	SNPstream
IRF5	rs4728142	Promoter	7q32	128361203	A/G	SNPstream
IRF5	rs2004640	The intron-exon border of exon 1B	7q32	128365537	G/T	SNPstream
IRF5	rs3807306	Intron 1	7q32	128367916	G/T	SNPstream
IRF5	rs10954213	3′ UTR	7q32	128376663	A/G	SNPstream
IRF5	rs11770589	Exon 10 3′ UTR	7q32	128376724	A/G	SNPstream
IRF5	rs2280714	3′flanking region	7q32	128381961	C/T	SNPstream
TRAF1-C5	rs3761847	5′ flanking region	9q33-q34	93307696	A/G	TaqMan
TGFB1	rs6957	Downstream 3′genomic region	19q13.1	46522446	C/T	TaqMan
TGFB1	rs2241715	Intron 1	19q13.1	46548726	G/T	TaqMan
TGFB1	rs1982073	Signal sequence of exone 1	19q13.1	46550761	C/T	TaqMan
TGFB1	rs1800469	Promoter	19q13.1	46552136	A/G	TaqMan

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Table 2. Allelic frequencies of *IRF5*, *STAT4* and *TRAF1-C5* polymorphisms in IgAN patients and controls.

Gene	SPNs	Association Allele	Control/Patient Ratio Counts	Control/Patient Frequencies	Chi Square	P Value*
STAT4	rs10181656	A	532:388, 206:178	0.578, 0.769	1.927	0.2
IRF5	rs729302	A	629:299, 257:123	0.678, 0.676	0.003	0.9
IRF5	rs4728142	G	505:425, 206:176	0.543, 0.539	0.015	0.9
IRF5	rs2004640	G	444:486, 169:207	0.477, 0.449	0.840	0.4
IRF5	rs3807306	G	455:471, 177:201	0.491, 0.468	0.574	0.4
IRF5	rs10954213	G	342:584, 135:241	0.369, 0.359	0.122	0.7
IRF5	rs11770589	G	475:453, 193:191	0.512, 0.503	0.093	0.8
IRF5	rs2280714	C	294:636, 112:266	0.316, 0.296	0.494	0.5
TRAF1-C5	rs3761847	C	739:197, 297:89	0.790, 0.769	0.651	0.4

*Uncorrected.

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model and recessive/dominant model between patients with lupus nephritis and SLE patients without nephritis. We detected no significant differences between these two groups. Moreover, there were no significant differences in allele frequencies of any investigated SNPs (Table 4). We found no differences in haplotype analyses in patients with SLE and healthy controls or between patients with lupus nephritis and SLE patients without nephritis.

Discussion

This is the first investigation to study the importance of *IRF5*, *STAT4* and *TRAF1-C5* gene polymorphisms in patients with IgAN. Our data show no evidence of an association of these genes with the development of IgAN or distinct risk alleles for lupus nephritis in this Swedish population.

According to recent findings, the *IRF5* gene is an important candidate gene in different chronic diseases, especially systemic diseases related to inflammation and autoimmunity. A meta-analysis which included 15 studies regarding *IRF5* gene polymorphism and SLE, confirmed the importance of rs2004640 for SLE susceptibility [4]. *TRAF1-C5* and *STAT4* polymorphisms have been shown to associate with RA and SLE, and also with some other autoimmune diseases [5,14,15].

Thus, one might speculate that susceptibility to IgA nephropathy may be due to common variations in *IRF5*, *TRAF1-C5* and *STAT4* genes. Our data do however not confirm this hypothesis. Since no single marker (for *IRF5*, *TRAF1-C5* and *STAT4*), no haplotype associations (for *IRF5*) were detected in patients with IgAN, it is reasonable to rule out a strong influence of these gene polymorphisms in IgAN development or disease progression. We noticed that we have almost 80% power to detect a 10% difference in minor allele frequency (MAF) in our cases and controls. However, due to limited sample size, we cannot exclude minor influences from the investigated gene polymorphism on IgAN, which may also differ in different populations. On the other hand, *TGFB1* polymorphisms were found previously in association with the susceptibility to IgAN [17] but did not show any association with SLE or lupus nephritis in the present study.

Table 3. Allelic frequencies of *STAT4*, *IFR5*, *TRAF1-C5* and *TGFB1* polymorphisms in SLE patients and controls.

