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# Differentially expressed lncRNAs and mRNAs identified by microarray analysis in GBS patients *vs* healthy controls

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**The aim of our present study was to determine whether message RNAs (mRNAs) and long noncoding RNAs (lncRNAs) are expressed differentially in patients with Guillain-Barré syndrome (GBS) compared with healthy controls. The mRNA and lncRNA profiles of GBS patients and healthy controls were generated by using microarray analysis. From microarray analysis, we listed 310 mRNAs and 114 lncRNAs with the mRMR software classed into two sample groups, GBS patients and healthy controls. KEGG mapping demonstrated that the top seven signal pathways may play important roles in GBS development. Several GO terms, such as cytosol, cellular macromolecular complex assembly, cell cycle, ligase activity, protein catabolic process, *etc.*, were enriched in gene lists, suggesting a potential correlation with GBS development. Co-expression network analysis indicated that 113 lncRNAs and 303 mRNAs were included in the co-expression network. Our present study showed that these differentially expressed mRNAs and lncRNAs may play important roles in GBS development, which provides basic information for defining the mechanism(s) that promote GBS.**

Guillain-Barré syndrome (GBS) is an acute inflammatory autoimmune disease affecting the peripheral nervous system. The characteristic features are progressive bilateral symmetric weakness and numbness in the legs and arms along with diminished or complete loss of deep tendon reflexes. In this presumed post-infectious, immune-mediated disease, cellular and humoral immune mechanisms probably play a vital developmental role. The production of autoantibodies or recruitment of inflammatory cells on the myelin sheath were thought to be responsible for the pathogenesis of GBS[1]. However, our present knowledge of the mechanism and epigenetic features of GBS remains insufficient.

Long noncoding RNAs (lncRNAs) are most commonly defined as transcripts longer than 200 nucleotides with little or no protein-coding capacity[2,3]. Since they cannot be completely dismissed as mere transcriptional "noise," lncRNAs have attracted increasing attention based on the development of lncRNA microarrays, high-throughput sequencing, and bioinformatics[4]. Although without protein coding capability, accumulating evidence has suggested that lncRNAs participate in a wide variety of biological processes, including genomic imprinting, cell differentiation, chromosome modification, X-chromosome silencing, organogenesis, chromosome dosage-compensation, transcriptional activation, *etc.*[5–8].

Currently, the role of lncRNA in autoimmune diseases has attracted considerable attention. Recent studies have reported that the activation, differentiation, and imbalanced expression of immune cells, including T cells, B cells, macrophages, and NK cells, may correlate directly with lncRNAs. Moreover, some specific lncRNAs also play a crucial role in autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), psoriasis, and autoimmune thyroid disease (AITD)[9].

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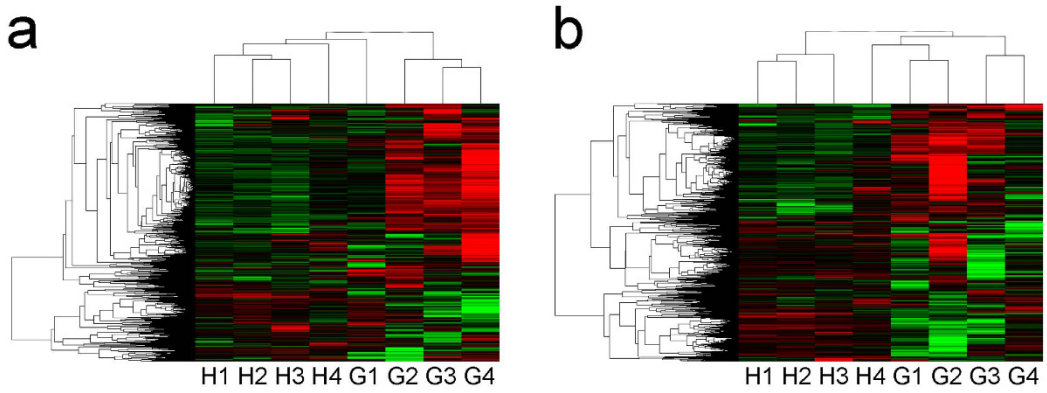


**Figure 1. Hierarchical clustering of lncRNAs and mRNAs in GBS patients and healthy controls.** G1-G4: GBS patients; H1-H4: healthy controls. The red and the green shades indicate the expression above and below the relative expression, respectively, across all samples. (**a**) lncRNA; (**b**) mRNA.

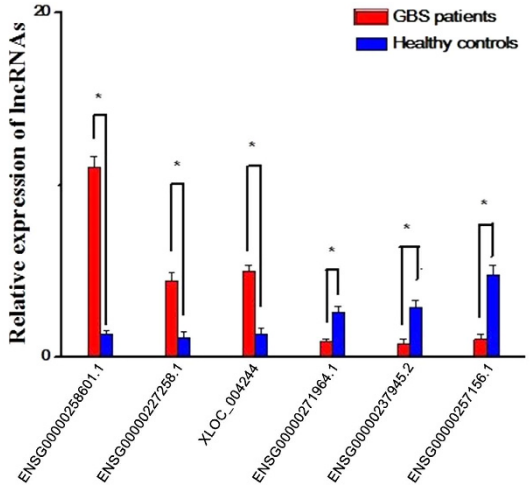


**Figure 2. Validation of lncRNA microarray data by qRT-PCR. Three upregulated and three downregulated lncRNAs were validated by qRT-PCR of RNA extracted from PBMCs of 15 GBS patients and 15 healthy controls.** The relative expression level of each lncRNA was normalized, and data displayed in histograms are expressed as means ± SD, $^{*}P < 0.05$ comparing GBS patients with healthy controls.

