Research Square

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IL-18 and CD14 variants in chronic HBV predisposition: an experimental–bioinformatics study focused on transcription and splicing

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Abstract

Background

Hepatitis B virus (HBV), a vaccine-avoidable infection, is a health concern worldwide, leading to liver disorders such as acute self-constraint and chronic hepatitis, liver failure, hepatic cirrhosis, and even hepatocellular carcinoma if untreated. *'Immunogeneticprofiling,'* genetic variations of the pro- and anti-inflammatory cytokines responsible for regulating the immune responses, cause person-to-person differences and impact the clinical manifestation of the disease. The current experimental–bioinformatics research was conducted to examine whether promoteric *IL-18*–rs187238 C > G and –rs1946518 T > G and intronic *CD14*–rs2569190 A > G variations are associated with chronic HBV.

Methods

A total of 400 individuals (200 in each case and control group) participated in the study and were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The data was also assessed bioinformatics-wise for conservation, genomic transcription and splicing, and protein interactions.

Results

Findings proposed that unlike the *IL-18*–rs1946518 T > G and *CD14*–rs2569190 A > G, the *IL-18*–rs187238 C > G is a protector against chronic HBV (odds ratio [OR] = 0.62, 95% confidence intervals [CI]: 0.46–0.83, and $p = 0.002$). The TG/CC/AA, TG/CC/AG, TT/CC/AG, and GG/CC/AA combined genotypes significantly increased chronic HBV risk ($p < 0.05$), while the *IL-18* G/T and G/G haplotypes lessened it ($p < 0.05$). Moreover, in contrast to the *IL-18*–rs1946518 T > G, *IL-18*–rs187238 C > G is likely to create novel binding sites for transcription factors, and the *CD14*–rs2569190 A > G presumably changed the ribonucleic acid splicing pattern.

Conclusions

The *IL-18*–rs187238 C > G might protect against chronic HBV and is likely to generate novel binding sites for transcription factors.

Introduction

Hepatitis B virus (HBV) infection continues to be a severe health concern globally, with over 296 million affected (most are in Asia and Africa), as the latest World Health Organization (WHO) reports [1]. Following decades of latency, it might transform into the hepatitis B e antigen-positive ($HBeAg^+$) chronic form labeled with constant or periodic augments in alanine aminotransferase (ALT) levels (so-called flares) and even proceeds to hepatocellular carcinoma (HCC). However, the virus in roughly 95% of HBV-infected elderly children and adults is wiped out spontaneously. Further, cytokines are a family of proteins that react to a broad spectrum of cellular stresses, including inflammation, infection, and damage. Their release is vital in regulating the immune system against oncogenesis and viral infections such as COVID-19 and hepatitis B viruses [2, 3]. The interleukin-18 (*IL-18*) gene, a newly identified cytokine with pro-inflammatory attributes in autoimmune and inflammatory diseases, is released from several cells such as monocytes, macrophages, dendritic and Kupffer cells, regulating immunological reactions by expressing cytokine-associated genes and discriminating T helper (Th) cells [4]. Additionally, the cluster of differentiation antigen 14 (CD14) is a surface lipopolysaccharide (LPS) in the cell membrane (mCD14) of the monocytes, macrophages, and neutrophils, or soluble (sCD14), and a multiple-function inflammatory cytokine chiefly produced by mature mononuclear macrophages [5, 6]. This antigen could transmit the activation signal to the downstream pathway through toll-like receptor 4 (TLR4) and bone marrow differentiation protein-2 (MD-2) [7]. Thusly, the

monocyte-macrophage system was commenced, and several pro-inflammatory cytokines began to emit – TNF-α, IL-1, and IL-6, to name a few–, provoking immunological and inflammatory reactions [8, 9].

Two key factors, host immunologic and genetic, vitally function in this disease [10]. The host genetic profile impacts cytokine function and production, which could be associated with gene variants. Coding sequences linked with cytokine configuration, 3´-untranslated regions (3´-UTR) capable of posttranscriptional effect on cytokine production, and the promoter local influencing gene transcription, are of these genetic alterations [11]. Owing to the genetic variants in the promoter or intron regions of the cytokines, the balance between pro- and anti-inflammatory of them is disordered, thereby altering gene expression [12]. Two single-nucleotide promoter variants of the *IL-18* gene are − 137 G/C (*IL-18*–rs187238 C > G) and − 607 G/T (*IL-18*–rs1946518 T > G) (**Supplementary Fig. 1**). These polymorphisms are defined by guanine/cytosine base replacement in the − 137 and thymine/guanine in the − 607 position of the gene, respectively, presumably influencing binding transcription factors and might regulate *IL-18* gene expression [13]. One of these deoxyribonucleic acid (DNA)-binding factors is the glucocorticoid receptor (GR), belonging to the nuclear receptor superfamily and regulating different physiological functions, such as cell growth, energy homeostasis, and inflammation, and plays a part in fast messenger ribonucleic acid (mRNA) degradation [14]. Two GR isoforms differ at their carboxyl termini: a cytoplasmic alpha form (GR_{α}), which modulates gene transcription; and a nuclear-localized beta isoform (GR_{β}) with a loss of known ligand-binding ability, which devitalizes GR_{α} activity [15–17]. GR_{β} is induced by TNF and regulates gluconeogenesis and inflammation in the liver [18]. Some experiments have analyzed the correlation of *IL-18* polymorphisms with chronic HBV. The *IL-18*–rs1946518 TT genotype was shown to ostensibly lessen transcriptional function and diminish IL-18 exudation from Kupffer cells, reducing HBV clearance [19, 20]. However, its capability as a diagnostic marker in HBV has yet to be identified. Furthermore, IL-18 attaches to its specific receptor on the cytoplasmic membrane, and through downstream signaling pathways, it triggers the nuclear factor-kappaB (NF-κB) mediator [21]. An endogenic inhibitor, IL-18-binding protein (IL-18BP), modulates IL-18 activity and prohibits IFN-gamma (γ). At the other end, the *CD14* gene is approximately 88 base pairs (bp) in size, encoding a 40 kilodalton (kDa) protein on chromosome 5q31.1 (**Supplementary Fig. 1**). *CD14*–rs2569190 A > G, a single-nucleotide polymorphism (SNP) of this gene, is situated close to the CCAAT/enhancer-binding protein site, playing a vital role during monocytic development [22]. Alongsides, alternative splicing is crucial in gene expression, resulting in phenotypic diversity and disease pathogenesis that is regulated through a complicated network of *trans*-factors and *cis*-regulatory elements. Not surprisingly, intronic variants such as those in the *CD14* gene might modulate splicing regulation [23].

