Significance of urine complement proteins in monitoring lupus activity

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Objectives: Complement activation is a critical feature in the development of systemic lupus erythematosus (SLE).Whether there are changes of complement components in the urine of SLE has not been reported. The aim of the study was to evaluate the complement-related proteins in the urine of SLE, verify differentially expressed proteins(DEPs) in the active phase of SLE, further explore their clinical application value. **Methods:** First, we used bioinformatics and functional enrichment to screen and identify the urine protein profile of SLE patients. Then, analyzed and verified the proteins related to the complement pathway by western-blot and Parallel Reaction Monitoring(PRM) technology. Further evaluated the relationship between urinary DEPs related to complement pathway and disease activity. **Results:** A total of 14 complement pathway-related proteins were screened for differences in expression between the active group and the stable group, eight of these DEPs were up-regulated and Six were down-regulated. These DEPs may play a key role in SLE disease activity. We used PRM technology to verify the eight up-regulated proteins, and found that four of these complement proteins, namely C9, C8A, C4B, and C8G, were significantly increased in active group. Furthermore, these four DEPs were highly correlated with disease activity. In the urine of SLE patients, AUCs of 0.750, 0.840, 0.757 and 0.736 were achieved with C9, C8A, C4B, and C8G, respectively. **Conclusions:** Complement-related DEPs in urine have a certain correlation with SLE disease activity. Urine C9, C8A, C4B and C8G present promising non-invasive biomarkers for monitoring lupus activity.

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18 **Abstract**

19 **Objectives.** Complement activation is a critical feature in the development of systemic lupus

20 erythematosus (SLE).Whether there are changes of complement components in the urine of SLE

21 has not been reported. The aim of the study was to evaluate the complement-related proteins in

22 the urine of SLE, verify differentially expressed proteins(DEPs) in the active phase of SLE,

23 further explore their clinical application value.

24 **Methods.** First, we used bioinformatics and functional enrichment to screen and identify the

25 urine protein profile of SLE patients. Then, analyzed and verified the proteins related to the

26 complement pathway by western-blot and Parallel Reaction Monitoring(PRM) technology.

27 Further evaluated the relationship between urinary DEPs related to complement pathway and

28 disease activity.

29 **Results.** A total of 14 complement pathway-related proteins were screened for differences in

30 expression between the active group and the stable group, eight of these DEPs were up-regulated

31 and Six were down-regulated. These DEPs may play a key role in SLE disease activity. We used

32 PRM technology to verify the eight up-regulated proteins, and found that four of these

33 complement proteins, namely C9, C8A, C4B, and C8G, were significantly increased in active

34 group. Furthermore, these four DEPs were highly correlated with disease activity. In the urine of

35 SLE patients, AUCs of 0.750, 0.840, 0.757 and 0.736 were achieved with C9, C8A, C4B, and

36 C8G, respectively.

37 **Conclusions.** Complement-related DEPs in urine have a certain correlation with SLE disease

38 activity. Urine C9, C8A, C4B and C8G present promising non-invasive biomarkers for

39   monitoring lupus activity.

40   **Keywords:** Urine; Complement; Differentially expressed proteins; Biomarker; Lupus.

41   **1 Introduction**

42   Systemic lupus erythematosus (SLE) is a global chronic autoimmune disease involving

43   multiple organs. With the development of diagnosis and treatment technology, the survival rate

44   of SLE has increased significantly, but it is still an irradicable disease[1]. SLE has the

45   characteristics of repeated recurrence and remission. Relapsing is a common clinical feature, a

46   sign of obvious disease activity, and the main cause of organ damage and poor prognosis[2].

47   Autoantibodies, immune complexes and complement system in the immune system play an

48   important role in the pathogenesis of SLE[3, 4]. Complement is an important effector system of

49   the immune response. On the one hand, complement is the ultimate effector of tissue damage in

50   autoimmune diseases; on the other hand, the lack of certain components of complement would

51   lead to autoimmune diseases[5].

52   It has been found that many complement components were closely related to the occurrence

53   and development of SLE. Serum low levels of complement C3, C4 and CH50 as indicators of

54   lupus activity have been included in the Systemic Lupus Erythematosus Disease Activity Index

55   2000(SLEDAI-2000) scoring system[6]. The relationship between serum C1q and the

56   development of lupus is well known, it is typically involved in the clearance of apoptotic cells,

57   and being a valuable biomarker of lupus activity[7, 8]. The genetic defects of complement C1,

58   C2, or C4 are strongly associated with the development of SLE[7, 8]. Since most complement

59   and activation products exist in plasma and are finally excreted in urine, these factors may be

60 used as non-invasive biomarkers for monitoring disease activity. In our early study, we used

61 label-free technology to analyze the urine proteins between the active and stable group of SLE,

62 and discovered many differentially expressed proteins (DEPs)[10].The changes of complement-

63 related proteins in the active phase of SLE aroused our interest.

64   Therefore, in order to analyze the expression of complement-related proteins in the urine of

65 SLE, we evaluated the urine protein expression profile during the active period of SLE.

66 Subsequently, we verified the changes of these complement proteins in the urine of lupus by

67 qualitative western-blot and quantitative proteomics technology, further explored the role of

68 different complement components in lupus activity to provide a basis for discovering urinary

69 biomarkers of lupus activity.