Gene	SPNs	Association Allele	Control/Patient Ratio Counts	Control/Patient Frequencies	Chi Square	P Value*
STAT4	rs10181656	C	481:127, 343:201	0.791, 0.631	36.363	1.64E-09
IRF5	rs729302	C	124:248, 105:285	0.333, 0.269	3.722	0.0537
IRF5	rs4728142	G	206:166, 160:230	0.554, 0.410	15.708	7.39E-05
IRF5	rs2004640	G	181:191, 134:256	0.487, 0.344	16.048	6.17E-05
IRF5	rs3807306	G	177:195, 139:251	0.476, 0.356	11.182	8.00E-04
IRF5	rs10954213	G	131:241, 102:288	0.352, 0.262	7.364	0.0067
IRF5	rs11770589	A	195:177, 203:187	0.524, 0.521	0.01	0.9
IRF5	rs2280714	C	115:257, 87:303	0.309, 0.223	7.239	0.007
TRAF1-C5	rs3761847	A	334:258, 277:253	0.564, 0.523	1.946	0.2
TGFB1	rs6957	T	511:89, 453:91	0.852, 0.833	0.772	0.4
TGFB1	rs2241715	G	187:419, 164:378	0.309, 0.303	0.048	0.8
TGFB1	rs1982073	T	224:384, 198:344	0.368, 0.365	0.012	0.9
TGFB1	rs1800469	A	191:417, 162:378	0.314, 0.300	0.269	0.6

*Uncorrected.

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Table 4. Allelic frequencies of *STAT4*, *IFR5*, *TRAF1-C5* and *TGFB1* polymorphisms in lupus nephritis against SLE patients without nephritis.

Gene	SPNs	Association Allele	Lupus without nephritis/ Lupus nephritis Ratio Counts	Lupus nephritis/none-nephritis Frequencies	Chi Square	P Value*
STAT4	rs10181656	C	210:122, 133:79	0.633, 0.627	0.015	0.9
IRF5	rs729302	C	65:165, 40:120	0.283, 0.250	0.510	0.5
IRF5	rs4728142	G	97:133, 63:97	0.422, 0.394	0.306	0.6
IRF5	rs2004640	G	87:143, 47:113	0.378, 0.294	2.988	0.1
IRF5	rs3807306	G	87:143, 52:108	0.378, 0.325	1.167	0.3
IRF5	rs10954213	G	65:165, 37:123	0.283, 0.231	1.289	0.3
IRF5	rs11770589	G	113:117, 74:86	0.491, 0.462	0.314	0.6
IRF5	rs2280714	C	59:171, 28:132	0.257, 0.175	3.618	0.06
TRAF1-C5	rs3761847	A	154:166, 99:111	0.481, 0.471	0.049	0.8

*Uncorrected.

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There are immunological and biochemical similarities between lupus nephritis and IgAN, and both conditions are associated with immune complex formation and mesangial immune deposits. There are also a number of reports on patients with SLE who develop IgAN [24,25]. However, there was no overlap in genetic risk factors in the here studied genes between SLE and IgAN patients or any specific genetic variants detected comparing lupus patients with or without nephritis.

An association of *TRAF1-C5* locus with SLE was recently detected in a relatively small cohort [14]. However, our data did not show an association between *TRAF1-C5* polymorphism neither in SLE nor in IgAN.

In conclusion, the findings in the present study do not support an overlap in genetic susceptibility between Swedish patients with IgAN or SLE and reveal no specific importance of SLE associated SNPs for presence of lupus nephritis.

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Author Contributions

Conceived and designed the experiments: MTV IG SHJ LP. Performed the experiments: MTV ACS LP. Analyzed the data: MTV LP. Contributed reagents/materials/analysis tools: IG SL ES LW AF ACS SHJ LP. Wrote the paper: MTV IG SL ES AF ACS LTD SHJ LP.