It is well comprehended that a host's genetic context, or *'Immunogeneticprofiling,'* is related to the proneness of some diseases. Thus far, several investigations have shed light on the correlation between *IL-18* and *CD14* variants with a predisposition to or development of various abnormalities and ailments, comprising non-alcoholic fatty liver disease, asthma, tuberculosis, chronic HBV, sepsis, inflammatory bowel disease, and gastric neoplasm [24–26]. Accordingly, this research hypothesizes that *IL-18* and *CD14* SNPs might change vulnerability to HBV infection by altering the protein expression of several cell signaling pathway constituents by changing the binding capability of transcription or RNA splicing factors. This research sought to evaluate the correlation between *IL-18*–rs187238 C > G and –rs1946518 T > G and *CD14*–rs2569190 A > G with chronic HBV infection experimentally and *via* bioinformatics approaches.

Methods

Characteristics of Participants

The current research recruited 400 participants (200 in each case and control group) for a total of 305 males and 95 females with an average age of 32.58 ± 9.32 in healthy and 41.13 ± 11.95 in patients referred to the Bu-Ali educational-therapeutic center, Zahedan, Iran. The patients were selected from those with hepatitis B surface antigen-positive ($HBsAg^{+}$) and immunoglobulin class G hepatitis B core antibody-positive (IgG $anti\text{-}HBc^{+}$) for at least six months utilizing commercial human enzyme-linked immunosorbent assay (ELISA) kits (Cell Biolabs®, Inc., San Diego, C.A., U.S.A.). According to their medical records, patients manifested clinical symptoms such as exhaustion, anorexia, icterus, hepatosplenomegaly, liver

hardness, and elevated aspartate aminotransferase and alanine aminotransferase enzymes. The control group was negative for HBsAg and IgG anti-HBc and had no history of inflammatory liver and kidney diseases. Human immunodeficiency virus (HIV) and hepatitis C and D viruses (HCV and HDV) co-infected participants were omitted from the research. All subjects in this research were of Baloch and Fars ethnicity, dwelling in Zahedan, Iran. The clinical and demographic datasets of all attendees are entered in Table 1. This study complies with the STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [27].

Table 1
Clinical and demographic characteristics of the study population

Parameter	Case (n = 200) (mean ± SD)		Control (n = 200) (mean ± SD)		*p*-value
Age (Year)	41.13 ± 11.95		32.58 ± 9.32		**< 0.001***
BMI (kg/m^2)	43.10 ± 28.32		26.40 ± 4.05		**< 0.001***
Gender					**< 0.001***
Male	122 (61.0%)		183 (91.5%)		
	Fars	Baloch	Fars	Baloch	0.455
	58 (47.5%)	64 (52.5%)	95 (51.9%)	88 (48.1%)	
Female	78 (39.0%)		17 (8.5%)		
	Fars	Baloch	Fars	Baloch	**0.007***
	41 (52.6%)	37 (47.4%)	15 (88.2%)	2 (11.8%)	
Ethnicity					0.271
Fars	99 (47.4%)		110 (52.6%)		
Baloch	101 (52.9%)		90 (47.1%)		
HBsAg	Positive		Negative		-
IgG anti-HBc	Positive		Negative		-
Anti-HIV	Negative		Negative		-
Anti-HCV	Negative		Negative		-
Anti-HDV	Negative		Negative		-
HBeAg					**< 0.001***
Negative	167 (83.5%)		191 (95.5)		
	Male	Female	Male	Female	0.385
	108 (64.7%)	59 (35.3%)	115 (60.0%)	76 (40.0%)	
Positive	33 (16.5%)		9 (4.5)		
	Male	Female	Male	Female	0.202
	14 (42.4%)	19 (57.6%)	6 (66.6%)	3 (33.4%)	
AST (U/L)	53.34 ± 9.53		33.42 ± 7.35		
	Male	Female	Male	Female	**< 0.001***
	52.75 ± 8.33	61.81 ± 12.65	34.79 ± 8.33	32.88 ± 8.37	

n represents the number of participants; SD, standard deviation; BMI, body mass index; kg/m^2, kilogram square meter; HBsAg, hepatitis B surface antigen; IgG anti-HBc, immunoglobulin class G hepatitis B core antibody; anti-HIV, human immunodeficiency virus antibody; anti-HCV, hepatitis C virus antibody; anti-HDV, hepatitis D virus antibody; HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; U/L, units per liter; ALT, alanine aminotransferase.

*$p < 0.05$ was considered statistically significant.

Parameter	Case (n = 200) (mean ± SD)		Control (n = 200) (mean ± SD)		*p*-value
	Fars	Baloch	Fars	Baloch	0.551
	57.14 ± 11.32	55.45 ± 10.92	34.49 ± 8.45	34.80 ± 8.24	
ALT (U/L)	71.73 ± 7.52		35.71 ± 8.57		
	Male	Female	Male	Female	**< 0.001***
	72.83 ± 7.59	72.81 ± 7.20	35.30 ± 8.31	35.83 ± 7.56	
	Fars	Baloch	Fars	Baloch	**0.034***
	71.59 ± 7.77	74.03 ± 6.89	35.31 ± 8.55	34.36 ± 8.04	

n represents the number of participants; SD, standard deviation; BMI, body mass index; kg/m^2, kilogram square meter; HBsAg, hepatitis B surface antigen; IgG anti-HBc, immunoglobulin class G hepatitis B core antibody; anti-HIV, human immunodeficiency virus antibody; anti-HCV, hepatitis C virus antibody; anti-HDV, hepatitis D virus antibody; HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; U/L, units per liter; ALT, alanine aminotransferase.

*$p < 0.05$ was considered statistically significant.