70 **2 Materials and methods**

71 2.1 Patients and sample collection

72   First of all, this study protocol was approved by the ethics committee of Beijing Shijitan

73 Hospital, Capital Medical University(sjtkyll-lx-2021(55)). The participants all gave written

74 informed consent of each subject, which was in accordance with the provisions of the Helsinki

75 Declaration. All subjects were adult females. Study participants included 24 patients with

76 diagnosed systemic lupus erythematosus (SLE group) and 12 controls that did not have SLE (NC)

77 with routine physical examination. Participants were recruited from Beijing Shijitan Hospital

78 from September 2020 until September 2021. SLE patients were diagnosed through the use of

79 1997 American College of Rheumatology classification criteria for systemic lupus erythematosus.

80 According to the SLEDAI-2K scoring system, SLEDAI-2K≥5 were classified as the active

81 group (SLE-A), and SLEDAI-2K<5 were classified as the stable group (SLE-S) . Exclusion

82 criteria included acute or chronic infections, tumors and other autoimmune diseases, and any

83 diagnoses of other severe liver and renal disease. The morning midstream urine samples were

84 collected into sterile polypropylene tubes. Immediately after collection, urine samples were

85 centrifuged at $400 \times g$ for 5 min to remove cell debris and casts and finally supernatants were

86 divided in aliquots and frozen at -80°C.

87 ## 2.2 Proteomic analysis and bioinformatics analysis

88 Base on SDS Polyacrylamide Gel Electrophoresis(SDS-PAGE) combined with nano-

89 upgraded reversed-phase liquid chromatography-tandem mass spectrometry (nanoRPLC-

90 MS/MS)method, we detected and analyzed the urinary proteins of SLE patients and controls

91 without SLE using Thermo Q-Exactive mass spectrometer. MaxQuant software was used to

92 process the original mass spectrometry files, and the significant difference in protein

93 quantification between SLE-A and SLE-S (Fold change=2.0, P<0.05) was defined as

94 differentially expressed proteins (DEPs). KEGG database and Metascape platform were used to

95 analyze the function and pathway enrichment of DEPs to determine the important pathways and

96 proteins related to SLE disease activity. Protein-protein interaction (PPI) network analysis is

97 performed by using a search tool (STRING) database for searching interacting genes/proteins.

98 ## 2.3 Qualitative identification by western blot.

99 To verify the previous screening results, we selected C9 and MASP2 with the most obvious

100 differences for western blot analysis. The total protein was enriched by a 10kD ultrafiltration

101 device (Millipore) and concentrated at 6000g for 15 minutes. The volume of the sample was

102 calculated according to the protein mass (7 μg). After SDS-PAGE gel electrophoresis, the protein

103 was transferred to the PVDF membrane and sealed with skim milk powder for 2 hours. The

104 PVDF membranes were incubated overnight at 1:1000 anti-C9 antibody and anti-MASP2

105 antibody at 4°C. The 1×TBST buffer solution was used to rinse the membranes three times, each

106 20 minutes. Then, 1:3000 second antibody was added and incubated at room temperature for 90

107 minutes. Finally, the PVDF membrane was washed with 1×TBST buffer and detected by the

108 enhanced chemiluminescence method.

109 ## 2.4 Quantitative identification by parallel reaction monitoring

110 The urinary samples were centrifuged at 2000g for 10min at 4°C, and urea particles were

111 added to the supernatant with a urea concentration of 8M, shaken until the urea particles were

112 fully dissolved. The volume of the samples were concentrated by ultrafiltration in a 10K

113 ultrafiltration tubes. After protein quantification, 60μg proteins were taken and placed in a

114 centrifuge tubes. 5μl of 1M DTT was added to the solution and mixed, and incubated at 37°C for

115 1h; 20μl of 1M IAA solution was added, and after mixing, the reaction was performed for 1h at

116 room temperature and avoided light. Trypsin was added to the ultrafiltration tube at 1:50

117 enzyme-to-substrate ratio, and digested at 37°C for more than 12 hours. The mix-sample was

118 fractionated in 100μl mobile phase A (100% ddH2O, 0.1% formic acid), centrifuged at 14000g

119 for 20min, and the supernatant was taken for separation at a flow rate of 0.7ml/min. Finally, the

120 eluates were monitored at UV 214 nm, collected for a tube per minute and merged into 3

121 fractions, which are lyophilized and waiting for Q Exactive HF-X mass spectrometer testing.

122 The Q Exactive HF-X mass spectrometer was operated in the data-dependent acquisition

123 mode and there was a single full-scan mass spectrum in the Orbitrap (350-1500 m/z, 120,000

124 resolution) followed by data dependent MS/MS scans in an Ion Routing Multipole at 27%

125 normalized collision energy (HCD). The single-sample was reconstituted in 0.1% FA, and

126 injected into the Q Exactive HF-X mass spectrometer (Thermo Fisher) operating in the Parallel

127 reaction monitoring (PRM) mode. For PRM acquisition, primary mass spectrum (MS1)

128 resolution was set to 120000, and secondary mass spectrum (MS2) resolution was set to 15000.