References

1. Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, et al. (2006) A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nat Genet 38: 550–555.
2. Graham RR, Kyogoku C, Sigurdsson S, Vlasova IA, Davies LR, et al. (2007) Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. Proc Natl Acad Sci U S A 104: 6758–6763.
3. Kozyrev SV, Alarcon-Riquelme ME (2007) The genetics and biology of Irf5-mediated signaling in lupus. Autoimmunity 40: 591–601.
4. Lee YH, Song GG (2008) Association between the rs2004640 functional polymorphism of interferon regulatory factor 5 and systemic lupus erythematosus: a meta-analysis. Rheumatol Int.
5. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, et al. (2005) Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. Am J Hum Genet 76: 528–537.
6. Han SW, Lee WK, Kwon KT, Lee BK, Nam EJ, et al. (2009) Association of Polymorphisms in Interferon Regulatory Factor 5 Gene with Rheumatoid Arthritis: A Metaanalysis. J Rheumatol.
7. Maalej A, Hamad MB, Rebai A, Teixeira VH, Bahloul Z, et al. (2008) Association of IRF5 gene polymorphisms with rheumatoid arthritis in a Tunisian population. Scand J Rheumatol 37: 414–418.
8. Shimane K, Kochi Y, Yamada R, Okada Y, Suzuki A, et al. (2009) A single nucleotide polymorphism in the IRF5 promoter region is associated with susceptibility to rheumatoid arthritis in the Japanese population. Ann Rheum Dis 68: 377–383.
9. Sigurdsson S, Padyukov L, Kurreeman FA, Liljedahl U, Wiman AC, et al. (2007) Association of a haplotype in the promoter region of the interferon regulatory factor 5 gene with rheumatoid arthritis. Arthritis Rheum 56: 2202–2210.
10. Kristjansdottir G, Sandling JK, Bonetti A, Roos IM, Milani L, et al. (2008) Interferon regulatory factor 5 (IRF5) gene variants are associated with multiple sclerosis in three distinct populations. J Med Genet 45: 362–369.
11. Nordmark G, Kristjansdottir G, Theander E, Eriksson P, Brun JG, et al. (2009) Additive effects of the major risk alleles of IRF5 and STAT4 in primary Sjogren's syndrome. Genes Immun 10: 68–76.
12. Dideberg V, Kristjansdottir G, Milani L, Libioulle C, Sigurdsson S, et al. (2007) An insertion-deletion polymorphism in the interferon regulatory Factor 5 (IRF5) gene confers risk of inflammatory bowel diseases. Hum Mol Genet 16: 3008–3016.
13. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, et al. (2007) TRAF1-C5 as a risk locus for rheumatoid arthritis–a genomewide study. N Engl J Med 357: 1199–1209.
14. Kurreeman FA, Goulielmos GN, Alizadeh BZ, Rueda B, Houwing-Duistermaat J, et al. (2009) The TRAF1-C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. Ann Rheum Dis.
15. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, et al. (2007) STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 357: 977–986.
16. Svenungsson E, Gustafsson J, Leonard D, Sandling J, Gunnarsson I, et al. (2009) A STAT4 risk allele is associated with ischemic cerebrovascular events and antiphospholipid antibodies in Systemic Lupus Erythematosus. Ann Rheum Dis.
17. Vuong MT, Lundberg S, Gunnarsson I, Wramner L, Seddighzadeh M, et al. (2009) Genetic variation in the transforming growth factor-{beta}1 gene is associated with susceptibility to IgA nephropathy. Nephrol Dial Transplant.
18. Gunnarsson I, Ronnelid J, Lundberg I, Jacobson SH (1997) Occurrence of anti-C1q antibodies in IgA nephropathy. Nephrol Dial Transplant 12: 2263–2268.
19. Potlukova E, Kralikova P (2008) Complement component c1q and anti-c1q antibodies in theory and in clinical practice. Scand J Immunol 67: 423–430.
20. Bhavnani SK, Eichinger F, Martini S, Saxman P, Jagadish HV, et al. (2009) Network analysis of genes regulated in renal diseases: implications for a molecular-based classification. BMC Bioinformatics 10 Suppl 9: S3.
21. Loscalzo J, Kohane I, Barabasi AL (2007) Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. Mol Syst Biol 3: 124.

22. Padyukov L, Hahn-Zoric M, Blomqvist SR, Ulanova M, Welch SG, et al. (2001) Distribution of human kappa locus IGKV2-29 and IGKV2D-29 alleles in Swedish Caucasians and Hong Kong Chinese. Immunogenetics 53: 22–30.
23. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265.
24. Basile C, Semeraro A, Montanaro A, Giordano R, De Padova F, et al. (1998) IgA nephropathy in a patient with systemic lupus erythematosus. Nephrol Dial Transplant 13: 1891–1892.
25. Horino T, Takao T, Terada Y IgA nephropathy in a patient with systemic lupus erythematosus. Lupus.