Further, the molecular mechanisms underlying the contributions of lncRNAs to GBS are not clear. Therefore, in the present study, we applied microarray technology to examine lncRNA and message RNA (mRNA) expression profiles in blood samples from GBS patients and healthy controls. Additionally, results from gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses predicted that these abnormally expressed mRNAs and lncRNAs function in the development of GBS.

## Results

**lncRNA and mRNA expression profile in GBS patients.** To investigate the expression levels of lncRNAs and mRNAs associated with GBS, lncRNA and mRNA microarray analyses were performed on the peripheral blood mononuclear cells (PBMCs) of 15 GBS patients and 15 healthy controls. Figure 1 was the hierarchical clustering that showed the differentially expressed lncRNAs (Fig. 1a) and mRNAs (Fig. 1b) between GBS patients and healthy controls. The red and the green shades indicate the expression above and below the relative expression, respectively, across all samples.

**Real-time quantitative PCR validation.** To validate our results independently and determine the role of lncRNAs in GBS, we randomly selected 6 lncRNAs. As shown in Fig. 2, differences in the expression of 6 lncRNAs were detected in GBS patients compared with healthy controls. LncRNA ENSG00000258601.1 was the most elevated (8.1-fold higher expression), followed by lncRNA ENSG00000227258.1 (3.94-fold higher expression), and lncRNA XLOC_004244 (3.64-fold higher expression). LncRNA ENSG00000257156.1, lncRNA ENSG00000237945.2, and lncRNA ENSG00000271964.1 exhibited 4.58-, 3.72- and 2.96- fold lower expression, respectively. These results were consistent with the results obtained from the microarray chip analyses.

**Minimum Redundancy Maximal Relevance (mRMR) Result.** After running the mRMR software, two outcomes were obtained. One was a MaxRel feature table ranking the 1246 mRNAs and 514 lncRNAs according to their relevance to the class of GBS patients or healthy controls (see File S1). The other, presented as the mRMR feature table, lists the top 310 mRNAs and 114 lncRNAs with the maximum relevance and minimum redundancy to the class of GBS patients or healthy controls (mRMR score equal 0 or 1, Table 1 and 2).

**GO and KEGG pathway analyses of differentially expressed mRNAs.** GO analysis was performed to investigate the over-representation of biological processes, cellular components, and specific molecular function associating protein-coding mRNAs, since no comprehensive annotation database is available for categorizing lncRNAs. A total of 310 filtered mRNAs (based on mRMR results) were included in GO analyses (see File S2). Figure 3 and Table 3 show the top 29 GO from the differentially expressed mRNAs ($-\lg^P > 2.5$); these include cytosol, cellular macromolecular complex assembly, cell cycle, ligase activity, and protein catabolic process.

Furthermore, from the data in mRMR, top seven KEGG pathways were listed, as Fig. 4 depicts, including "Proteasome", "Spliceosome", "Citrate cycle (TCA cycle)", "NOD-like receptor signaling pathway", "Primary immunodeficiency", "Endocytosis" and "T cell receptor signaling pathway." Among them, "Proteasome" was the most significant, because it also appeared in the previous study[10].

**lncRNA-mRNA co-expression network.** Co-expression network analysis was performed between the 114 differentially expressed lncRNAs and the 310 differentially expressed mRNAs based on the mRMR results. In total, 113 lncRNAs and 303 mRNAs were included in the co-expression network. Moreover, our data showed that the co-expression network was composed of 5391 network nodes and 420 connections. The co-expression network indicated that one mRNA may correlate with 1–53 lncRNAs, and one lncRNA may correlate with 1 to 140 mRNAs (see File S3). Moreover, Fig. 5 reveals that 92 lncRNAs interacting with 6 mRNAs participated in the meaningful "Proteasome" pathway.

## Discussion

LncRNAs had long been considered as simply transcriptional noise[11]. However, recent studies showed that lncRNAs can regulate basal transcription, posttranscriptional processes, epigenetic modifications, DNA methylation, histone modification and even directly bind proteins, and regulate protein function[12–15]. Not until the last decade, however, has the discovery emerged that lncRNAs play an important role in diseases of the immune and nervous systems.

The first study implicating lncRNAs as regulators of the innate immune response showed that lincRNA-Cox2 is upregulated in mouse macrophages following exposure to lipopolysaccharide[16]. Subsequently, more lncRNAs were found to regulate the production of inflammatory mediators, such as LETHE, THRIL, NEAT1, PACER and IL-1β-RBT46[17,18]. A previous study focused on the involvement of lncRNA in modulating innate and adaptive immune responses, immune cell development, and differential expression of lncRNAs in autoimmune diseases[9]. In that context, although the pathogenesis of GBS has been extensively investigated, the exact molecular mechanism and epigenetic feature of this disease are still unclear. Therefore, establishing that lncRNA profiles are expressed differentially in GBS patients compared to their healthy counterparts is necessary and important.

In the present study, we investigated lncRNA and mRNA expression profiles in clinical samples from 15 GBS patients and 15 healthy controls using a microarray analysis. With mRMR software, we then ranked the mRNAs and lncRNAs according to their relevance to the class of GBS patients or healthy controls. The top 310 mRNAs and 114 lncRNAs were then identified according to their relevance to the class of GBS patients or healthy controls. These results indicated that these differentially expressed mRNAs and lncRNAs may be potential biomarkers for the diagnosis of GBS.