129 The m/z range covered from 350 to 1500 m/z. The maximum capacity of C-trap was set to 3×10^6,

130 and the maximum injection was 80 ms. The dynamic exclusion range was set to 16 s and allow

131 the mass spectrometer always operating in the parallel ion filling and detection mode. The raw

132 data of mass spectrometry (MS) was generated and processed by Skyline software. The false

133 discovery rate was set to 1% for proteins and peptides. The enzyme specificity was set to trypsin,

134 and a maximum of three missed cleavages were allowed in the database search. Parameter

135 settings for screening differential proteins: difference ratio >1.2 or difference ratio <1/1.2, *P*

136 value<0.05.

137 ## 2.5 Statistical analysis

138 Statistical analysis was performed using SPSS 21.0 software and the visualization of the

139 data carried out by using GraphPad Prism 9.0 software. The results of quantitative data were

140 expressed as mean ± standard deviation(SD) or median (interquartile range). The P-values were

141 calculated by Student's t test and Mann-Whitney U test for two groups, or ANOVA (more than

142 two groups). The correlation analysis was performed to examine the relationship between

143 continuous variables by Spearman's correlation coefficient. Receiver operating characteristic

144 (ROC) curve analysis and area under the curve (AUC) calculations were used to analyze the

145 diagnostic value of urine candidate DEPs in the disease activity of patients with SLE. In all cases

146 two-tailed P<0.05 was accepted as statistically significant.

147 **3. Results**

148 3.1 Comparison of complement-related proteins between active group (SLE-A)

149 and stable group (SLE-S).

150 Screening and identifying the urine protein profile of SLE patients demonstrated that

151 complement-related proteins showed varying degrees of difference between the active group

152 (SLE-A) and stable group (SLE-S).To classify the functional annotations of the identified

153 proteins, pathway analysis was performed by kyoto encyclopedia of genes and genomes (KEGG)

154 database. The most representative pathway was complement and coagulation pathways

155 (map04610). Among the urine protein profile, there were 16 proteins involved in the

156 complement pathway, 14 of these proteins were differentially expressed, 8 differentially

157 expressed proteins (DEPs) were up-regulated, and 6 DEPs were down-regulated (Figure 1A).

158 These 14 DEPs played different roles in the complement pathway, 10 of these DEPs were

159 key factors of the complement pathway. The expressions of Complement C5 (C5), Complement

160 component C9 (C9), Complement C3 (C3), Complement component C7 (C7), Complement C4-

161 B (C4B), Complement component C8 alpha chain (C8A) and Complement component C8

162 gamma chain (C8G) were up-regulated between SLE-A and SLE-S, while the expressions of

163 Mannan-binding lectin serine protease 2 (MASP2), Complement component C6 (C6) and

164 Complement factor D (CFD) are down-regulated. The other 4 DEPs were the inhibitory

165 molecules of complement pathway, namely Vitronectin (VTN) whose expression was up-

166 regulated and Complement receptor type 1(CR1), Plasma protease C1 inhibitor (SERPING1) and

167 V-set and immunoglobulin domain-containing protein 4(VSIG4) whose expression were down-

168 regulated (Figure 1B). The relative abundance of these DEPs among SLE-A, SLE-S and controls

169 that did not have SLE (NC) were shown in Figure 1C, and detailed information were shown in

170 Table 1. These DEPs may play key roles in disease activity.

171 3.2 Bioinformatics analysis of complement-related proteins

172   In order to predict the interactions of complement-related proteins, protein–protein

173 interaction (PPI) network was conducted by STRING database. The PPI network involved in the

174 complement pathway were tight and these proteins had strong interactions(Figure 2A). Based on

175 the Metascape platform, the functional analysis of these proteins revealed that the main

176 enrichment pathways involved in complement and coagulation cascades, complement cascade,

177 complement activation, regulation of complement cascade and other pathways. These findings

178 suggested that these proteins play important roles in the activation and regulation of the

179 complement pathway (Figure 2B).

180 3.3 Preliminary verification of urine C9 and MASP2 among SLE-A, SLE-S and

181 controls group (NC)

182   To verify the screening results, we selected the two most different proteins from the DEPs,

183 namely C9 and MASP2, performing western blot analysis. The results showed that the

184 expression of C9 in the urine was significantly increased in most patients with SLE-A compared

185 with SLE-S and NC. But the changes of MASP2 in the three groups were not obvious, and even

186   the detection rate of MASP2 in the urine of individual SLE patients and control groups was low

187   (Supplementary Figure 1). It may be due to the limitations of the western blot method in the

188   detection of urine proteins, and the sensitivity is not enough. Therefore, we focused on the up-

189   regulated proteins for further verification.

190   3.4 Quantitative verification of up-regulated complement proteins in urine

191   Parallel reaction monitoring (PRM) technology was used to identified 8 up-regulated

192   proteins related to the complement pathway. The results revealed that the expression of 4

193   proteins were consistent with the previous mass spectrometry screening results. The detailed

194   information of the four proteins were shown in Table 2. Compared with the SLE-S and NC, the

195   expression levels of C9, C8A, C4B, and C8G exhibited a significant increase in urine of SLE-A.