Genomic DNA Isolation and Genotyping

Five milliliters of whole blood were collected from each entrant in an ethylenediaminetetraacetic acid (EDTA)-containing tube, and subsequently, genomic DNA (gDNA) was separated utilizing a FlexiGene DNA Kit (QIAGEN© Sciences Inc., Washington, D.C., U.S.A.) as advised by the manufacturer. A microspectrophotometer (BOECO®, Hamburg, Germany) was used to assess the purity and concentration of the extracted DNA. Samples with 1.7 ≤ optical density (OD)260/OD280 ratio ≤ 2 were used for the study. Data of the studied SNPs (with a minor allele frequency (MAF) > 0.2 according to the 1000 Genomes Project) were extracted from the National Center for Biotechnology Information (*NCBI*) databank. Allele-specific primers for genotyping *IL-18* and *CD14* variations (**Supplementary Table 1**) were designed utilizing Gene Runner® v.6.5.52 Beta and Oligo7® and synthesized by TAG Copenhagen A/S® Co. (Frederiksberg, Denmark).

Genotypic discrimination for the studied SNPs was performed by applying the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The reactions were as follows: a total volume of 20 µl containing 0.9 µl of genomic DNA (~ 70 ng/ml), 0.7 µl of each primer (7 pmol), 10 µl of Taq 2x Master Mix Red-$MgCl_2$ 1.5mM (AMPLIQON© A/C, Denmark), and 7.7 µl of distilled water. Each mixture was heated to 95°C for 5 min for initial denaturation and underwent 35 cycles at 94°C for 30 s, annealing at different temperatures (based on **Supplementary Table 1** for each SNP) for 30 s, and an extension step at 72°C for 30 s, followed by a final extension stage at 72°C for 5 min. *MboII* (for *IL-18*–rs187238 C > G), *DraI* (for *IL-18*–rs1946518 T > G), and *BsuRI* (also known as *HaeIII*) (for *CD14*–rs2569190 A > G) restriction enzymes (purchased from ThermoFisher® SCIENTIFIC Inc., Massachusetts, U.S.A.) were used to digest amplicons at 37°C incubation for 14 hours. Digested products were electrophoresed on 2% agarose gel (3% for *CD14*–rs2569190 A > G to better separate immediate bands) stained with loading dye (SINACLON® Co., Tehran, Iran) and visualized under ultraviolet light of a gel documentation system (UVTech Biosciences© Ltd., South Wales, U.K.) (**Supplementary Fig. 2**). At least 30% of the samples were regenotyped at random, indicating genotyping precision of 99%.

Statistical Analyses

This study's statistical analyses were calculated by recruiting the R Project for Statistical Computing version 4.2.0 (http://www.r-project.org). Data were provided by mean ± standard deviation (SD), and the Hardy–Weinberg equilibrium was applied to scrutinize the deviation of genotype distribution. Where appropriate, Chi-square, independent samples *t*-test, and Mann-Whitney-Wilcoxon tests were utilized to compare differences between groups for quantitative variables. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated by binary logistic regression analysis for the relationship

between allele/genotype frequencies of *IL-18* and *CD14* SNPs and the proneness of chronic HBV. Furthermore, the *SHEsis* web-based platform was utilized to conduct haplotype analysis [28]. Notably, *p*-values ≤ 0.05 were considered statistically significant.

Bioinformatics Analyses

The *WebLogo v.2.8.2* server assisted in identifying conserved sequences of all three studied variants [29]. The PROMO v.3.0.2, one of the main projects of the algorithmics and genetics group (ALGGEN) of the Technical University of Catalonia, is a virtual laboratory for identifying putative transcription factor binding sites (TFBS) in DNA sequences, which was also applied in the current study [30, 31]. In this respect, the *TRANSFAC v.8.3.* (TRANScription FACtor) database was recruited to develop specific binding site weight matrices for TFBS prediction in *IL-18*–rs187238 C > G and –rs1946518 T > G promoteric variations. Accordingly, *Homo sapiens* factors and their connection recognition sites were selected. The maximum percentage difference between the actual binding site of the transcription factor and the predicted one was determined to be 5% (factors predicted within a dissimilarity margin ≤ 5%), and the gene sequence related to the promoter position extracted from the *NCBI* databank was inserted. Besides, to determine the effect of the intronic *CD14*–rs2569190 variation on the splicing site model, the *SpliceAid2* server (freely available at http://www.introni.it/spliceaid.html) was recruited [32]. Accordingly, by simultaneously comparing wild-type and mutated sequences, splicing factors related to the SNP position were filtered.

Furthermore, to predict the protein-protein interaction (PPI) network of the *IL-18* and *CD14* genes, the latest version (04-01-2022) of the *inBio Discover*™ database, a high-coverage map of human protein biology [33], was used. The information was obtained by entering the related *UniProt*[1] IDs (Q14116 and P08571 for *IL-18* and *CD14* genes, respectively). The network expansion mode provided neighboring and related proteins regarding expression, regulation, and function. To display proteins with a closer connection to the CD14 protein, the relevance score cutoff was set to 0.33.

[1] *UniProt* is a reservoir of protein information developed by recruiting the *Swiss-Prot*, *TrEMBL,* and *PIR-PSD* databases altogether.

Results

Genotypic Distribution, Haplotype, and Linkage Analyses

The results unveiled that the *IL-18*–rs187238 C > G variant significantly preserves against chronic HBV risk under codominant CG (OR = 0.49, 95% CI: 0.31–0.76, and $p = 0.001$), codominant GG (OR = 0.49, 95% CI: 0.26–0.93, and $p = 0.030$), dominant CG + GG (OR = 0.49, 95% CI: 0.33–0.74, and $p = 0.001$), and over-dominant CG (OR = 0.58, 95% CI: 0.38–0.87, and $p = 0.008$). Moreover, the G allele of this variant (OR = 0.62, 95% CI: 0.46–0.83, and $p = 0.002$) was related to the protection against chronic HBV in the study population. However, no significant relationship was realized with this disease in the *IL-18*–rs1946518 T > G and *CD14*–rs2569190 A > G variations. There were inconsequential alterations in *p*-values following adjustment for age. Table 2 outlines the genotype and allele frequencies of *IL-18* and *CD14* variations in controls and chronic HBV sufferers.

