

Potential Markers of Autoimmune Diseases, Alleles rs115662534(T) and rs548231435(C), Disrupt the Binding of Transcription Factors STAT1 and EBF1 to the Regulatory Elements of Human *CD40* Gene

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Abstract—CD40 receptor is expressed on B lymphocytes and other professional antigen-presenting cells. The binding of CD40 to its ligand CD154 on the surface of T helper cells plays an important role in the activation of B lymphocytes required for production of antibodies, in particular, against autoantigens. Association of several single nucleotide polymorphisms (SNPs) located in the non-coding areas of human *CD40* locus with the elevated risk of autoimmune diseases has been demonstrated. The most studied of these SNPs is rs4810485 located in the first intron of the *CD40* gene. Expression of the *CD40* gene in B lymphocytes of donors homozygous for the common allelic variant of this polymorphism (G) is higher than in B cells from donors carrying the minor (T) variant. We investigated the enhancer activity of this fragment of the *CD40* locus in human B cell lines and showed that it is independent on the rs4810485 alleles. However, the minor allelic variants of the rs4810485-linked SNPs rs548231435 and rs115662534 were associated with a significant decrease in the activity of the *CD40* promoter due to the impairments in the binding of EBF1 and STAT1 transcription factors, respectively.

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The *CD40* gene encodes CD40 receptor protein belonging to the tumor necrosis factor (TNF) superfamily. CD40 is expressed on the surface of B cells, macrophages, dendritic cells, fibroblasts, endothelial and smooth muscle cells, etc. [1]. Interactions between CD40 and its cognate ligand CD154 expressed on the T cells play an important role in the B cell activation, antibody isotype switching, and formation of memory B cells [2,

3]. CD40 expressed on dendritic cells is essential for the primary T cell immune response against a broad range of various immunogens [4, 5]. CD40 expressed on the surface of macrophages ensures efficient cell response against intracellular bacteria [6, 7]. Similarly to various regulators of adaptive immunity, CD40 also plays a role in the development of autoimmune disorders, such as autoimmune thyroiditis, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, and psoriasis [1]. Blocking the CD154–CD40 axis interferes with the development of autoimmune pathologies in mice [5, 8] and non-human primates [9, 10]. However,

Abbreviations: SNP, single nucleotide polymorphism; TNF, tumor necrosis factor.

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clinical trials with CD40-suppressing antibodies have been terminated mainly due to the adverse side effects of the tested preparations [11].

The relationship between single nucleotide polymorphisms (SNPs) in the non-coding region of the *CD40* gene and the risk of autoimmune disorders has been demonstrated. Moreover, for some of the SNPs, molecular mechanisms underlying this relationship were proposed. For instance, the rs1883832 polymorphism associated with the risk of Graves' disease is located within the Kozak sequence, so that the CD40 mRNA carrying the minor T allele is translated 15% less efficiently than the mRNA with the C allele [12, 13]. On the other hand, the polymorphism rs4810485 found in the first intron of the *CD40* gene was shown to be associated with a risk of multiple autoimmune diseases [14-16]. Donors homozygous by the major rs4810485 variant (G) have an elevated level of *CD40* mRNA in the peripheral blood cells [17], as well as an increased number of B cells with upregulated CD40 expression [16].

Here, we used the B lymphoblastoid MP1 cell line bearing the reporter gene to demonstrate that the activity of the regulatory element within the first intron of the *CD40* gene does not depend on the rs4810485 allelic variant. We also searched for the linked polymorphisms that potentially influence the *CD40* gene expression using an approach earlier applied to examine the *CD25* gene [18]. Two rs4810485-linked candidate SNPs (rs548231435 and rs115662534) in the *CD40* gene were identified whose allelic variants significantly affected the activity of the *CD40* gene promoter by altering the binding of the EBF1 and STAT1 transcription factors, respectively.

MATERIALS AND METHODS

Cell line, transfection, *EBF1* and *STAT1* gene knock-down, luciferase reporter assay. MP1 B lymphoblastoid cells [19] (kindly provided by E. A. Clark, University of

Table 2. Primers for cloning DNA regulatory elements and for introduction of minor polymorphic variants

Primer	Nucleotide sequence 5'-3'
CD40pr-H3F	5'-ATATAAGCTTCCCGAGTAGCTGGGATTACAGG-3'
CD40pr2-Pag1R	5'-TAATTCATGACGAGGTGAGAC-CAGGC-3'
CD40enh1-BH1F	5'-TTAAGGATCCTCTGCAGTGCG-TCCCTCG-3'
CD40enh1-SalR	5'-TAATGTGCGACAGGCAGTGTTT-GGCTCAGAG-3'
Control-enh-BH1F	5'-AGGATCCGGATTACAGGTGTA-TTTCACCAT-3'
Control-enh-SalR	5'-TATGTGCGACGTTGATGTAATT-CCTTTAAATCTAT-3'
rs4810485(T)F	5'-TGTAGATTCTGCCTGAAGCC-TGGCA-3'
rs4810485(T)R	5'-GCTTCAGGCAGGAATCTACAGCCCTC-3'
Prs548231435(C)F	5'-ACTCCCGGCGGAATTAGAC-3'
Prs548231435(C)R	5'-TCTAATCCGCCGGGAGTTTA-3'
Ers576918178(T)F	5'-CCCGTTTTCTGTCCCCTTC-3'
Ers576918178(T)R	5'-GGGACAGAAAACGGGCCAG-3'
Prs115662534(T)F	5'-AAACTCTTCTTTGAAACGCCT-CC-3'
Prs115662534(T)R	5'-GGCGTTTCAAAGAAGAGTTT-CC-3'
Prs11569301(T)F	5'-CCTCGTTCGGGCGCCCA-3'
Prs11569301(T)R	5'-CGCCGAACGAGGCCTC-3'