Based on the results of mRMR, GO and KEGG pathways, we proceeded to obtain detailed information on the biological functions and potential mechanisms of these mRNAs in GBS. GO analysis showed that these differentially expressed mRNAs based on mRMR results were enriched in top 29 GO ($-\lg^P > 2.5$), including the cytosol, cellular macromolecular complex assembly, cell cycle, ligase activity, and protein catabolic process, etc (Fig. 3 and Table 3). As shown in Fig. 4, the top 310 mRNAs were associated with top seven major pathways, of which the "Proteasome" pathway was the most significant, as previously implicated in autoimmune diseases, especially GBS. The first report describing the role of proteasomes in autoimmune diseases noted that sera from patients with SLE contained specific autoantibodies against several polypeptide components of the proteasome[19]. Since then, patients with such autoimmune diseases as polymyositis-myositis and primary Sjogren's syndrome also had autoantibodies against proteasomes[20,21]. Mengual *et al.* had shown that patients with multiple sclerosis (MS) presented with B and T cell autoreactivity against the proteasome in glial and neuronal cells[22]. Mayo *et al.* later wrote that both serum and cerebrospinal fluid (CSF) of MS patients had antibodies to almost all the polypeptide components of the proteasome. Additionally, their titres of these antibodies were 5-10-fold higher in the sera than in the CSF. Moreover, the incidence of anti-proteasome seroreactivity samples from MS patients was significantly higher than that in those from individuals with other inflammatory diseases, such as SLE, Sjogren's syndrome, or sarcoidosis[23]. The previous study indicated that proteasome may be an antigenic target that evokes the cell-mediated immune response in MS patients and, possibly more generally, in several systemic inflammatory diseases.

GBS, as an acute inflammatory autoimmune disease affecting the peripheral nervous system, has attracted growing attention. Previous study showed that both the MB1 (X) and delta (Y) proteasome subunits were expressed in Schwann cells. Moreover, staining of the proteasome subunit delta (Y) was more abundant in peripheral nerves from GBS patients compared with those from inflammation-free controls[10]. Our present results from assessing the KEGG pathway in patients with GBS also indicated meaningful emphasis on the "Proteasome" pathway, an outcome that coincided with the previous studies[10] and reinforced the veracity of our results.

| Order | mRNA | Order | mRNA | Order | mRNA |
|---|---|---|---|---|---|
| 1 | SLC35C1 | 35 | CYTH4 | 69 | FAM190B |
| 2 | LOC100507448 | 36 | STK24 | 70 | XRCC6 |
| 3 | SLC35D1 | 37 | SPTAN1 | 71 | SEPW1 |
| 4 | SLC35F2 | 38 | STAT4 | 72 | FAM160B1 |
| 5 | SLC31A1 | 39 | DCXR | 73 | FAM115C |
| 6 | ELFN1 | 40 | DDOST | 74 | SEPHS1 |
| 7 | ELF2 | 41 | SNURF | 75 | SH3KBP1 |
| 8 | ELMO1 | 42 | B4GALT3 | 76 | FER |
| 9 | ELL | 43 | SNRPD2 | 77 | RYK |
| 10 | DTX3L | 44 | SNRPC | 78 | SACM1L |
| 11 | AUP1 | 45 | SPOCK2 | 79 | FBXO7 |
| 12 | SLC9A6 | 46 | DHX8 | 80 | ZBTB2 |
| 13 | ELMO2 | 47 | SPATA21 | 81 | RUFY2 |
| 14 | ATP5O | 48 | SOX13 | 82 | FGFRL1 |
| 15 | ESCO1 | 49 | FRYL | 83 | ASL |
| 16 | ESYT2 | 50 | ZC3HAV1 | 84 | SCNN1D |
| 17 | SLA2 | 51 | ARNTL | 85 | FAM40A |
| 18 | EXOC3L1 | 52 | ZBTB6 | 86 | SDHD |
| 19 | SHOC2 | 53 | RPS14 | 87 | FBXO31 |
| 20 | SHISA5 | 54 | RRP1 | 88 | GMEB1 |
| 21 | EWSR1 | 55 | FMN1 | 89 | CCDC12 |
| 22 | SKP1 | 56 | RPL27 | 90 | CCDC23 |
| 23 | SLC25A39 | 57 | RPN1 | 91 | TSEN54 |
| 24 | ATP6V1E1 | 58 | ARPC4 | 92 | TSPAN14 |
| 25 | ATP8A1 | 59 | RPS11 | 93 | TSHZ1 |
| 26 | EPB41 | 60 | FNDC9 | 94 | CASP5 |
| 27 | DTNBP1 | 61 | ZDHHC4 | 95 | TUBA1B |
| 28 | SSBP4 | 62 | ZDHHC20 | 96 | CASP10 |
| 29 | SSNA1 | 63 | RNF113B | 97 | CARD10 |
| 30 | DEFB1 | 64 | RNASE3 | 98 | CAPZA1 |
| 31 | DDX19B | 65 | RIN3 | 99 | C12orf57 |
| 32 | DDX17 | 66 | GDPD5 | 100 | CD3E |
| 33 | SREBF1 | 67 | CYP11A1 | 101 | CD48 |
| 34 | SRP14 | 68 | FAM178B | 102 | CD244 |
| Order | mRNA | Order | mRNA | Order | mRNA |
| 103 | TRIM56 | 139 | CXCL5 | 175 | LOC100130542 |
| 104 | CCNK | 140 | SUSD1 | 176 | NR3C1 |
| 105 | CACNA2D4 | 141 | XLOC_012444 | 177 | ADAM12 |
| 106 | C22orf46 | 142 | CTAGE15P | 178 | ADD1 |
| 107 | VCPIP1 | 143 | TAP1 | 179 | OBSCN |
| 108 | VAMP5 | 144 | BIN1 | 180 | OGDH |
| 109 | C21orf91 | 145 | TACO1 | 181 | LLPH |
| 110 | VDR | 146 | BROX | 182 | ADAR |
| 111 | VAMP2 | 147 | XLOC_006443 | 183 | PARP3 |
| 112 | C3orf36 | 148 | CEP350 | 184 | PHC3 |
| 113 | USP4 | 149 | TOR1AIP1 | 185 | PGRMC2 |
| 114 | USP47 | 150 | TP73 | 186 | PFDN5 |
| 115 | UTP18 | 151 | CDK5RAP2 | 187 | PI4K2B |
| 116 | VWCE | 152 | TOE1 | 188 | KANSL2 |
| 117 | VPRBP | 153 | CLASP1 | 189 | KIF2A |
| 118 | C20orf201 | 154 | CLIC5 | 190 | KIF22 |
| 119 | C19orf66 | 155 | BRWD3 | 191 | KIAA0947 |
| 120 | USP39 | 156 | TMEM104 | 192 | KIAA1715 |
| 121 | UBE2F | 157 | TM9SF2 | 193 | KIAA1267 |
| 122 | C9orf173 | 158 | TLE4 | 194 | ABCD3 |
| 123 | UBP1 | 159 | CHCHD3 | 195 | MADD |
| 124 | UBE2E4P | 160 | CHMP4A | 196 | MPZL2 |
| Continued | | | | | |