196   The levels of these four proteins among the three groups are shown in Figure 3A.

197   3.5 Differentially expressed proteins (DEPs) were closely associated with lupus

198   activity

199   Spearman correlation analysis was used to analyze the correlation between four DEPs and

200   disease activity. The results shown that the levels of urine C9 (r=0.599, P=0.002), C8A (r=0.669,

201   P<0.001), C4B (r=0.588, P=0.003), and C8G (r=0.597, P=0.002) were significantly positively

202   correlated with SLEDAI-2K. The higher the score of SLEDAI-2K, the greater the concentration

203   of these four proteins (Figure 3B).

204   ROC curve analysis was performed to determine the cutoff value of the four DEPs for

205   distinguishing SLE-A from SLE-B. The areas under the curves(AUCs) of urine C9, C8A, C4B

206   and C8G were 0.750 (95% CI: 0.554-0.947), 0.840 (95% CI: 0.674-1.00), 0.757(95% CI: 0.556-

207 0.958) and 0.736 (95% CI: 0.526-0.946), respectively(Figure 3C). These four proteins all have

208 high discriminatory capacity for disease activity. Among the four urinary proteins, C8A had the

209 highest judgment value for lupus activity, with an AUCs of 0.840. When the cutoff level was

210 0.640 ng/ml, the sensitivity and specificity were 91.67% and 75%, respectively.

211 **4. Discussion**

212   Although the pathogenesis of SLE is not fully understood, it is widely accepted that the

213 complement system in the blood is involved in the pathogenesis of SLE and has important roles

214 in the pathophysiology of SLE, including promotion of inflammatory processes, clearance of

215 immune complexes, cellular and apoptotic debris[11-13].The complement system is composed of

216 more than 30 plasma proteins and cell surface receptors. There are three pathways of

217 complement activation: classical pathway, alternative pathway and mannose-binding lectin

218 pathways, which may be related to inflammation and tissue damage in SLE[14-16].Therefore,

219 activating components or products of complement in SLE patients may serve as biomarkers for

220 diagnosis or monitoring disease activity of SLE.

221   In the past few decades, most studies have focused on the detection of complement proteins

222 (C3, C4) and hemolytic activity. Although known as markers of disease activity, the limitations

223 of these indicators have also caused some controversy[17-19].In recent years, some studies have

224 found that detection of complement split products more accurately reflected complement

225 activation, such as C3a, C4a, C5a, C3d, C4d, Ba, Bb and soluble C5b-9 complexes[20-

226 22].However, these split products have a short half-life in plasma and are easily activated in vitro,

227 so there are many challenges in detection technology[23].The content of proteins in urine is

228 relatively stable, and urine is easy to collect and available in large quantities[24].Therefore, the

229 detection of complement components and split products in urine is a convenient and good choice.

230 Several investigators reported that the complement split products like C3 fragment, C4d,

231 C5a and C5b-9 could be detected in the urine of lupus nephritis (LN) patients, and found that the

232 levels of C3d in urine was closely related to LN disease activity[25, 26]. Whereas, the changes of

233 other complement pathway-related proteins and fragments in the urine of SLE patients are

234 unknown. Therefore, in order to explore the changes of complement components in the urine of

235 SLE patients and the relationship with SLE disease activity, and search the potential urine

236 markers of monitoring SLE activity, we analyzed the urine protein profiles of patients with

237 active lupus and stable lupus, and screened out the urinary proteins involved in the complement

238 pathway, based on label-free mass spectrometry technology. Subsequently, we further

239 investigated the expression levels and functions of the complement related DEPs between the

240 active group (SLE-A) and stable group (SLE-S). Then, the up-regulated DEPs were selected for

241 quantitative verification using targeted proteomics technology to explore the clinical application

242 value of these urinary proteins as biomarkers of disease activity.

243 In this study, a total of 16 complement pathway-related proteins were identified by

244 analyzing the urine protein profiles of SLE, 14 of these proteins were abnormally expressed in

245 the active and stable SLE groups. Through functional analysis, we found that these differential

246 proteins have different roles in the complement pathway, mainly involved in complement

247 activation and the regulation of complement activation pathway, and these DEPs may as crucial

248 markers for SLE disease activity. To verify the expression of these DEPs in the urine of SLE

249   patients, we focused on the verification of 7 up-regulated proteins relating to complement

250   activation pathway (C5, C9, C8A, C3, C7, C4B and C8G). Our results showed that the

251   expression of urinary C9, C8A, C4B and C8G in SLE patients was consistent with previous mass

252   spectrometry data.

253   C9, C8 (C8A, C8G) are both the final components of the complement system, major

254   components of the membrane attack complex (MAC), which served as part of innate and

255   adaptive immune responses[27]. C8 contains three polypeptides, (α, β, and γ), consisting of

256   disulfide-linked heterodimers (α-γ) and non-covalently bound β chains[28]. C9 binds C8 in the

257   C5b-8 complex and forms the MAC that causes cellular and organ damage, therefore, the

258   complement system is involved in the occurrence and development of various diseases[29].