Table 2
Genotypic and allelic distribution of *IL-18* and *CD14* polymorphisms in patients with chronic HBV and healthy participants

SNP	**Type**	**HBV (%)**			**Non-HBV (%)**			**Model**	***OR (95% CI)**	***p-value**
IL-18–**rs1946518 T > G**	TT	102 (51.0)	Male	61 (59.8)	89 (44.5)	Male	81 (91.0)	1 [reference]		-
			Female	41 (40.2)		Female	8 (9.0)			
	TG	87 (43.5)	Male	54 (62.0)	92 (46.0)	Male	84 (91.4)	Codominant 1 TG vs. TT	0.81 (0.53–1.22)	0.314
			Female	33 (38.0)		Female	8 (8.6)			
	GG	11 (5.5)	Male	7 (63.6)	19 (9.5)	Male	18 (94.7)	Codominant 2 GG vs. TT	0.46 (0.20–1.06)	0.068
			Female	4 (36.4)		Female	1 (5.3)			
	HWE	0.169			0.493			Dominant TG + GG vs. TT	0.75 (0.50–1.12)	0.157
								Recessive GG vs. TT + TG	0.52 (0.24–1.16)	0.111
								Over-dominant TG vs. TT + GG	0.89 (0.59–1.33)	0.569
	T	291 (72.7)	Male	176 (60.5)	270 (67. 5)	Male	246 (91.0)	1 [reference]		-
			Female	115 (39.5)		Female	24 (9.0)			
	G	109 (27.25)	Male	68 (62.4)	130 (32.5)	Male	120 (92.4)	Allelic G vs. T	0.76 (0.55–1.03)	0.080
			Female	41 (37.6)		Female	10 (7.6)			
IL-18-**rs187238 C > G**	CC	107 (53.5)	Male	68 (63.5)	73 (36.5)	Male	66 (90.4)	1 [reference]		**-**
			Female	39 (36.5)		Female	7 (9.6)			
	CG	71 (35.5)	Male	45 (63.4)	96 (48.0)	Male	90 (93.7)	Codominant 1 CG vs. CC	0.49 (0.31–0.76)	**0.001**
			Female	26 (36.6)		Female	6 (6.3)			

Codominant 1 and 2 represent the heterozygous and homozygous codominant models, respectively. *p-value and OR (95% CI) were age-adjusted, and $p < 0.05$ was considered statistically significant and indicated in **bold**. SNP, single-nucleotide polymorphism; HBV, hepatitis B virus; OR, odds ratio; CI, confidence intervals; *IL-18*, interleukin 18; rs, reference SNP; HWE, Hardy–Weinberg equilibrium; *CD14*, cluster of differentiation antigen 14.

SNP	Type	HBV (%)			Non-HBV (%)			Model	*OR (95% CI)	*p-value
	GG	22 (11.0)	Male	9 (40.9)	31 (15.5)	Male	27 (87.1)	Codominant 2 GG vs. CC	0.49 (0.26–0.93)	**0.030**
			Female	13 (59.1)		Female	4 (12.9)			
	HWE	0.059			0.951			Dominant CG + GG vs. CC	0.49 (0.33–0.74)	**0.001**
								Recessive GG vs. CC + CG	0.70 (0.38–1.27)	0.236
								Over-dominant CG vs. CC + GG	0.58 (0.38–0.87)	**0.008**
	C	285 (71.25)	Male	181 (63.5)	242 (60.5)	Male	222 (91.7)	1 [reference]		-
			Female	104 (36.5)		Female	20 (8.3)			
	G	115 (28.75)	Male	63 (54.8)	158 (39.5)	Male	144 (91.1)	Allelic G vs. C	0.62 (0.46–0.83)	**0.002**
			Female	52 (45.2)		Female	14 (8.9)			
CD14–**rs2569190 A > G**	AA	77 (38.5)	Male	48 (62.3)	78 (39.0)	Male	71 (91.0)	1 [reference]		-
			Female	29 (37.7)		Female	7 (9.0)			
	AG	100 (50.0)	Male	56 (56.0)	88 (44.0)	Male	82 (93.2)	Codominant 1 AG vs. AA	1.07 (0.69–1.65)	0.756
			Female	44 (44.0)		Female	6 (6.8)			
	GG	23 (11.5)	Male	18 (78.3)	34 (17.0)	Male	30 (88.2)	Codominant 2 GG vs. AA	0.67 (0.36–1.25)	0.205

Codominant 1 and 2 represent the heterozygous and homozygous codominant models, respectively. *p-value and OR (95% CI) were age-adjusted, and $p < 0.05$ was considered statistically significant and indicated in **bold**. SNP, single-nucleotide polymorphism; HBV, hepatitis B virus; OR, odds ratio; CI, confidence intervals; *IL-18*, interleukin 18; rs, reference SNP; HWE, Hardy–Weinberg equilibrium; *CD14*, cluster of differentiation antigen 14.

SNP	Type	HBV (%)			Non-HBV (%)			Model	*OR (95% CI)	*p-value
			Female	5 (21.7)		Female	4 (11.8)			
	HWE	0.266			0.287			Dominant AG + GG vs. AA	0.96 (0.64–1.44)	0.838
								Recessive GG vs. AA + AG	0.64 (0.36–1.14)	0.132
								Over-dominant AG vs. AA + GG	1.19 (0.80–1.78)	0.386
	A	254 (63.5)	Male	152 (59.8)	244 (61.0)	Male	224 (91.8)	1 [reference]		-
			Female	102 (40.2)		Female	20 (8.2)			
	G	146 (36.5)	Male	92 (63.0)	156 (39.0)	Male	142 (91.0)	Allelic G vs. A	0.87 (0.65–1.17)	0.358
			Female	54 (37.0)		Female	14 (9.0)			

Codominant 1 and 2 represent the heterozygous and homozygous codominant models, respectively. **p*-value and OR (95% CI) were age-adjusted, and $p < 0.05$ was considered statistically significant and indicated in **bold**. SNP, single-nucleotide polymorphism; HBV, hepatitis B virus; OR, odds ratio; CI, confidence intervals; *IL-18*, interleukin 18; rs, reference SNP; HWE, Hardy−Weinberg equilibrium; *CD14*, cluster of differentiation antigen 14.

Additionally, compared to the $TG_{IL\text{-}18\text{–}rs1946518\ T>G}/CG_{IL\text{-}18\text{–}rs187238\ C>G}/AG_{CD14\text{–}rs2569190\ A>G}$ combined genotype (reference), the statistical analyses revealed that TG/CC/AA (OR = 2.51, 95% CI: 1.01−6.24, and $p = 0.046$), TG/CC/AG (OR = 2.72, 95% CI: 1.08−6.85, and $p = 0.032$), TT/CC/AG (OR = 3.95, 95% CI: 1.58−9.88, and $p = 0.002$), and GG/CC/AA (OR = 7.77, 95% CI: 0.83–72.13, and $p = 0.042$) made a significant augment in the chronic HBV risk. Table 3 indicates the interaction analyses of the studied variants.