| Order | mRNA | Order | mRNA | Order | mRNA |
|---|---|---|---|---|---|
| 125 | C5orf56 | 161 | CIAPIN1 | 197 | MAP2K7 |
| 126 | C17orf85 | 162 | CHRM4 | 198 | MR1 |
| 127 | C18orf25 | 163 | ARHGAP30 | 199 | LRP8 |
| 128 | C6orf136 | 164 | L2HGDH | 200 | LPGAT1 |
| 129 | TCEB1 | 165 | OR2A12 | 201 | MAP3K4 |
| 130 | COX17 | 166 | P4HA2 | 202 | LSM14A |
| 131 | TBC1D7 | 167 | ZNF622 | 203 | MIS12 |
| 132 | TBCA | 168 | KLRB1 | 204 | 9-Sep |
| 133 | CNGB1 | 169 | KRTAP10-3 | 205 | MDH2 |
| 134 | THRAP3 | 170 | P4HB | 206 | MDM1 |
| 135 | CORO7 | 171 | PAPD7 | 207 | METTL23 |
| 136 | CLPS | 172 | NSMCE1 | 208 | MED15 |
| 137 | CWF19L1 | 173 | LOC100127946 | 209 | MESDC1 |
| 138 | CTU2 | 174 | LOC100130342 | 210 | LOC731932 |
| Order | mRNA | Order | mRNA | Order | mRNA |
| 211 | MAPKAP1 | 247 | PSMD11 | 283 | ILDR1 |
| 212 | ABCA2 | 248 | HCST | 284 | PPA1 |
| 213 | MAPRE2 | 249 | PVRL1 | 285 | POLR2L |
| 214 | MAP3K7 | 250 | HEATR7B1 | 286 | PLXNA4 |
| 215 | AAGAB | 251 | AP2A1 | 287 | IDS |
| 216 | LOC100506191 | 252 | Q9EPR2 | 288 | ZNF350 |
| 217 | LOC100506047 | 253 | PSMD4 | 289 | PLEKHA2 |
| 218 | LOC100506906 | 254 | PRKCB | 290 | IL10RA |
| 219 | NEK9 | 255 | HLA-F | 291 | IKBKG |
| 220 | NGDN | 256 | HNRNPA1L2 | 292 | AMPD2 |
| 221 | NIPA2 | 257 | HNRNPD | 293 | AMOTL1 |
| 222 | ACTR3 | 258 | APH1A | 294 | IL12RB1 |
| 223 | NEUROG1 | 259 | PSMB1 | 295 | ANAPC13 |
| 224 | ZNF728 | 260 | HIST1H3C | 296 | ISG20L2 |
| 225 | NCK1 | 261 | PRR5 | 297 | PPP1R2 |
| 226 | LOC401480 | 262 | PSMC3 | 298 | PPP1R11 |
| 227 | LOC644285 | 263 | PSMC5 | 299 | ITCH |
| 228 | LOC400128 | 264 | PRPF6 | 300 | ZNF24 |
| 229 | MYBBP1A | 265 | HK1 | 301 | PPP2R1A |
| 230 | MVP | 266 | ZNF207 | 302 | HSP90AA2 |
| 231 | MSH6 | 267 | ARFGAP2 | 303 | HSD17B10 |
| 232 | MSRA | 268 | RB1CC1 | 304 | ZNF26 |
| 233 | LOC648044 | 269 | GPR108 | 305 | AMH |
| 234 | MYEOV2 | 270 | GPN1 | 306 | ISCU |
| 235 | NBAS | 271 | RBL2 | 307 | PPIB |
| 236 | NAP1L4 | 272 | GOLPH3 | 308 | PMF1 |
| 237 | ACOX2 | 273 | GMPS | 309 | PPP2R5D |
| 238 | LOC200726 | 274 | GNGT2 | 310 | C1QL2 |
| 239 | N4BP2 | 275 | RASA3 | | |
| 240 | HERC6 | 276 | GSTP1 | | |
| 241 | HERPUD2 | 277 | GSPT2 | | |
| 242 | HIPK2 | 278 | RAB11B | | |
| 243 | HEG1 | 279 | ZMYND11 | | |
| 244 | HINT2 | 280 | RANGRF | | |
| 245 | PSME1 | 281 | RAB8A | | |
| 246 | HBS1L | 282 | RAC1 | | |