259   Recent evidence reported that the decrease of eGFR in patients with diabetic nephropathy was

260   significantly associated with the excretion of the urinary membrane attack complex[30]. Our

261   results revealed that urinary complement C9, C8A and C8G were significantly increased during

262   active SLE, and correlated well with SLEDAI-2K, suggesting complement activation and the

263   formation of membrane attack complexes during SLE disease activity.

264   C4 is a non-enzymatic component of C3 and C5 convertases and is necessary for

265   complement activation[31]. After complement was activated through the classical or lectin-

266   activated pathway, C4 was activated and hydrolyzed to generate C4B. C4B was an opsonin that

267   covalently binds complement-activating targets[32]. Recent studies have reported that diversities

268   of C4A and C4B proteins and their gene copy number variations (CNVs) in healthy subjects and

269   patients with autoimmune diseases[33]. Our results demonstrated that the expression of C4B was

270 also significantly elevated in the urine of active SLE, and was positively correlated with disease

271 activity.

272   Furthermore, in the ROC curve analysis of urine C9, C8A, C8G and C4B on SLE disease

273 activity, we found that these four proteins all have good ability to judge SLE activity. Among

274 these proteins, urine C8A had the best judgment ability, with an AUC of 0.840. When the cut-off

275 value was 0.640 ng/ml, the sensitivity and specificity for judging disease activity were 91.67%

276 and was 75%, respectively. These four complement related DEPs undertook critical roles and

277 performed different functions in the complement cascade (Figure 4). As the important

278 component of C3 convertase and C5 convertase, C4b played a very important role in the entire

279 complement cascade. While C9, C8A, and C8G were mainly involved in the last step of the

280 complement cascade, which participated in the formation of membrane attack complexes,

281 resulting in cell lysis and tissue damage. According to our results, urine C9, C8A, C8G, and C4B

282 were significantly increased during active SLE, the four complement-related proteins may be

283 potential urinary biomarkers of SLE disease activity.

284 **5. Conclusions**

285   In conclusion, we analyzed and verified the expression of complement pathway-related

286 proteins in the urine of SLE patients with different activity states by using proteomic technology.

287 Then, we further explored the association between these complement-related proteins and

288 disease activity. Higher abundance of urinary C9, C8A, C8G, and C4B were strongly associated

289 with higher scores of SLEDAI-2K. These findings suggested that activation of distinct

290 components of the complement pathway may be associated with disease activity. Consequently,

291 therapeutically targeting the complement pathway may improve progression of SLE and these

292 four urinary proteins may be potential biomarkers to assist in monitoring lupus activity.

Statement of Ethics

294 This study was approved by the ethics committee of Beijing Shijitan Hospital, , Capital

295 Medical University. The participants all gave written informed consent of each subject, which

296 was in accordance with the provisions of the Helsinki Declaration.

Conflicts of Interest

298 Upon manuscript submission, all authors completed the author disclosure form. The authors

299 declare no competing interests.

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370

371   **Figures and Legends**

372

373   **Figure 1. Comparison of urine proteins related to complement pathway.** (A) Heatmap of
374   candidate urine proteins among active group (SLE-A), stable group (SLE-S) and controls
375   without SLE (NC). (B) Different roles of differential proteins in the complement pathway
376   between SLE-A and SLE-S. (C) The abundance of these complement related proteins in arbitrary
377   units among three groups.

378

379   **Figure 2. Functional analysis of complement-related proteins.** (A) Protein–protein
380   interaction(PPI) network of complement-related proteins. The confidence level was set to
381   medium of 0.4. (B) Pathway analysis of complement-related proteins performing by Metascape
382   platform.

383

384   **Figure 3. The comparison of selected protein expression by PRM.** (A) The expression levels
385   of C9, C8A, C4B and C8G among active group (SLE-A), stable group (SLE-S) and controls
386   without SLE (NC). An asterisk (*) indicated a significant change in protein abundance between
387   the two groups ($P<0.05$) and two asterisk (*) meant $P<0.01$. (B) The correlation between DEPs
388   and SLEDAI-2K, including C9, C8A, C4B and C8G, was determined by Spearman correlation
389   analysis in SLE patients. (C) Receiver operating characteristic (ROC) curve analysis of the four
390   DEPs to distinguish SLE disease activity from SLE stable. PRM: Parallel reaction monitoring;
391   DEPs: differentially expressed proteins; SLEDAI-2K: Systemic Lupus Erythematosus Disease
392   Activity Index 2000; AUC: area under the curve.

393

394   **Figure 4. Simplified complement cascade featuring complement-related proteins.** C9, C8A,
395   C4B and C8G play different roles in the complement pathway. Red indicating the four
396   significantly up-regulated proteins or protein complex.

397

398   **Supplementary Figure 1. The expression of urine C9 and MASP2 determined by western**
399   **blotting among active group (SLE-A), stable group (SLE-S) and controls without SLE (NC).**
400   The total protein were enriched from the urine of each subject. A1-A12: Western blot bands in
401   the urine from 12 SLE-A patients. S1-S12: Western blot bands in the urine from 12 SLE-S
402   patients. N1-N12: Western blot bands in the urine from 12 controls without SLE.

403

The expression of complement pathway associated proteins between active group (SLE-A) and stable group (SLE-S).