Table 1. RNA oligonucleotides bearing two 3'-deoxyribonucleotides for generating siRNAs

Primer	Nucleotide sequence 5'-3'
EBF1siRNA-F*	5'-CCACGAGCAUGAACGGAUATT-3'
EBF1siRNA-R*	5'-UAUCCGUUCAUGCUCGUGGTG-3'
EBF1siRNA-scF	5'-GCAAAUGACACGCGUAGCATT-3'
EBF1siRNA-scR	5'-UGCUACGCGUGUCAUUUGCTG-3'
STAT1siRNA-F**	5'-CAGAAAGAGCTTGACAGTAAA-3'
STAT1siRNA-R**	5'-UUUACUGUCAAGCUCUUUCTG-3'
STAT1siRNA-scF	5'-GAGAGAACAACAATTGAGTA-3'
STAT1siRNA-scR	5'-UACUCAAGUUGUUGUUCUCTC-3'

* See [22].

** See [21].

Washington, USA) were grown in RPMI1640 medium (PanEko, Russia) supplemented with 10% fetal bovine serum (Biosera, France), 2 mM L-glutamine, penicillin/streptomycin, and 1 mM sodium pyruvate (Thermo Fisher Scientific, USA). *EBF1* and *STAT1* gene knock-downs were performed as published in [20] using short interfering RNAs (siRNAs) described in [21, 22] and control siRNAs designed using the Invivogen siRNA Wizard v3.1 tool (Table 1). Electroporation and luciferase reporter assay were performed as described in [18].

Plasmid constructs bearing the reporter gene.

Plasmids containing the promoter (886 bp) and putative enhancer (1353 bp) sequences from the *CD40* gene and the control element from the first intron of the *STAT3* gene (~1100 bp) lacking the enhancer activity [18] were amplified with the primers shown in Table 2 using MP1 cell genomic DNA as a template. Genomic DNA was iso-

Table 3. Primers for generation and quantitative analysis of amplicons used in immunoprecipitation

Primer	Nucleotide sequence 5'-3'
rs548231435-PD-F	5'-ATGGGATCTCTCTCCGGTCG-3'
rs548231435-PD-R	5'-ATTCACCGCGCAGGAGTTTC-3'
rs115662534-PD-F	5'-GAGACGACGCGCCCAC-3'
rs115662534-PD-R	5'-TCCGCTGCCTCTAGGTATCC-3'
PD-Control-F*	5'-AGGAAGGTTTTTCAATGTGAT-TTCTACATC-3'
PD-Control-R*	5'-TTCCCCTGCTCCCTCCAAGAC-3'

* See [18].

Table 4. Primers for analysis of transcription factor expression

Primer	Nucleotide sequence 5'-3'
STAT1-RT-F	5'-CCTCCTGTCACAGCTGGATG-3'
STAT1-RT-R	5'-TCCCCGACTGAGCCTGATTA-3'
EBF1-RT-F	5'-AGCCAACAGCGAAAAGACCA-3'
EBF1-RT-R	5'-TTCGGTTGCCACAGCTTTTC-3'

lated with a Genomic DNA Purification Kit (Thermo Fisher Scientific). The promoter sequence was cloned into the pGL3-basic luciferase reporter vector (Promega, USA) by the HindIII and PstI sites. The putative enhancer and the control element were cloned by the BamHI and SalI sites into the pGL3-basic vector bearing the *CD40* gene promoter. Single nucleotide substitutions were introduced into the promoter and putative enhancer sequences by the two-stage PCR with polymorphism-specific internal overlapping primers and common external primers (CD40pr-H3F/PstI and CD40enh1-BH1F/SalI) (Table 2). The regimes for the single- and two-stage PCRs were described in [18, 23]. All mutant PCR products were used to replace the original sequences in the corresponding luciferase reporter vectors. The plasmids were isolated using NucleoBond Xtra Midi Kit (Macherey-Nagel, Germany) and verified by Sanger sequencing (Genome Common Use Center, Moscow).

In vitro immunoprecipitation of DNA–protein complexes (pull-down assay). To evaluate the effect of various allelic polymorphisms on the binding of transcription factors, we studied immunoprecipitation of the obtained PCR products as described in [20]. For this, we amplified DNA sequences containing both variants of the rs548231435 and rs115662534 polymorphisms with the rs548231435-PD-F/R and rs115662534-PD-F/R primer pairs (Table 3) using the plasmids carrying these polymorphic variants as templates. A fragment of the *IL2RA* gene lacking the binding sites for EBF1 and STAT1 was