**Table 1. Significant mRNAs based on mRMR result.**

The co-expression network analysis cited here was constructed based on the 114 differentially expressed lncRNAs and the 310 differentially expressed mRNAs, *i.e.,* in comparisons between GBS patients and healthy controls. Results showed that a total of 113 lncRNAs and 303 mRNAs were included in the co-expression network. This co-expression network, which was composed of 5391 network nodes and 420 connections, indicated that one

| Order | lncRNA | Order | lncRNA | Order | lncRNA |
|---|---|---|---|---|---|
| 1 | ENSG00000262967.1 | 35 | XLOC_004629 | 69 | FAM190B |
| 2 | ENSG00000263069.1 | 36 | ENSG00000260194.1 | 70 | XRCC6 |
| 3 | DL492557 | 37 | AX748067 | 71 | SEPW1 |
| 4 | ENSG00000234494.2 | 38 | AX747809 | 72 | FAM160B1 |
| 5 | XLOC_001920 | 39 | ENSG00000260550.2 | 73 | FAM115C |
| 6 | ENSG00000234953.2 | 40 | LOC644554 | 74 | SEPHS1 |
| 7 | XLOC_003501 | 41 | HIX0014588 | 75 | SH3KBP1 |
| 8 | XLOC_003669 | 42 | LOC729164 | 76 | FER |
| 9 | XLOC_003365 | 43 | HIX0032156 | 77 | RYK |
| 10 | ENSG00000262721.1 | 44 | LOC400027 | 78 | SACM1L |
| 11 | CR936829 | 45 | nc082 | 79 | FBXO7 |
| 12 | XLOC_001869 | 46 | ENSG00000225407.3 | 80 | ZBTB2 |
| 13 | ENSG00000233044.1 | 47 | LIT3556 | 81 | RUFY2 |
| 14 | ENSG00000232959.1 | 48 | ENSG00000225886.1 | 82 | FGFRL1 |
| 15 | ENSG00000267121.1 | 49 | LIT3584 | 83 | ASL |
| 16 | ENSG00000266963.1 | 50 | ENSG00000226266.2 | 84 | SCNN1D |
| 17 | ENSG00000266947.1 | 51 | LIT3611 | 85 | FAM40A |
| 18 | ENSG00000266936.1 | 52 | LOC100129203 | 86 | SDHD |
| 19 | ENSG00000233138.1 | 53 | ENSG00000272700.1 | 87 | FBXO31 |
| 20 | ENSG00000261609.1 | 54 | HIX0213194 | 88 | GMEB1 |
| 21 | ENSG00000266677.1 | 55 | ENSG00000227258.1 | 89 | CCDC12 |
| 22 | XLOC_000741 | 56 | HOTAIRM1 | 90 | CCDC23 |
| 23 | ENSG00000167117.4 | 57 | LOC100653021 | 91 | TSEN54 |
| 24 | ENSG00000150316.7 | 58 | ENSG00000226849.1 | 92 | TSPAN14 |
| 25 | ENSG00000237416.2 | 59 | LOC100652739 | 93 | TSHZ1 |
| 26 | XLOC_007231 | 60 | ENSG00000269609.1 | 94 | CASP5 |
| 27 | ENSG00000259260.1 | 61 | ENSG00000203875.6 | 95 | TUBA1B |
| 28 | ENSG00000259115.1 | 62 | ENSG00000203875.5 | 96 | CASP10 |
| 29 | ASO3749 | 63 | ENSG00000269371.1 | 97 | CARD10 |
| 30 | AX747758 | 64 | uc.263+ | 98 | CAPZA1 |
| 31 | ENSG00000235609.3 | 65 | uc.46- | 99 | C12orf57 |
| 32 | BC041623 | 66 | uc.454- | 100 | CD3E |
| 33 | ENSG00000235586.1 | 67 | ENSG00000267827.1 | 101 | CD48 |
| 34 | XLOC_005449 | 68 | ENSG00000196364.7 | 102 | CD244 |
| Order | lncRNA | | | | |
| 103 | ENSG00000242973.2 | | | | |
| 104 | ENSG00000244030.1 | | | | |
| 105 | AK311257 | | | | |
| 106 | XLOC_011769 | | | | |
| 107 | XLOC_011339 | | | | |
| 108 | ENSG00000249614.1 | | | | |
| 109 | ENSG00000249478.1 | | | | |
| 110 | ENSG00000243558.1 | | | | |
| 111 | AL833150 | | | | |
| 112 | ENSG00000255191.1 | | | | |
| 113 | AK289390 | | | | |
| 114 | XLOC_002473 | | | | |

**Table 2. Significant lncRNAs based on mRMR result.**

lncRNA could target at most 140 mRNAs and one mRNA could correlate with at most 53 lncRNAs (see File S3). We also found that 92 lncRNAs interacted with 6 mRNAs involved in the meaningful "Proteasome" pathway (Fig. 5). This outcome suggests that the inter-regulation of lncRNAs and mRNAs is involved in the development of GBS and warrants further study.