1 **Table 1.** The expression of complement pathway associated proteins between active group
2 (SLE-A) and stable group (SLE-S).

Uinprot-ID	Protein Name	Gene Name	(SLE-A/SLE-S)Ratio	P-value	Form of expression
P01031	Complement C5	C5	37.79	5.32E-03	up
P02748	Complement component C9	C9	20.46	5.28E-04	up
P07357	Complement component C8 alpha chain	C8A	19.94	3.82E-02	up
P01024	Complement C3	C3	15.39	1.33E-04	up
P10643	Complement component C7	C7	13.44	1.90E-05	up
P0C0L5	Complement C4-B	C4B	5.321	4.00E-06	up
P04004	Vitronectin	VTN	3.866	2.11E-04	up
P07360	Complement component C8 gamma chain	C8G	3.405	3.38E-03	up
P08603	Complement factor H	CFH	1.785	6.33E-03	n.s.
P10909	Clusterin	CLU	0.850	2.51E-02	n.s.
O00187	Mannan-binding lectin serine protease 2	MASP2	0.029	1.69E-04	down
P17927	Complement receptor type 1	CR1	0.035	8.46E-04	down
P13671	Complement component C6	C6	0.039	1.21E-02	down
P00746	Complement factor D	CFD	0.167	3.34E-02	down
P05155	Plasma protease C1 inhibitor	SERPING1	0.348	5.62E-04	down
Q9Y279	V-set and immunoglobulin domain-containing protein 4	VSIG4	0.4009	3.68E-03	down

The four differentially expressed proteins (DEPs) to be verified by parallel reaction monitoring (PRM).

1   **Table 2.** The four differentially expressed proteins (DEPs) to be verified by parallel reaction
2   monitoring (PRM).

Uinprot-ID	peptide	Gene Name	M/Z	SLE-A/SLE-S	P-value	Form of expression
P02748	LSPIYNLVPVK	C9	621.88	38.51	0.0377	up
P07357	LGSLGAACEQTQTEGAK	C8A	860.91	18.80	0.0047	up
P0C0L5	FSDGLESNSSTQFEVK	C4B	887.91	8.30	0.0327	up
P07360	VQEAHLTEDQIFYFPK	C8G	655.66	15.83	0.0496	up

Figure 1

Comparison of urine proteins related to complement pathway.

(A) Heatmap of candidate urine proteins among active group (SLE-A), stable group (SLE-S) and normal controls (NC). (B) Different roles of differential proteins in the complement pathway between SLE-A and SLE-S. (C) The abundance of these complement related proteins in arbitrary units among three groups.
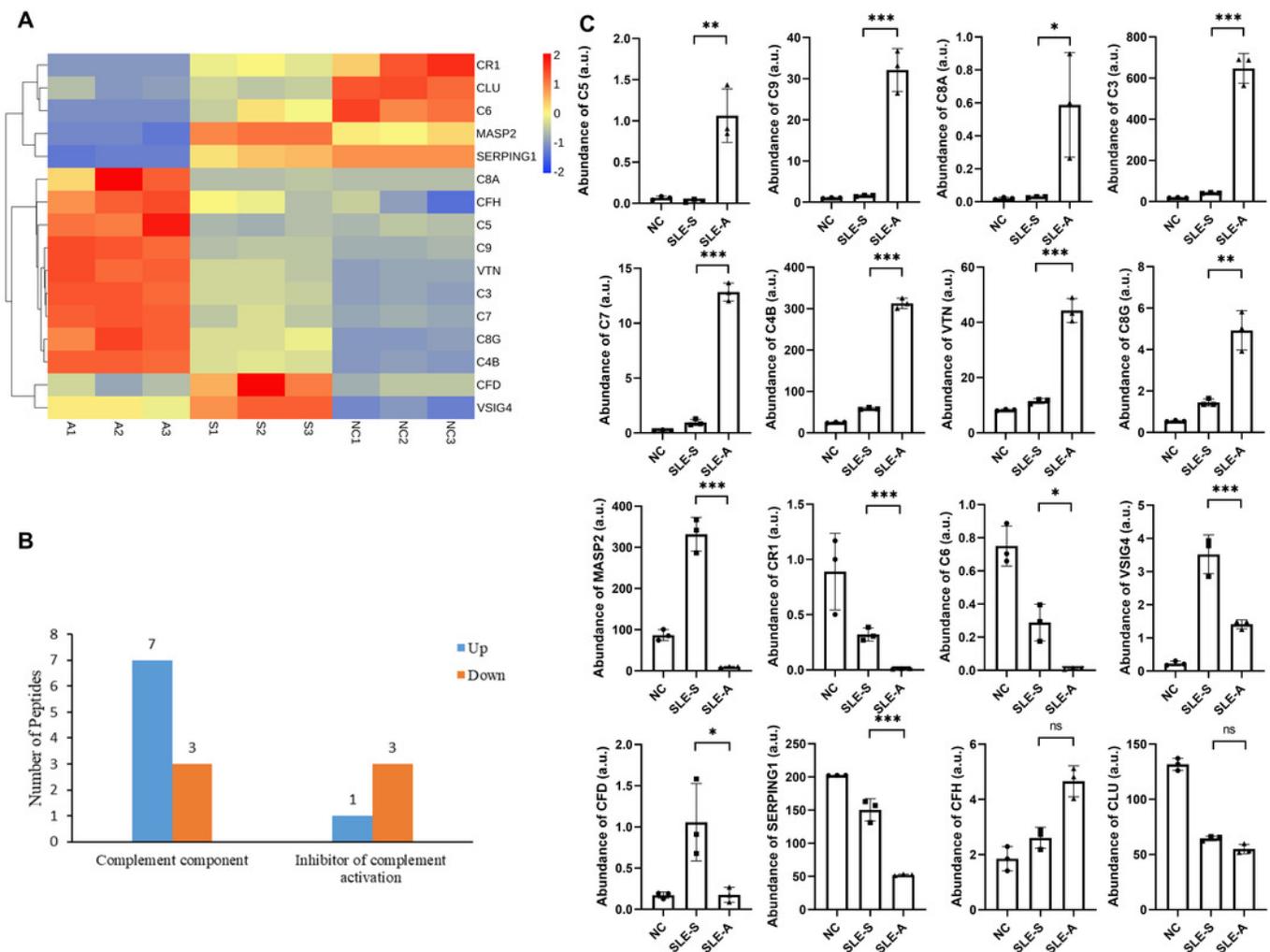
Figure 2