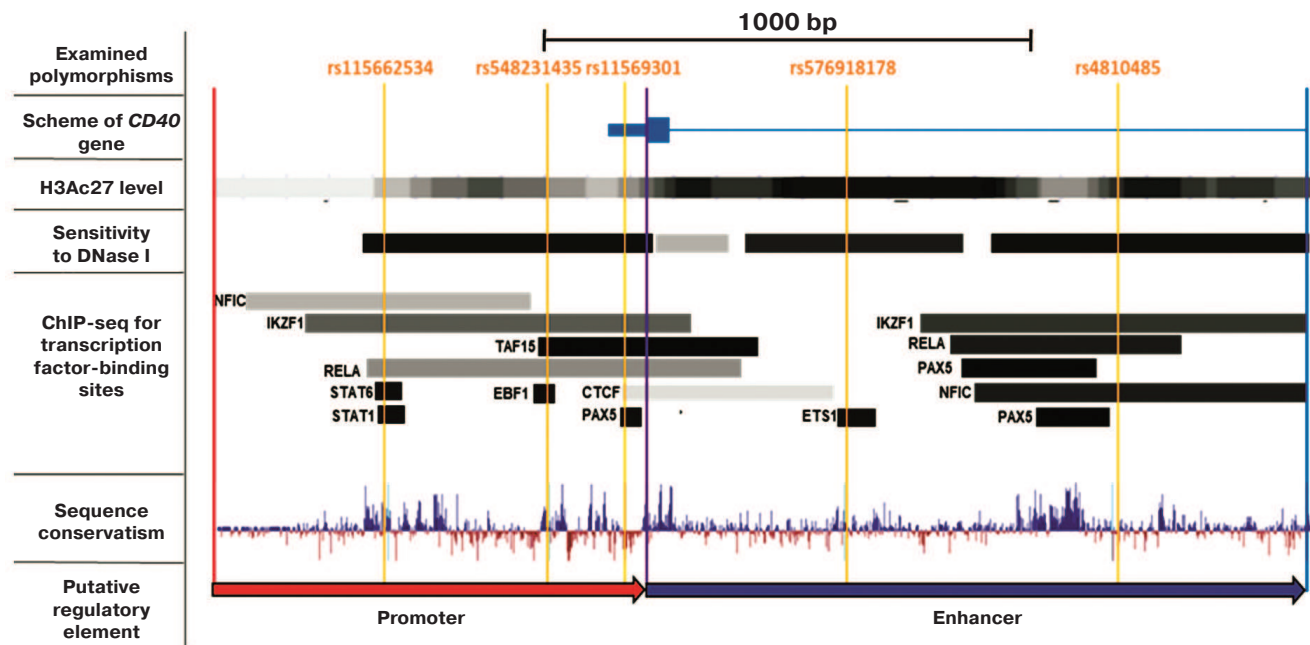


Fig. 1. Positions of the examined polymorphisms and putative regulatory elements within the *CD40* gene locus (based on data visualized by the UCSC and GTRD genome browser). Red and blue arrows denote the promoter and putative enhancer in the *CD40* gene, respectively. Orange lines mark position of the examined polymorphisms. Based on data visualized by UCSC and GTRD genome browser.

amplified with the PD-Control-F/R primers ([18], Table 3) and MP1 cell genomic DNA as a template to be used as a control. All amplified fragments had similar length: amplicon with the rs548231435 polymorphism – 196 bp, amplicon with the rs115662534 polymorphism – 197 bp, control amplicon – 189 bp. DNA–protein complexes were immunoprecipitated from the MP1 cell nuclear extracts as described in [20] using the following antibodies: rabbit polyclonal IgGs against EBF1 (Antibodies-online Inc., USA) and ESR2 (Thermo Fisher Scientific), anti-STAT1 and anti-ESR1 mouse IgGs (Abcam, UK).

RNA isolation, cDNA synthesis, and quantitative real-time PCR. Total RNA from MP1 cells was isolated using the ExtractRNA reagent (Evrogen, Russia).

Reverse transcription was performed by using 2 μ g total RNA and an MMLV RT Kit (Evrogen). Quantitative PCR was performed as published before [20] using the primers shown in Table 4.

RESULTS

The enhancer activity of the rs4810485-containing region of the *CD40* gene first intron does not depend on the SNP allelic variant. The effect of the rs4810485 polymorphism on the *CD40* gene expression was examined by cloning the *CD40* promoter (–886/0 bp upstream of the translation initiation site) and its putative enhancer