In conclusion, the present study using microarray data provides newfound information regarding the potential role of mRNAs and lncRNAs in GBS patients. By using mRMR software, we also found top seven supposed KEGG pathways, especially a "Proteasome" pathway, and top 29 GO during GBS development. The co-expression network identified here also indicated the inter-regulation of lncRNAs and mRNAs in GBS patients. These

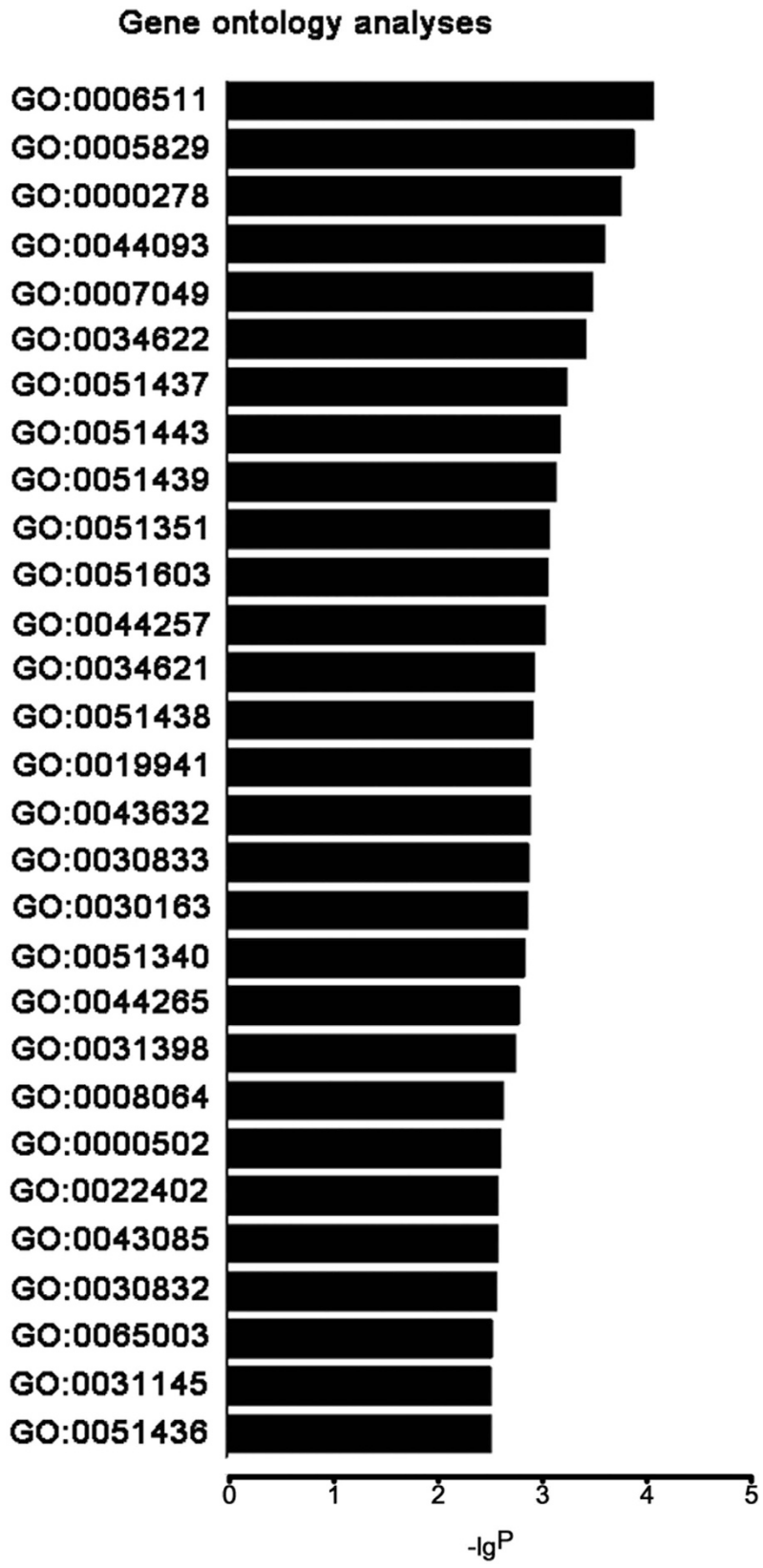


**Figure 3. Top 29 gene ontology analysis.** A total of 310 differentially expressed mRNAs were chosen based on the results of mRMR. The column graphs represent the enrichment of these mRNAs. The $(-\lg^P)$ value was a positive correlation with GO. The $(-\lg^P)$ values above 2.5 are presented. The top 29 GO are shown in detail in Table 3.