Functional analysis of complement-related proteins.

(A) Protein–protein interaction(PPI) network of complement-related proteins. The confidence level was set to medium of 0.4. (B) Pathway analysis of complement-related proteins performing by Metascape platform.
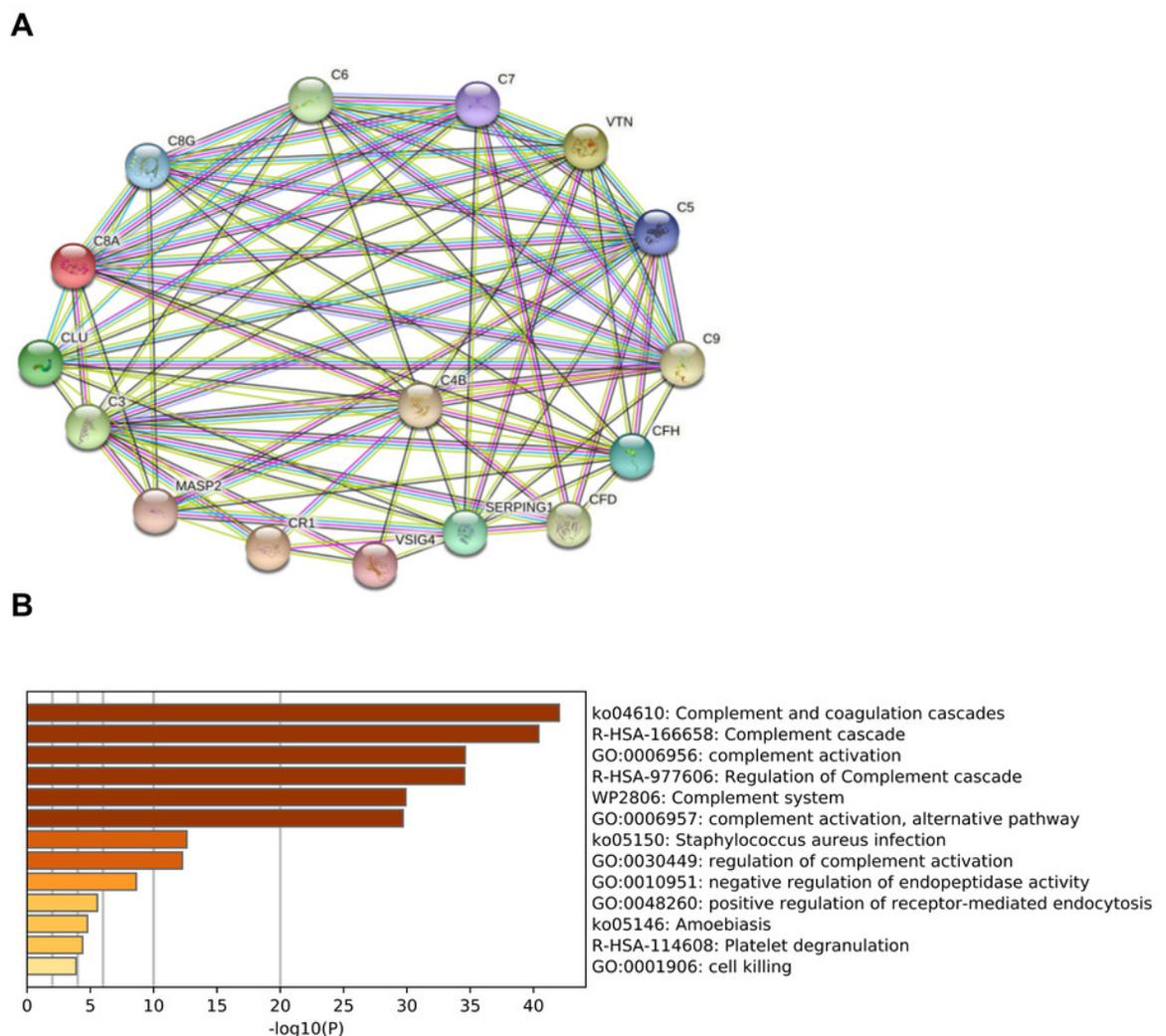
Figure 3

The comparison of selected protein expression by PRM.