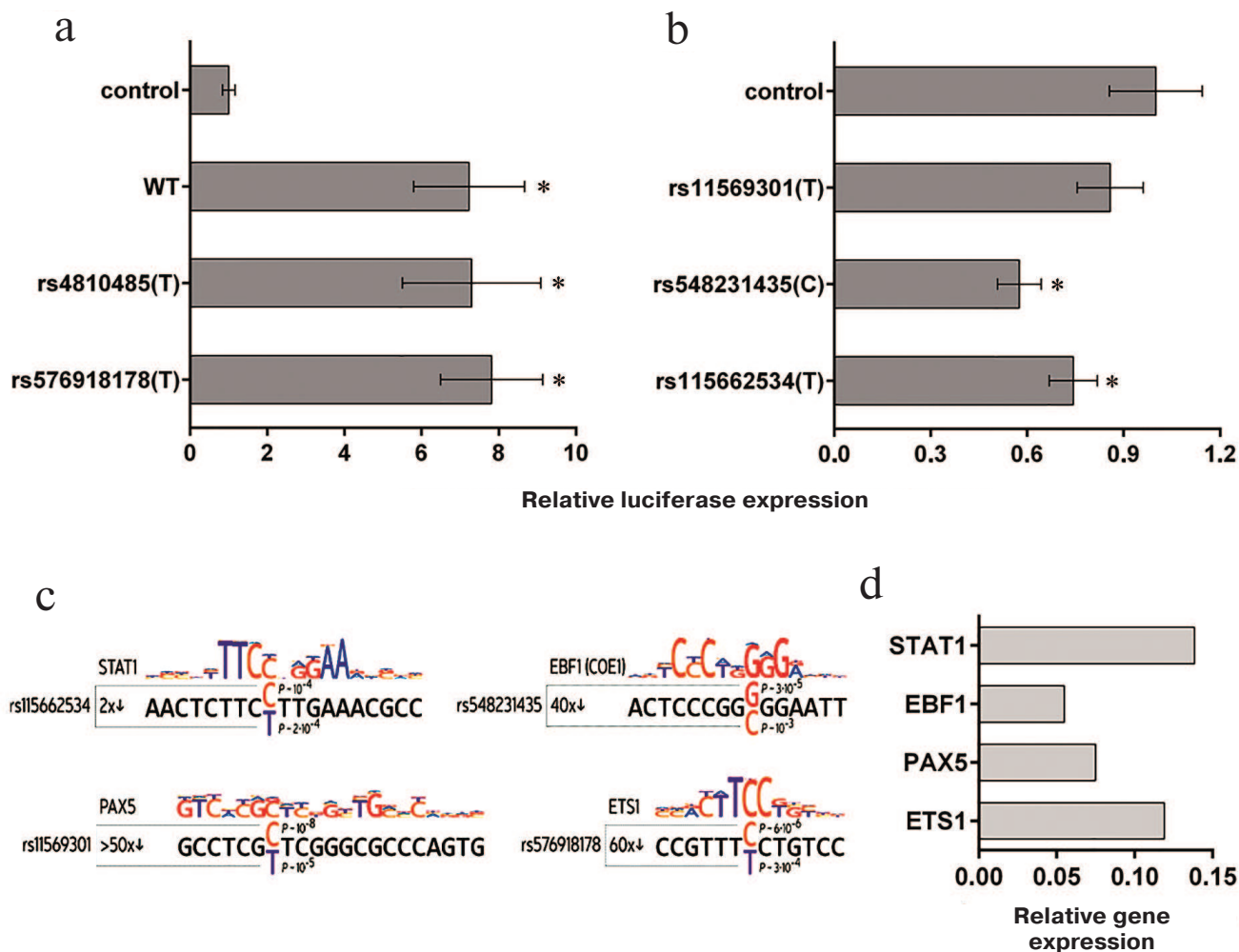


Fig. 2. Effect of the examined polymorphisms in the *CD40* gene locus on the activity of the *CD40* putative enhancer (a) and promoter (b) in MP1 cells. Activity of the reporter plasmids was normalized to the activity of the control plasmid containing the *CD40* promoter with major examined polymorphic variants and a fragment of the *STAT3* gene locus lacking the enhancer activity (control). The WT plasmid contained the *CD40* promoter and putative enhancer bearing all major examined polymorphic variants. The rs4810485(T) and rs576918178(T) vectors carried minor variants of enhancer polymorphisms. Plasmids rs11569301(T), rs548231435(C), and rs115662534(T) differed from the control plasmid by carrying the minor variants of the corresponding polymorphisms. The data from at least seven experiments are presented as mean \pm SEM in both plots; *, significant difference between the plasmid with minor polymorphic variants and WT control ($p < 0.05$, Student's *t*-test). c) Examined polymorphic variants and visualization of the sequences involved in the binding of transcription factors (modality of the changes in the affinity and *p*-values for the alternative alleles) are shown [27]. d) mRNAs levels for transcription factors in MP1 cells normalized to the amount of β -actin mRNA ($p < 0.05$, Student's *t*-test).

(0/1353 bp upstream of the translation initiation site) containing this SNP into the reporter gene plasmid coding for firefly luciferase (FLUC). According to the ENCODE project data [24], similar regions in the genome of the GM12878 B cell line display features typical for promoter and enhancer elements, e.g., high level of acetylation of lysine 27 in histone H3 (H3K27Ac), hypersensitivity to DNase I, the presence of transcription factor-binding motifs, and sequence conservatism [24] (Fig. 1). Although the region $-711/0$ bp was described earlier as the *CD40* promoter [25], here, we used the $-886/0$ bp region containing all transcription factor-binding sites identified by ChIP-Seq assay [24, 26] (Fig. 1).

We found that the putative enhancer element was highly efficient in the MP1 cells and upregulated activity of the *CD40* gene promoter approximately 7-fold (Fig. 2a). However, no significant difference in the activity of enhancer elements bearing alternative rs4810485 allelic polymorphisms was found (Fig. 2a).

Identification and functional analysis of rare rs4810485-linked polymorphic alleles. We suggested that the association between the minor allele of the rs4810485 polymorphism and reduced risk of autoimmune disorders might be related to the effects caused by the minor variants of other linked SNPs that have been overlooked in the current genome-wide studies due to a low population prevalence (less than 5%) and insignificantly elevated morbidity (less than 3-fold) [28]. We have searched for SNPs that were located in the active chromatin region and resided directly within the transcription factor-bind-

ing sites, whose interaction with the corresponding transcription factors in B cells have been verified experimentally. The information on the interactions between the examined motifs and various transcription factors was taken from the earlier study [18], ENCODE project [24], and new GTRD database containing results from more than 12,000 experiments [26]. Analysis of these data resulted in the identification of another enhancer polymorphism (rs576918178) located in the ETS1-binding site and three polymorphisms (rs11569301, rs548231435, and rs115662534) located in the binding sites for PAX5, EBF1, and STAT1, respectively, in the *CD40* promoter (Figs. 1 and 2c).

The effect of alternative alleles of selected polymorphisms on the regulatory activity of the corresponding *CD40* gene regions was tested in MP1 B lymphoblastoid cell line. As demonstrated earlier [20], all genes encoding the above-mentioned transcription factors are expressed in these cells at relatively high levels (Fig. 2d). We found that the activity of the reporter plasmid bearing the *CD40* gene promoter and putative enhancer did not depend on the allelic variant of rs576918178 (Fig. 2a), whereas the activity of the vector bearing the *CD40* gene promoter only was independent of the rs11569301 allelic variants (Fig. 2b). On the other hand, introduction of the minor allelic variants rs548231435(C) and rs115662534(T) substantially reduced the promoter activity (Fig. 2b).

Minor variants rs548231435(C) and rs115662534(T) impair the binding of the EBF1 and STAT transcription factors to the *CD40* gene promoter *in vitro*.