| Category | Term | Count | % | $-lg^P$ |
|---|---|---|---|---|
| GO:0006511 | Ubiquitin-dependent protein catabolic process | 14 | 4.73 | 4.044 |
| GO:0005829 | Cytosol | 39 | 13.18 | 3.859 |
| GO:0000278 | Mitotic cell cycle | 17 | 5.74 | 3.737 |
| GO:0044093 | Positive regulation of molecular function | 22 | 7.43 | 3.589 |
| GO:0007049 | Cell cycle | 26 | 8.78 | 3.469 |
| GO:0034622 | Cellular macromolecular complex assembly | 15 | 5.07 | 3.409 |
| GO:0051437 | Positive regulation of ubiquitin-protein ligase activity during mitotic cell cycle | 7 | 2.36 | 3.227 |
| GO:0051443 | Positive regulation of ubiquitin-protein ligase activity | 7 | 2.36 | 3.159 |
| GO:0051439 | Regulation of ubiquitin-protein ligase activity during mitotic cell cycle | 7 | 2.36 | 3.126 |
| GO:0051351 | Positive regulation of ligase activity | 7 | 2.36 | 3.062 |
| GO:0051603 | Proteolysis involved in cellular protein catabolic process | 21 | 7.09 | 3.046 |
| GO:0044257 | Cellular protein catabolic process | 21 | 7.09 | 3.019 |
| GO:0034621 | Cellular macromolecular complex subunit organization | 15 | 5.07 | 2.918 |
| GO:0051438 | Regulation of ubiquitin-protein ligase activity | 7 | 2.36 | 2.911 |
| GO:0019941 | Modification-dependent protein catabolic process | 20 | 6.76 | 2.882 |
| GO:0043632 | Modification-dependent macromolecule catabolic process | 20 | 6.76 | 2.882 |
| GO:0030833 | Regulation of actin filament polymerization | 6 | 2.02 | 2.862 |
| GO:0030163 | Protein catabolic process | 21 | 7.09 | 2.857 |
| GO:0051340 | Regulation of ligase activity | 7 | 2.36 | 2.826 |
| GO:0044265 | Cellular macromolecule catabolic process | 23 | 7.77 | 2.772 |
| GO:0031398 | Positive regulation of protein ubiquitination | 7 | 2.36 | 2.744 |
| GO:0008064 | Regulation of actin polymerization or depolymerization | 6 | 2.03 | 2.625 |
| GO:0000502 | Proteasome complex | 6 | 2.03 | 2.602 |
| GO:0022402 | Cell cycle process | 19 | 6.42 | 2.576 |
| GO:0043085 | Positive regulation of catalytic activity | 18 | 6.08 | 2.574 |
| GO:0030832 | Regulation of actin filament length | 6 | 2.03 | 2.563 |
| GO:0065003 | Macromolecular complex assembly | 21 | 7.09 | 2.519 |
| GO:0031145 | Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process | 6 | 2.03 | 2.504 |
| GO:0051436 | Negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle | 6 | 2.03 | 2.504 |

**Table 3. Top 29 GO analyses.**

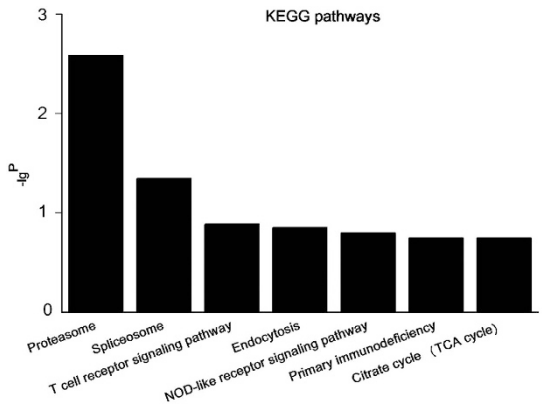


**Figure 4. KEGG pathways.** A total of 310 differentially expressed mRNAs were chosen based on the results of mRMR. The column graphs represent the enrichment of these mRNAs. The top seven significantly enriched KEGG pathways were calculated when plotted as the $-lg^P$.

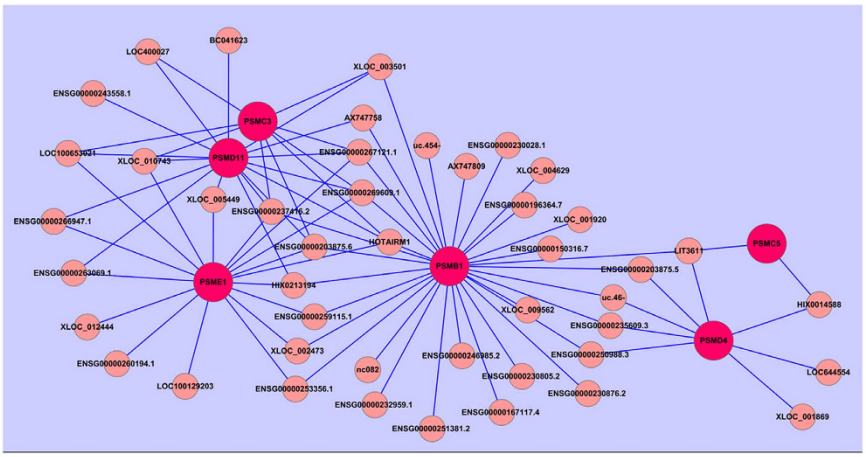


**Figure 5. LncRNA-mRNA co-expression network in the "Proteasome" pathway.** Here, 92 lncRNAs were interacting with 6 mRNAs in the meaningful "Proteasome" pathway.

findings may provide basic mechanistic information, possible biomarkers, and novel treatment strategies for patients afflicted with GBS.