Table 3
Interaction analysis of the studied variations on chronic HBV risk.

IL-18–rs1946518 T > G	*IL-18*–rs187238 C > G	*CD14*–rs2569190 A > G	HBV (%)	Control (%)	OR (95% CI)	*p*-value
TG	CG	AG	18 (9.0)	28 (14.0)	1 [reference]	
TG	CG	AA	6 (3.0)	17 (8.5)	0.55 (0.18–1.65)	0.287
TT	CC	AA	19 (9.5)	17 (8.5)	1.74 (0.72–4.20)	0.220
TT	CG	AG	20 (10.0)	15 (7.5)	2.07 (0.85–5.07)	0.110
TT	CG	AA	13 (6.5)	15 (7.5)	1.35 (0.52–3.49)	0.540
TG	CC	AA	21 (10.5)	13 (6.5)	2.51 (1.01–6.24)	**0.046***
TG	CC	AG	21 (10.5)	12 (6.0)	2.72 (1.08–6.85)	**0.032***
TT	CC	AG	28 (14.0)	11 (5.5)	3.95 (1.58–9.88)	**0.002***
TT	GG	AG	6 (3.0)	8 (4.0)	1.16 (0.34–3.92)	0.804
TT	CC	GG	4 (2.0)	7 (3.5)	0.88 (0.22–3.47)	0.866
TG	CC	GG	7 (3.5)	7 (3.5)	1.55 (0.46–5.18)	0.473
TT	GG	AA	6 (3.0)	6 (3.0)	1.55 (0.43–5.57)	0.499
TG	CG	GG	4 (2.0)	6 (3.0)	1.03 (0.25–4.19)	0.959
GG	CG	AA	2 (1.0)	6 (3.0)	0.51 (0.09–2.85)	0.449
TG	GG	AG	3 (1.5)	5 (2.5)	0.93 (0.19–4.39)	0.931
TT	CG	GG	6 (3.0)	4 (2.0)	2.33 (0.57–9.43)	0.230
GG	CC	AG	2 (1.0)	4 (2.0)	0.77 (0.12–4.69)	0.785
GG	CG	AG	2 (1.0)	4 (2.0)	0.77 (0.12–4.69)	0.785
TG	GG	AA	5 (2.5)	3 (1.5)	2.59 (0.55–12.20)	0.221
GG	CC	AA	5 (2.5)	1 (0.5)	7.77 (0.83–72.13)	**0.042***

*$p < 0.05$ was considered statistically significant and shown in **bold**. HBV, hepatitis B virus; *IL-18*, interleukin 18; rs, reference single-nucleotide polymorphism; *CD14*, cluster of differentiation antigen 14; OR, odds ratio; CI, confidence intervals.

IL-18–rs1946518 T > G	*IL-18*–rs187238 C > G	*CD14*–rs2569190 A > G	HBV (%)	Control (%)	OR (95% CI)	*p*-value
TG	GG	GG	2 (1.0)	1 (0.5)	3.11 (0.26–36.87)	0.352

*$p < 0.05$ was considered statistically significant and shown in **bold**. HBV, hepatitis B virus; *IL-18*, interleukin 18; rs, reference single-nucleotide polymorphism; *CD14*, cluster of differentiation antigen 14; OR, odds ratio; CI, confidence intervals.

As illustrated in **Supplementary Table 2**, compared to the $C_{IL-18\text{–rs187238 C>G}}/T_{IL-18\text{–rs1946518 T>G}}$ (reference), the G/T haplotype significantly reduced predisposition to chronic HBV by virtually 39% (OR = 0.61, 95% CI: 0.43–0.87, and $p = 0.005$). Similarly, the G/G haplotype remarkably lessened the risk of this infection by approximately 55% (OR = 0.45, 95% CI: 0.26–0.76, and $p = 0.002$). (**Supplementary Fig. 3**).

Bioinformatics Prediction

The *WebLogo* server demonstrated that unlike *IL-18*–rs187238 C > G and *CD14*–rs2569190 A > G, *IL-18*–rs1946518 T > G is the only variant situated in the protected regions across multiple mammalian species (**Supplementary Fig. 4**). Interestingly, the PROMO tool predicted that at *IL-18*–rs187238 C > G, the promoteric allelic polymorphism makes a more suitable factor site available for transcription factors such as GR_{β} [T01920], forkhead box P3 protein (FOXP3 [T04280]), and progesterone receptor forms A and B (PR A [T01661] and PR B [T00696], respectively). GR_{β} binds to the sequence of interest with more certainty *in silico* (dissimilarity index = 0); thus, the *IL-18* gene is likely more regulated (Fig. 1). Additionally, this database predicted that polymorphic allelic changes in *IL-18*–rs1946518 T > G caused an inappropriate binding site for transcription factors such as GR_{β} [T01920], transcription factor IID (TFIID [T00820]), homeobox D9 and D10 protein (HOXD9 [T01424] and HOXD10 [T01425], respectively) (Fig. 2). Alongsides, genomic splicing analyses revealed that a 20-nucleotide (nt) flanking region containing *CD14*–rs2569190 A > G is presumably to modify the pattern of splicing factor sites. The A allele of this SNP makes a recognition site for serine/arginine-rich splicing factor (SC35), whereas the G allele develops a new site for the RNA-binding protein 5 (RBM5) (Fig. 3).

The *inBio Discover™* database output provided 12 proteins interacting directly or mediating with the IL-18 protein. Based on the known interactions (from curated databases and experimentally determined), interleukin 18-binding protein (IL-18BP), interleukin 18 receptor 1 (IL-18R1), interleukin 18 receptor accessory protein (IL-18RAP), and caspase 1 (CASP1) proteins have a high relationship with IL-18 in *Homo sapiens* (**Supplementary Fig. 5**). Further on, by applying the 0.33 cutoff for CD14, *"sticky proteins"* and other likely irrelevant proteins from the network were removed. Thus, 15 proteins were shown to have a closer link with CD14 (**Supplementary Fig. 6**). Accordingly, lipopolysaccharide-binding protein (LBP), toll-like receptor 1, 2, 4, and 6 (TLR1, TLR2, TLR4, and TLR6, respectively) proteins have a high relationship with CD14 in *Homo sapiens*.