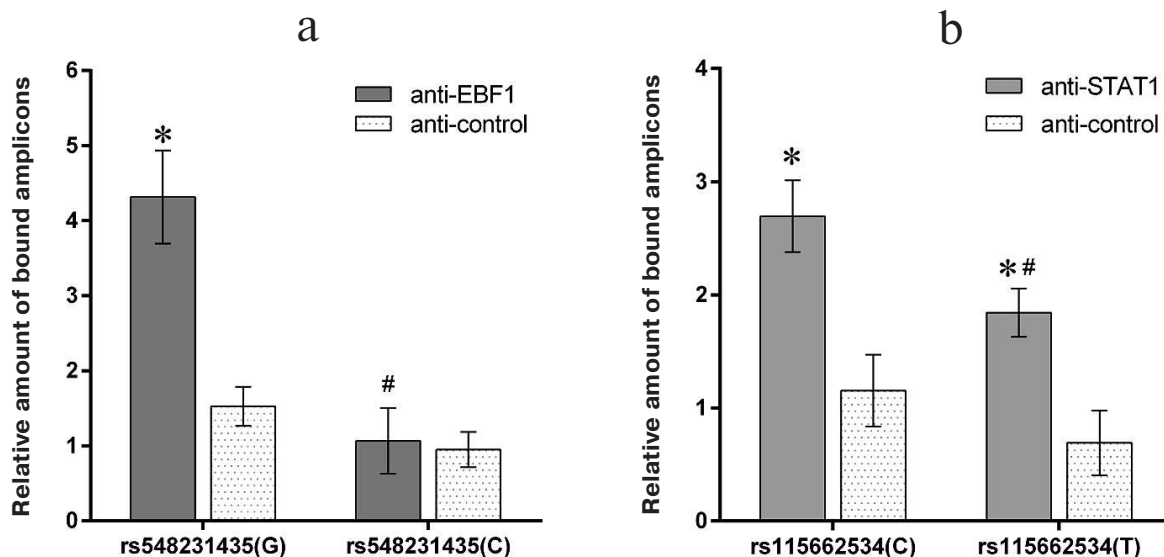


Fig. 3. Effect of the rs548231435 (a) and rs115662534 (b) polymorphisms on the ability of the *CD40* gene promoter to bind transcription factors STAT1 and EBF1 in MP1 cells. Amplicons containing various polymorphic variants were precipitated from the nuclear extracts of MP1 cells using anti-STAT1 and anti-EBF1 antibodies. The amount of the amplicons pulled down with the antibodies was estimated by real-time PCR and normalized to the amount of bound amplicon lacking the EBF1- and STAT1-binding sites. Samples treated with the isotype control antibodies were used as a negative control. The data from at least three experiments are presented as mean \pm SEM in both plots; *, significant difference between the examined sample and negative control; #, significant difference between vectors bearing minor polymorphic variants and wild-type sequence ($p < 0.05$, Student's *t*-test).