## Experimental Procedures

**Patients and sample collection.** For this study, we enrolled 15 GBS patients who fulfilled the standard diagnostic criteria for GBS in Tianjin Medical University General Hospital between 2014 and 2015[24]. When their blood was sampled, these patients were within the peak timing of manifesting GBS and before treatment with intravenous immune globulin (IVIG), plasma exchange or glucocorticoid. We also recruited 15 age- and gender-matched healthy controls for the comparative study. Informed consent was obtained at enrollment from all patients or legally acceptable surrogates. The study was carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. The present study was approved by the ethical review committees of Tianjin Medical University General Hospital. Peripheral blood anticoagulated by ethylene diamine tetraacetic acid (EDTA) was obtained from all GBS patients and healthy controls. Human PBMCs were isolated with Ficoll-Hypaque gradients.

**RNA extraction.** For RNA purification, we used Trizol reagent (Invitrogen) according to the manufacturer's instructions followed by application of PBMC to RNeasy spin columns (Qiagen, Venlo, Limburg, Netherlands). The RNA was quantified and the quality evaluated using a Nanodrop and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively. The individual RNA samples were stored at −80 °C until further use.

**Arraystar human lncRNA Microarray V3.0.** The labeled cRNAs were hybridized onto the human LncRNA Expression Microarray V3.0 (Arraystar, Rockville, MD), which was designed for the global profiling of human lncRNAs and protein-coding transcripts. The third lncRNA microarray generated for each sample detected approximately 30586 lncRNAs and 26109 coding transcripts. Then, lncRNAs were carefully constructed using well-respected public transcriptome databases (Refseq, UCSC Known Genes, and Genecode), as well as landmark publications.

**Quantitative Real-time PCR validation.** Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) is the gold standard for data verification. For the reverse transcriptase (RT) reaction, SYBR Green RT reagents (Bio-Rad, USA) were used. In brief, the RT reaction was performed for 60 min at 37 °C, followed by 60 min at 42 °C, using oligo (dT) and random hexamers. PCR amplifications were performed using SYBR Green Universal Master Mix. In brief, reactions were performed in duplicate containing 2× concentrated Universal Master Mix, 1 μL of template cDNA, and 100 nM of primers in a final volume of 12.5 μL, followed by analysis in a 96-well optical reaction plate (Bio-Rad). The lncRNA PCR results were quantified using the 2ΔΔct method against β-actin for normalization. The data represent the means of three experiments.

**mRMR method.** The mRMR method was used to rank the importance of all features[25–27]. The mRMR method ranks these features based on not only their relevance to the target, but also the redundancy between features. A smaller index of a feature indicates that the latter index provides a better trade-off between maximum relevance to the target and minimum redundancy. The mutual information (MI) function, which estimates the extent to which one vector is related to another, quantifies both relevance and redundancy. The MI is defined as:

$$I(x, y) = \iint p(x, y) \log \frac{p(x, y)}{p(x)p(y)} dxdy \quad (1)$$

In equation (1), x and y are vectors, p(x, y) is their joint probabilistic density, and p(x) and p(y) are the marginal probabilistic densities. V supposedly denotes the entire feature set. Vs denotes the already-selected feature set containing m features, and Vt is used to denote the to-be-selected feature set containing n features. The relevance D between the target c and the feature f in Vt can be calculated by:

$$D = I(f, c) \tag{2}$$

The redundancy R between all the features in Vs and the feature f in Vt can be calculated by:

$$R = \frac{1}{m} \sum_{f_i \in \Omega_s} I(f, fi) \tag{3}$$

To determine the feature fj in Vt with maximum relevance and minimum redundancy, the mRMR function combines equation (2) and equation (3) and is defined as:

$$\max_{f_j \in \Omega_t} \left[ I(f_j, c) - \frac{1}{m} \sum_{f_i \in \Omega_s} I\left(f_j, f_i\right) \right] (j = 1, 2, \ldots, n) \tag{4}$$

Then, the mRMR feature evaluation will continue N rounds when given a feature set with N (N = m+n) features. After evaluating the mRMR feature, a feature set S is obtained:

$$S = \{f_1', f_2', \ldots, f_h', \ldots, f_N'\} \tag{5}$$

In this feature set S, the index h of each feature indicates at which round the feature is selected. The smaller the index h, the earlier the feature satisfies equation (4) and the better the feature is.

**GO and KEGG pathway analysis.** GO was used to describe genes and gene product attributes, including cellular components, molecular functions, and biological processes. GO not only organizes genes into hierarchical categories but also uncovers the gene regulatory network on the basis of biologic processes and molecular functions[28]. KEGG mapping was used to predict the main pathways of the differentially expressed genes. DAVID method was used to select the main pathway with the significance threshold defined with *P* value and FDR[29].

**Analysis of the lncRNA-mRNA co-expression network.** Based on the correlation between the differentially expressed lncRNAs and mRNAs, the lncRNA-mRNA co-expression network was built. The network was constructed according to the normalized signal intensities of specific expression levels of mRNAs and lncRNAs. We used Pearson's correlation coefficients, equal to or greater than 0.8, to identify the lncRNAs and coding genes. Then, the lncRNA-mRNA co-expression network was constructed by Cytoscape software (The Cytoscape Consortium, San Diego, CA, USA).

**Statistical analysis.** All statistical data were analyzed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Differences in lncRNA expression between the GBS patients and healthy controls were analyzed using mRMR software. Statistical differences were considered significant at $P < 0.05$.

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## Author Contributions

J.H., J.X. and C.G. designed the project and wrote the paper. J.X., C.G., F.Z., X.M., X.P., G.L., R. Z., D.K., A.S. and L.H. conducted the experiments. All authors reviewed and approved the final manuscript.

## Additional Information

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