Discussion

HBV, a vaccine-avoidable pathogen, is one of the principal causes of liver abnormalities, namely, acute self-limiting and chronic hepatitis, liver failure, hepatic cirrhosis, and HCC [34]. Iran, with a prevalence rate of virtually 1% in the general populace, is deemed a relatively modest nation for this infection [35]. Owing to the crucial activity of *IL-18* and *CD14* genes in the immunoregulatory reaction against HBV, this investigation set out with the aim of assessing the association of the *IL-18*–rs187238 C > G and –rs1946518 T > G and *CD14*–rs2569190 A > G variations with this disease in a population in Zahedan, southeast Iran. Summarily, the current study identified *IL-18*–rs187238 C > G as a protector against chronic HBV in the investigated populace, unlike the rs1946518 T > G of the same gene and *CD14*–rs2569190 A > G. The TG/CC/AA, TG/CC/AG, TT/CC/AG, and GG/CC/AA combined genotypes significantly enhanced predisposition to this disease, whereas the *IL-18* G/T and G/G haplotypes diminished it. Other significant findings were bioinformatics-wise: *IL-18*–rs1946518 T > G is located in the conserved DNA regions across mammalian species; intriguingly, in contrast to the *IL-18*–rs1946518 T > G promoteric variant, *IL-18*–rs187238 C > G developed new binding sites for transcription factors; the *CD14*–rs2569190 A > G intronic

variant created new sites for RNA splicing factors; and 12 and 15 proteins most interacting with IL-18 and CD14, respectively, were recognized.

Experimental Standpoint

Salehi et al. (2018) revealed no significant difference between the *IL-18*–rs1946518 T > G and chronic HBV risk [36]. Similarly, in a meta-analysis study, Zhu et al. (2016) showed that this position had nothing to do with the risk of liver neoplasm [37]. These studies agree with the present study. Additionally, in 2019, Maroufi et al. indicated that patients with breast cancer were 37.86% GG, 47.14% GT, and 15% TT, whereas controls were 40.72% GG, 42.85% GT, and 16.43% TT. They concluded that this variation did not significantly differ between people with breast cancer and healthy controls ($p > 0.05$), and the significant difference between the case and control group was merely correlated with family health history ($p = 0.023$) [38]. On the contrary, Li et al. (2012) unveiled that the TT genotype was further manifested in the sufferers than in the control group. The OR of the TT genotype to that of the TG and GG was 1.537 (95% CI: 1.116–2.218 and $p = 0.009 < 0.025$) [39]. Scrutinizing genotypes of this variant in the same year, Ezzat et al. illuminated that the prevalence of the T allele and GT genotype was considerably more in children with asthma than in controls ($p < 0.001$). Further on, these sufferers with the TT/TG genotype for this SNP were linked to a higher risk of asthma (OR = 6.417 and CI: 2.432–17.289). Moreover, the incidence of the heterozygous GT genotype and T allele in this SNP was higher in asthmatic kids, and there was also no relationship between the intensity of asthma and this variant [40].

In addition, Ramazi et al. (2014) assessed the association between the serum IgE level and the above-discussed SNP as a risk factor for allergic rhinitides; the TG genotype was linked to elevated levels of serum IgE, indicating this variant's impact on the commencement of the illness [41]. A meta-analysis study by Zhang et al. (2020) reported that *IL-18*–rs1946518 T > G and –rs187238 C > G variants might cause proneness to some chronic liver diseases [42]. Moreover, Celik et al. (2018) revealed that the genotype distribution of *IL-18*–rs1946518 T > G statistically significantly differed among alopecia areata sufferers and controls ($p = 0.0008$). The GG + GT genotype distribution and G allele frequency in this SNP were higher in the case group ($p = 0.001$ for both) [43]. Although current results differ from the abovementioned studies, they are consistent with those evaluating the association between this SNP and liver-related aberrations.

The genotype distribution of *IL-18*–rs187238 C > G was statistically meaningfully different in patients with alopecia areata and controls ($p = 0.0014$). The GG genotype and G allele prevalence were also higher in these sufferers ($p = 0.0003$ and $p = 0.0010$, respectively) [43]. In other relevant studies, Migita et al. (2008) assessed the relationship between *IL-18*–rs187238 C > G and –rs1946518 T > G with liver disease progression in patients with chronic HBV. Their findings showed that the C allele of *IL-18*–rs187238 C > G and the TT genotype of *IL-18*–rs1946518 T > G in healthy individuals were higher than in chronic HBV cases [44]. Teixeira et al. (2013) examined the roles of *IL-18*–rs187238 C > G and –rs1946518 T > G in the proneness of HCC. The allele and genotype frequency of the *IL-18*–rs1946518 T > G variation in HCC cases and controls were approximately similar, but the C allele in *IL-18*–rs187238 C > G was more frequent in people with HCC than in controls [45].

Mustarim et al. (2019) reported no meaningful correlation between *CD14*–rs2569190 A > G and the incidence of neonatal sepsis ($p > 0.05$) [46]. Moreover, Jia et al., in the same year, aimed to determine whether this variation is correlated with autoimmune thyroid diseases, especially Graves' disease (GD) and Hashimoto's thyroiditis (HT) [20]. The consequences concluded a significant relationship between this variant and GD. The G allele frequency in these patients was also significant ($p = 0.027$). The allelic, recessive, and homozygous genetic model of *CD14*–rs2569190 A > G also embodied strong associations with GD following age- and gender-matching ($p = 0.014$, $p = 0.015$, and $p = 0.009$, respectively) [47].

Bioinformatics Standpoint

GR plays an essential role in the pathogenesis of acute-on-chronic hepatitis B. The alpha isoform upregulates anti-inflammatory genes in the nucleus and suppresses pro-inflammatory cytokines in the cytosol, thereby lowering inflammation. Unlike GR_{α}, GR_{β} does not bind to glucocorticoids and, in turn, competitively attaches to the glucocorticoid response elements (GREs) in the promoters of steroid-responsive genes and can positively or negatively modulate them, resulting in cellular

interactions, angiogenesis, and consequently an increase in inflammation [48]. In accordance with the above attitudes, the present bioinformatics analyses revealed that *IL-18*–rs187238 C > G develops a more appropriate binding site for $GR_β$ as well as forkhead box P3 protein and progesterone receptor forms A and B, presumably augmenting *IL-18* gene expression. Furthermore, *IL-18*–rs1946518 T > G perhaps attenuates the affinity of transcription factors such as $GR_β$, transcription factor IID, and homeobox D9 and D10 proteins to the promoter.