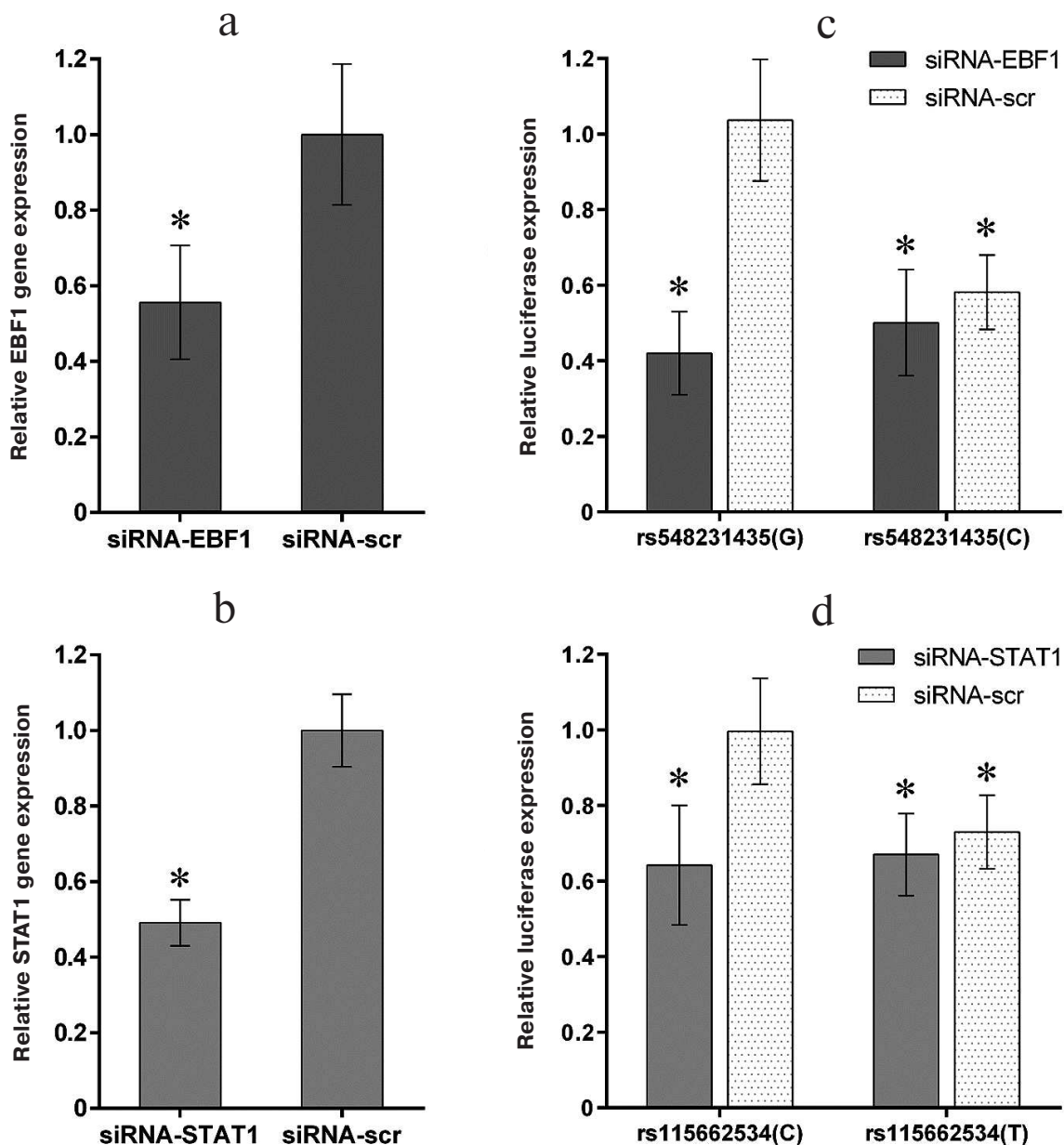


Fig. 4. Suppression of *STAT1* and *EBF1* gene expression in MP1 B lymphoblastoid cell line reduces the activity of polymorphic variants of the *CD40* promoter bearing rare rs548231435 and rs115662534 alleles: suppression of *EBF1* (a) and *STAT1* (b) mRNA expression by specific siRNAs in MP1 cells; activity of *CD40* gene promoter variants bearing rs548231435 and rs115662534 alleles upon decreased expression of *EBF1* (c) and *STAT1* (d); siRNA-scr, control siRNA with scrambled nucleotide sequence. The data from at least five experiments are presented as mean \pm SEM; * in (a) and (b), significant difference with the control siRNA-scr; * in (c) and (d), significant difference with the wild-type promoter in the absence of specific siRNA ($p < 0.05$, Student's *t*-test).

Immunoprecipitation of DNA–protein complex (pull-down assay) demonstrated that the amplicon bearing the more common rs548231435(G) allele binds EBF1 three times better compared to the amplicon containing the minor rs548231435(C) allele or the control amplicon fully lacking the EBF1-binding sites (Fig. 3a). Moreover, the binding of STAT1 to the amplicon containing the less common allele rs115662534(T) was lower by 30% that the

binding of the more frequent rs115662534(C) polymorphic variant. Both variants, however, bound to STAT1 significantly better than the control amplicon (Fig. 3b), which might be due to the fact that the rs115662534 polymorphism is located outside of the conserved STAT1-binding site (Fig. 2c). The lack of ESR1/2-binding site in all the examined amplicons allowed us to use anti-ESR2 and anti-ESR1 antibodies as isotype controls. All the

amplicons were precipitated with control antibodies with a similar efficiency (Fig. 3).

Suppression of the EBF1 and STAT1 expression affects the activity of the CD40 gene promoter depending on the polymorphic allelic variants of rs548231435 and rs115662534. To verify the specificity of EBF1 and STAT1 effects on the transcriptional activity of the examined CD40 gene promoter variants in B cells, expression of the EBF1 and STAT1 genes were downregulated using short interfering RNAs (siRNAs) that reduced the expression of these genes ~2-fold (according to real-time quantitative PCR) (Fig. 4, a and b). Suppression of the EBF1 expression by 2-fold resulted in 50–60% decrease in the promoter activity of the wild-type CD40 gene but had virtually no effect on the activity of the promoter bearing the minor allele rs548231435(C) (Fig. 4c), which correlates well with weak interaction between this rare polymorphic variant of the CD40 gene promoter and EBF1 protein.

Downregulation of the STAT1 expression decreased the promoter activity of the wild-type CD40 gene by 30–40%, whereas introduction of the minor rs115662534(T) variant attenuated this effect (Fig. 4d). Because the used siRNAs were strictly specific to the STAT1 gene sequence [21], it can be suggested that the observed effect was due to the binding of STAT1, and not of other STAT proteins, to the site bearing the rs115662534 polymorphism.

DISCUSSION

It has been demonstrated before that expression of the CD40 protein is elevated during the development of various autoimmune diseases [8, 29]. The upregulation of CD40 expression in lymphocytes and antigen-presenting cells in autoimmune processes may be related to the increased activity of the EBF1 and STAT1 transcription factors [25, 30, 31]. Therefore, it is reasonable to suggest that rare alleles of rs548231435 and rs115662534 polymorphisms could play a protective role in the development of autoimmune diseases via affecting the binding of EBF1 and STAT1 to the CD40 gene promoter. Low frequency of these such protective alleles may result from their rejection in the process of evolution, since low body capacity to efficiently combat infectious diseases poses serious threat, especially in the pre-reproductive period. On the other hand, alleles increasing the risk of autoimmune disorders exhibit activity in adulthood and, therefore, affect less the ability to produce an offspring [32]. Hence, it can be expected that the majority of people possess alleles associated with an increased immune response.

At present, the incidence rate and the threat of numerous infectious diseases have been markedly decreased due to the wide use of vaccines and antibiotics. On the contrary, the morbidity of diseases associated with autoimmune processes (e.g., type I and II diabetes melli-

tus, rheumatoid arthritis, neurodegenerative diseases, etc.) has substantially increased over the last centuries primarily due to the life expectancy increase [33–35]. Because of this, identification of rare polymorphisms in genes encoding immune checkpoint regulators associated with a decreased risk of various autoimmune responses is of special importance. With the increasing efficacy and safety of genome editing techniques, introduction of such alleles into the genome of human hematopoietic cells might become an attractive approach for the therapy of autoimmune pathologies.