(A) The expression levels of C9, C8A, C4B and C8G among active group (SLE-A), stable group (SLE-S) and normal controls (NC). An asterisk (*) indicated a significant change in protein abundance between the two groups ($P<0.05$) and two asterisk (*) meant $P<0.01$. (B) The correlation between DEPs and SLEDAI-2K, including C9, C8A, C4B and C8G, was determined by Spearman correlation analysis in SLE patients. (C) Receiver operating characteristic (ROC) curve analysis of the four DEPs to distinguish SLE disease activity from SLE stable. PRM: Parallel reaction monitoring; DEPs: differentially expressed proteins; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; AUC: area under the curve.
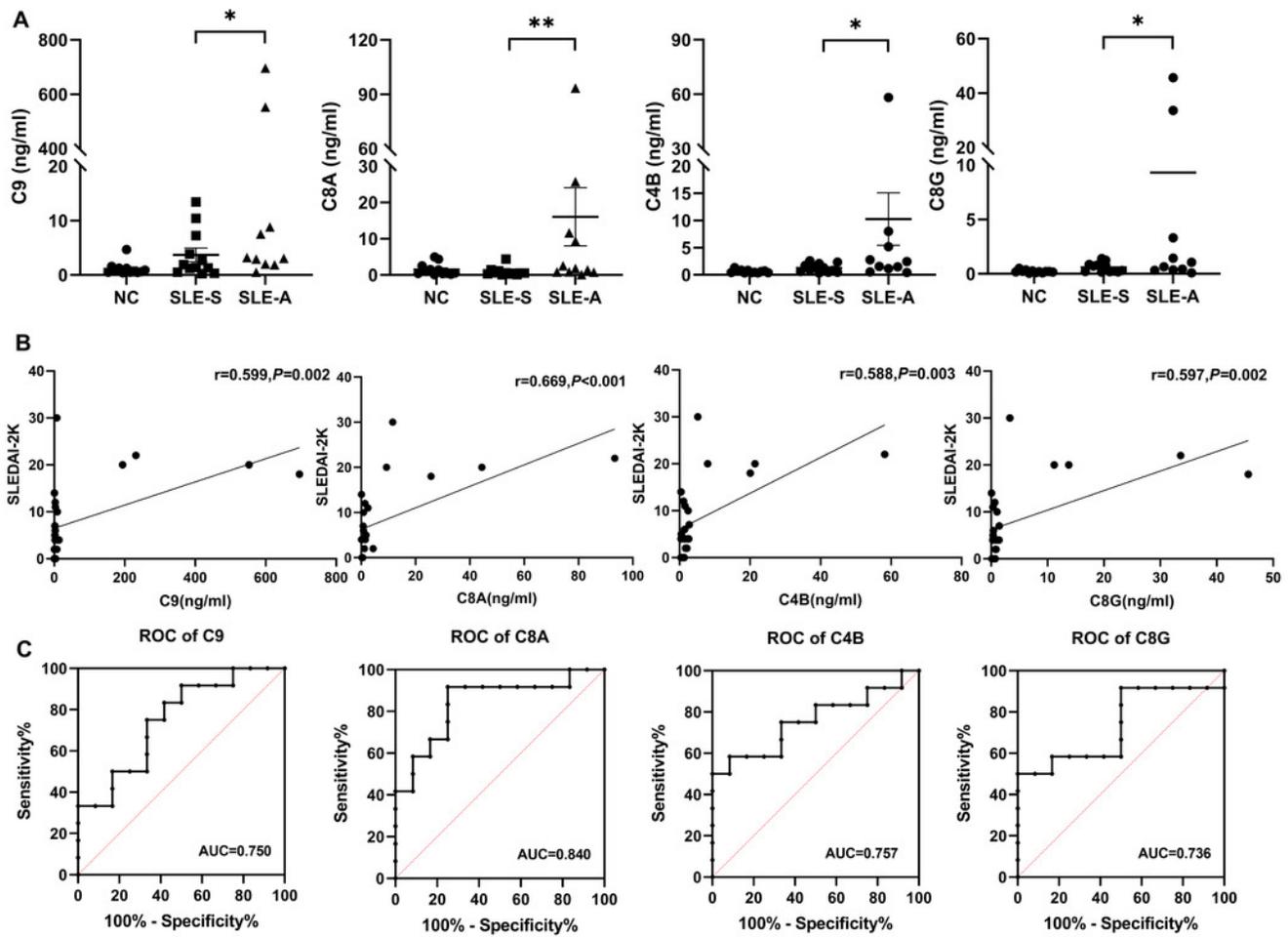
Figure 4

Simplified complement cascade featuring complement-related proteins.

C9, C8A, C4B and C8G play different roles in the complement pathway. Red indicating the four significantly up-regulated proteins or protein complex.