SC35, newly named serine/arginine-rich splicing factor 2 (SRSF2), is a crucial constituent of the nuclear structure speckle that binds to purine-rich RNA sequences. This protein regulates genomic stability, gene transcription, mRNA stability, and translation necessary for splicing pre-mRNA [49]. Besides, RNA binding motif 5 (*RBM5*) is a tumor suppressor gene encoding a transcriptional regulator and modifies the expression of cell cycle- and apoptosis-associated genes, including Fas cell surface death receptor (*FAS*) and caspase 2 (*CASP2*) *via* alternative splicing of some mRNAs. It might modulate splice site pairing after recruiting U1 and U2 small nuclear ribonucleoprotein particles (snRNPs) to the intron's 5' and 3' splice sites. In the case of *FAS*, it promotes the exclusion of exon 6, thereby producing a soluble form of Fas that inhibits apoptosis. In respect of *CASP2*, it efficaciously excludes exon 9, developing a catalytically active form of CASP2 that induces apoptosis [50, 51]. In the present investigation, the A allele of the *CD14*–rs2569190 A > G variation creates a binding site for this splicing regulator, probably modifying *CD14* gene expression at the RNA level, and the G allele generates a binding site for RBM5. Although the function of RBM5 in apoptosis-related genes is somehow elucidated, there appears to be a need for more research on its roles in pro- and anti-inflammatory cytokines.

Furthermore, deviations from the basic pattern can indicate DNA distortion or base flipping in analyzing DNA sequence conservation [29]. In this parallel, the present research found that *IL-18*–rs1946518 T > G is in the conserved regions across multiple mammalian species. Notably, exome-sequencing projects and genome-wide association research that have employed integrated protein-protein interaction data to unveil non-obvious molecular pathways perturbed by somatic mutations in malignancies demonstrate the significant importance of integrated protein networks for the analysis of massive genomic data sets in metabolic, psychological, and immunological diseases [33]. From this perspective, the current computational analyses reveal that interleukin 18-binding protein, interleukin 18 receptor 1, interleukin 18 receptor accessory protein, and caspase 1 are the most relevant proteins interacting with IL-18. In addition, lipopolysaccharide-binding protein and toll-like receptors 1, 2, 4, and 6 are deemed the most interacting proteins with CD14. More research on the association between these proteins and the studied immunological cytokine is recommended.

As with any other study, this investigation was not constraint-free. In light of the significance of single-nucleotide variations in the vulnerability to diseases and given the dissimilar results in different populations and races, further studies with larger sample sizes and in other variants related to these cytokines and among individuals capable of utterly eradicating the virus during the acute phase of the disease are needed.

In conclusion, the *IL-18*–rs187238 C > G might be a protector against chronic HBV. The TG/CC/AA, TG/CC/AG, TT/CC/AG, and GG/CC/AA combined genotypes and *IL-18* G/T and G/G haplotypes presumably increase and lessen vulnerability to this disease, respectively. From a bioinformatics standpoint, *IL-18*–rs1946518 T > G supposedly is in the protected genomic regions. Moreover, *IL-18*–rs187238 C > G is likely to generate novel binding sites for transcription factors, and the *CD14*–rs2569190 A > G probably altered the RNA splicing model. The results of this study might vary in different populations and from race to race. Thus, more investigations with larger sample sizes and diverse populations are required to prove these findings.

Declarations

Conflict of interest: The authors assert that there is no conflict of interest.

Ethics approval and consent to participate: The ethics committee of Zahedan University of Medical Sciences authorized the study protocol (ethical code: IR.ZAUMS.REC.1395.105). Human-involved procedures were under the 1964 Helsinki declaration

and its later amendments or equivalent moral principles. Before entering the research, informed consent was also obtained from all study attendees or their legal guardians

Consent for publication: All subjects have written informed consent.

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Author Contributions: E.P. and M.M. designed the study; N.H.R. and Z.A. collected demographic information and clinical samples; M.M. designed primers and experiments; F.G., S.S., and E.P. conducted the experiments. M.M. and M.S. analyzed the data. M.S., E.P., and N.H.R. wrote the manuscript; M.S. and N.H.R. revised the manuscript; M.M. supervised the project. All authors read and consented to the last version of the article.

Availability of data and materials

Upon rational demand, the data for this manuscript will be accessible through the corresponding authors.

References

1. WHO’s latest reports on hepatitis B virus infection-2019 [Internet]. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
2. Yoshio S, Mano Y, Doi H, Shoji H, Shimagaki T, Sakamoto Y, et al. Cytokine and chemokine signatures associated with hepatitis B surface antigen loss in hepatitis B patients. JCI Insight [Internet]. 2018;3. Available from: https://insight.jci.org/articles/view/122268.
3. Rokni M, Sarhadi M, Heidari Nia M, Mohamed Khosroshahi L, Asghari S, Sargazi S, et al. Single nucleotide polymorphisms located in TNFA, IL1RN, IL6R, and IL6 genes are associated with COVID-19 risk and severity in an Iranian population. Cell Biol Int [Internet]. 2022; Available from: https://onlinelibrary.wiley.com/doi/10.1002/cbin.11807.
4. Harel M, Fauteux-Daniel S, Girard-Guyonvarc’h C, Gabay C. Balance between Interleukin-18 and Interleukin-18 binding protein in auto-inflammatory diseases. Cytokine [Internet]. England; 2022;150:155781. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043466621003707.
5. Mendel I, Feige E, Yacov N, Salem Y, Levi I, Propheta-Meiran O, et al. VB-201, an oxidized phospholipid small molecule, inhibits CD14- and Toll-like receptor-2-dependent innate cell activation and constrains atherosclerosis. Clin Exp Immunol. 2014;175:126–37.
6. Zhao L, Bracken MB. Association of CD14-260 (-159) C > Tand asthma: a systematic review and meta-analysis. BMC Med Genet. 2011;12:1–10.
7. Estruch M, Bancells C, Beloki L, Sanchez-Quesada JL, Ordóñez-Llanos J, Benitez S. CD14 and TLR4 mediate cytokine release promoted by electronegative LDL in monocytes. Atherosclerosis. 2013;229:356–62.
8. Fernández-Real JM, Broch M, Richart C, Vendrell J, López-Bermejo A, Ricart W. CD14 monocyte receptor, involved in the inflammatory cascade, and insulin sensitivity. J Clin Endocrinol Metab. 2003;88:1780–4.
9. Abdelmoaty MM, Yeapuri P, Machhi J, Olson KE, Shahjin F, Kumar V, et al. Defining the Innate Immune Responses for SARS-CoV-2-Human Macrophage Interactions. Front Immunol [Internet]. 2021;12:741502. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.741502/full.
10. Stättermayer AF, Scherzer T, Beinhardt S, Rutter K, Hofer H, Ferenci P. Review article: Genetic factors that modify the outcome of viral hepatitis. Aliment Pharmacol Ther. 2014;39:1059–70.