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Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

1. Chatzigeorgiou, A., Lyberi, M., Chatzilimperis, G., Nezos, A., and Kamper, E. (2009) CD40/CD40L signaling and its implication in health and disease, *Biofactors*, **35**, 474–483.
2. Grewal, I. S., and Flavell, R. A. (1998) CD40 and CD154 in cell-mediated immunity, *Annu. Rev. Immunol.*, **16**, 111–135.
3. Lane, P., Traunecker, A., Hubele, S., Inui, S., Lanzavecchia, A., and Gray, D. (1992) Activated human T cells express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes, *Eur. J. Immunol.*, **22**, 2573–2578.
4. Cella, M., Scheidegger, D., Palmer-Lehmann, K., Lane, P., Lanzavecchia, A., and Alber, G. (1996) Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation, *J. Exp. Med.*, **184**, 747–752.
5. Summers deLuca, L., and Gommerman, J. L. (2012) Fine-tuning of dendritic cell biology by the TNF superfamily, *Nat. Rev. Immunol.*, **12**, 339–351.
6. Stout, R. D., Suttles, J., Xu, J., Grewal, I. S., and Flavell, R. A. (1996) Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice, *J. Immunol.*, **156**, 8–11.
7. Suttles, J., and Stout, R. D. (2009) Macrophage CD40 signaling: a pivotal regulator of disease protection and pathogenesis, *Semin. Immunol.*, **21**, 257–264.

8. Peters, A. L., Stunz, L. L., and Bishop, G. A. (2009) CD40 and autoimmunity: the dark side of a great activator, *Semin. Immunol.*, **21**, 293-300.
9. Choi, E. W., Lee, K. W., Park, H., Kim, H., Lee, J. H., Song, J. W., Yang, J., Kwon, Y., Kim, T. M., Park, J. B., and Kim, S. (2018) Therapeutic effects of anti-CD154 antibody in cynomolgus monkeys with advanced rheumatoid arthritis, *Sci. Rep.*, **8**, 2135.
10. 't Hart, B. A., Blezer, E. L., Brok, H. P., Boon, L., de Boer, M., Bauer, J., and Laman, J. D. (2005) Treatment with chimeric anti-human CD40 antibody suppresses MRI-detectable inflammation and enlargement of pre-existing brain lesions in common marmosets affected by MOG-induced EAE, *J. Neuroimmunol.*, **163**, 31-39.
11. Croft, M., Benedict, C. A., and Ware, C. F. (2013) Clinical targeting of the TNF and TNFR superfamilies, *Nat. Rev. Drug Discov.*, **12**, 147-168.
12. Jacobson, E. M., Concepcion, E., Oashi, T., and Tomer, Y. (2005) A Graves' disease-associated Kozak sequence single-nucleotide polymorphism enhances the efficiency of CD40 gene translation: a case for translational pathophysiology, *Endocrinology*, **146**, 2684-2691.
13. Jacobson, E. M., Huber, A. K., Akeno, N., Sivak, M., Li, C. W., Concepcion, E., Ho, K., and Tomer, Y. (2007) A CD40 Kozak sequence polymorphism and susceptibility to antibody-mediated autoimmune conditions: the role of CD40 tissue-specific expression, *Genes Immun.*, **8**, 205-214.
14. Lee, Y. H., Bae, S. C., Choi, S. J., Ji, J. D., and Song, G. G. (2015) Associations between the functional CD40 rs4810485 G/T polymorphism and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis, *Lupus*, **24**, 1177-1183.
15. Raychaudhuri, S., Remmers, E. F., Lee, A. T., Hackett, R., Guiducci, C., Burtt, N. P., Gianniny, L., Korman, B. D., Padyukov, L., Kurreeman, F. A., Chang, M., Catanese, J. J., Ding, B., Wong, S., van der Helm-van Mil, A. H., Neale, B. M., Coby, J., Cui, J., Tak, P. P., Wolbink, G. J., Crusius, J. B., van der Horst-Bruinsma, I. E., Criswell, L. A., Amos, C. I., Seldin, M. F., Kastner, D. L., Ardlie, K. G., Alfredsson, L., Costenbader, K. H., Altshuler, D., Huizinga, T. W., Shadick, N. A., Weinblatt, M. E., de Vries, N., Worthington, J., Seielstad, M., Toes, R. E., Karlson, E. W., Begovich, A. B., Klareskog, L., Gregersen, P. K., Daly, M. J., and Plenge, R. M. (2008) Common variants at CD40 and other loci confer risk of rheumatoid arthritis, *Nat. Genet.*, **40**, 1216-1223.
16. Smets, I., Fiddes, B., Garcia-Perez, J. E., He, D., Mallants, K., Liao, W., Dooley, J., Wang, G., Humblet-Baron, S., Dubois, B., Compston, A., Jones, J., Coles, A., Liston, A., Ban, M., Goris, A., and Sawcer, S. (2018) Multiple sclerosis risk variants alter expression of co-stimulatory genes in B cells, *Brain*, doi: 10.1093/brain/awx372.
17. Vazgiourakis, V. M., Zervou, M. I., Choulaki, C., Bertsias, G., Melissourgaki, M., Yilmaz, N., Sidiropoulos, P., Plant, D., Trouw, L. A., Toes, R. E., Kardassis, D., Yavuz, S., Boumpas, D. T., and Goulielmos, G. N. (2011) A common SNP in the CD40 region is associated with systemic lupus erythematosus and correlates with altered CD40 expression: implications for the pathogenesis, *Ann. Rheum. Dis.*, **70**, 2184-2190.
18. Schwartz, A. M., Demin, D. E., Vorontsov, I. E., Kasyanov, A. S., Putlyaeva, L. V., Tatosyan, K. A., Kulakovskiy, I. V., and Kuprash, D. V. (2017) Multiple single nucleotide polymorphisms in the first intron of the IL2RA gene affect transcription factor binding and enhancer activity, *Gene*, **602**, 50-56.
19. Pistillo, M. P., Tanigaki, N., Chua, R., Mazzoleni, O., and Ferrara, G. B. (1989) Human anti-HLA-DQw2 monoclonal antibody secreted by an Epstein-Barr-virus-transformed lymphoblastoid cell line: assessment of the monoclonality, allospecificity, and target, *Hum. Immunol.*, **24**, 253-263.
20. Schwartz, A. M., Putlyaeva, L. V., Covich, M., Klepikova, A. V., Akulich, K. A., Vorontsov, I. E., Korneev, K. V., Dmitriev, S. E., Polanovsky, O. L., Sidorenko, S. P., Kulakovskiy, I. V., and Kuprash, D. V. (2016) Early B-cell factor 1 (EBF1) is critical for transcriptional control of SLAMF1 gene in human B cells, *Biochim. Biophys. Acta*, **1859**, 1259-1268.
21. Bachmann, S. B., Frommel, S. C., Camicia, R., Winkler, H. C., Santoro, R., and Hassa, P. O. (2014) DTX3L and ARTD9 inhibit IRF1 expression and mediate in cooperation with ARTD8 survival and proliferation of metastatic prostate cancer cells, *Mol. Cancer*, **13**, 125.
22. Hasson, S. A., Kane, L. A., Yamano, K., Huang, C. H., Sliter, D. A., Buehler, E., Wang, C., Heman-Ackah, S. M., Hessa, T., Guha, R., Martin, S. E., and Youle, R. J. (2013) High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy, *Nature*, **504**, 291-295.
23. Putlyaeva, L. V., Schwartz, A. M., Korneev, K. V., Covic, M., Uroshlev, L. A., Makeev, V. Y., Dmitriev, S. E., and Kuprash, D. V. (2014) Upstream open reading frames regulate translation of the long isoform of SLAMF1 mRNA that encodes costimulatory receptor CD150, *Biochemistry (Moscow)*, **79**, 1405-1411.
24. Rosenbloom, K. R., Armstrong, J., Barber, G. P., Casper, J., Clawson, H., Diekhans, M., Dreszer, T. R., Fujita, P. A., Guruvadoo, L., Haeussler, M., Harte, R. A., Heitner, S., Hickey, G., Hinrichs, A. S., Hubley, R., Karolchik, D., Learned, K., Lee, B. T., Li, C. H., Miga, K. H., Nguyen, N., Paten, B., Raney, B. J., Smit, A. F., Speir, M. L., Zweig, A. S., Haussler, D., Kuhn, R. M., and Kent, W. J. (2015) The UCSC Genome Browser database: 2015 update, *Nucleic Acids Res.*, **43**, D670-681.
25. Nguyen, V. T., and Benveniste, E. N. (2000) Involvement of STAT-1 and Ets family members in interferon-gamma induction of CD40 transcription in microglia/macrophages, *J. Biol. Chem.*, **275**, 23674-23684.
26. Yevshin, I., Sharipov, R., Valeev, T., Kel, A., and Kolpakov, F. (2017) GTRD: a database of transcription factor binding sites identified by ChIP-seq experiments, *Nucleic Acids Res.*, **45**, D61-D67.
27. Kulakovskiy, I. V., Vorontsov, I. E., Yevshin, I. S., Sharipov, R. N., Fedorova, A. D., Rumynskiy, E. I., Medvedeva, Y. A., Magana-Mora, A., Bajic, V. B., Papatsenko, D. A., Kolpakov, F. A., and Makeev, V. J. (2018) HOCOMOCO: towards a complete collection of transcription factor binding models for human and mouse via large-scale ChIP-Seq analysis, *Nucleic Acids Res.*, **46**, D252-D259.
28. McCarthy, M. I., Abecasis, G. R., Cardon, L. R., Goldstein, D. B., Little, J., Ioannidis, J. P., and

- Hirschhorn, J. N. (2008) Genome-wide association studies for complex traits: consensus, uncertainty and challenges, *Nat. Rev. Genet.*, **9**, 356-369.
29. Toubi, E., and Shoenfeld, Y. (2004) The role of CD40–CD154 interactions in autoimmunity and the benefit of disrupting this pathway, *Autoimmunity*, **37**, 457-464.
30. Dong, L., Chen, X., Shao, H., Bai, L., Li, X., and Zhang, X. (2018) Mesenchymal stem cells inhibited dendritic cells via the regulation of STAT1 and STAT6 phosphorylation in experimental autoimmune uveitis, *Curr. Mol. Med.*, **17**, 478-487.
31. Luo, S., Liu, Y., Liang, G., Zhao, M., Wu, H., Liang, Y., Qiu, X., Tan, Y., Dai, Y., Yung, S., Chan, T. M., and Lu, Q. (2015) The role of microRNA-1246 in the regulation of B cell activation and the pathogenesis of systemic lupus erythematosus, *Clin. Epigenetics*, **7**, 24.
32. Ramos, P. S., Shedlock, A. M., and Langefeld, C. D. (2015) Genetics of autoimmune diseases: insights from population genetics, *J. Hum. Genet.*, **60**, 657-664.
33. Itariu, B. K., and Stulnig, T. M. (2014) Autoimmune aspects of type 2 diabetes mellitus – a mini-review, *Gerontology*, **60**, 189-196.
34. Mammanna, S., Fagone, P., Cavalli, E., Basile, M. S., Petralia, M. C., Nicoletti, F., Bramanti, P., and Mazzon, E. (2018) The role of macrophages in neuroinflammatory and neurodegenerative pathways of Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis: pathogenetic cellular effectors and potential therapeutic targets, *Int. J. Mol. Sci.*, **19**, E831.
35. Neshar, G., and Moore, T. L. (1993) Rheumatoid arthritis in the aged. Incidence and optimal management, *Drugs Aging*, **3**, 487-501.