11. Ben Selma W, Alibi S, Smach MA, Saad A, Boukadida J. IL-18 variant increases risk of enhanced HBV DNA replication in chronic hepatitis. Immunol Lett. 2020;228:70–5.
12. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y, Ali F. Atherosclerotic cardiovascular disease: a review of initiators and protective factors. Inflammopharmacology. 2016;24:1–10.
13. Kim Y, Lee C. The gene encoding transforming growth factor β1 confers risk of ischemic stroke and vascular dementia. Stroke. 2006;37:2843–5.
14. Cho H, Park OH, Park J, Ryu I, Kim J, Ko J, et al. Glucocorticoid receptor interacts with PNRC2 in a ligand-dependent manner to recruit UPF1 for rapid mRNA degradation. Proc Natl Acad Sci [Internet]. National Acad Sciences; 2015;112:E1540–9. Available from: https://pnas.org/doi/full/10.1073/pnas.1409612112.
15. Martins CS, de Castro M. Generalized and tissue specific glucocorticoid resistance. Mol Cell Endocrinol [Internet]. Elsevier; 2021;530:111277. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0303720721001210.
16. Scheschowitsch K, Leite JA, Assreuy J. New Insights in Glucocorticoid Receptor Signaling—More Than Just a Ligand-Binding Receptor. Front Endocrinol (Lausanne) [Internet]. Frontiers Media SA; 2017;8:16. Available from: http://journal.frontiersin.org/article/10.3389/fendo.2017.00016/full.
17. Sarapultsev A, Sarapultsev P, Dremencov E, Komelkova M, Tseilikman O, Tseilikman V. Low glucocorticoids in stress-related disorders: the role of inflammation. Stress [Internet]. Taylor & Francis; 2020;23:651–61. Available from: https://www.tandfonline.com/doi/full/10.1080/10253890.2020.1766020.
18. He B, Cruz-Topete D, Oakley RH, Xiao X, Cidlowski JA. Human Glucocorticoid Receptor β Regulates Gluconeogenesis and Inflammation in Mouse Liver. Mol Cell Biol [Internet]. Am Soc Microbiol; 2016;36:714–30. Available from: https://journals.asm.org/doi/10.1128/MCB.00908-15.
19. Liu W, Tang Q, Jiang H, Ding X, Liu Y, Zhu R, et al. Promoter polymorphism of interleukin-18 in angiographically proven coronary artery disease. Angiology. 2009;60:180–5.
20. Khripko OP, Sennikova NS, Lopatnikova JA, Khripko JI, Filipenko ML, Khrapov EA, et al. Association of single nucleotide polymorphisms in the IL-18 gene with production of IL-18 protein by mononuclear cells from healthy donors. Mediators Inflamm. 2008;2008.
21. Liu Z, Yao X, Sun B, Jiang W, Liao C, Dai X, et al. Pretreatment with kaempferol attenuates microglia-mediate neuroinflammation by inhibiting MAPKs–NF–κB signaling pathway and pyroptosis after secondary spinal cord injury. Free Radic Biol Med [Internet]. United States; 2021;168:142–54. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0891584921001970.
22. Livingston B, Crimi C, Newman M, Higashimoto Y, Appella E, Sidney J, et al. A Rational Strategy to Design Multiepitope Immunogens Based on Multiple Th Lymphocyte Epitopes. J Immunol. 2002;168:5499–506.
23. Amoah K, Hsiao Y-HE, Bahn JH, Sun Y, Burghard C, Tan BX, et al. Allele-specific alternative splicing and its functional genetic variants in human tissues. Genome Res. 2021;31:359–71.
24. Kapil S, Duseja A, Sharma BK, Singla B, Chakraborti A, Das A, et al. Genetic polymorphism in CD14 gene, a co-receptor of TLR4 associated with non-alcoholic fatty liver disease. World J Gastroenterol. 2016;22:9346–55.
25. Wang J, Guo X, Yu S, Song J, Zhang J, Cao Z, et al. Association between CD14 gene polymorphisms and cancer risk: A meta-analysis. PLoS ONE. 2014;9:e100122.
26. Wu Q, Xu X, Ren J, Liu S, Liao X, Wu X, et al. Association between the – 159C/T polymorphism in the promoter region of the CD14 gene and sepsis: A meta-analysis. BMC Anesthesiol. 2017;17:1–11.
27. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, Von Elm E, et al. STrengthening the REporting of genetic association studies (STREGA)-an extension of the strobe statement [Internet]. PLoS Med. 2009. p. 0151–63. Available from: https://dx.plos.org/10.1371/journal.pmed.1000022.
28. YONG Y, HE L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res [Internet]. England; 2005;15:97–8. Available from: http://www.nature.com/articles/7290272.

29. Crooks GE, Hon G, Chandonia J-M, Brenner SE. WebLogo: A Sequence Logo Generator: Fig. 1. Genome Res [Internet]. Cold Spring Harbor Lab; 2004;14:1188–90. Available from: http://genome.cshlp.org/lookup/doi/10.1101/gr.849004.
30. Messeguer X, Escudero R, Farré D, Nuñez O, Martínez J, Albà MM. PROMO: detection of known transcription regulatory elements using species-tailored searches. 18. Bioinformatics: Oxford University Press; 2002. pp. 333–4.
31. Farré D, Roset R, Huerta M, Adsuara JE, Roselló L, Albà MM, et al. Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. Nucleic Acids Res. Oxford University Press; 2003;31:pp. 3651–3.
32. Piva F, Giulietti M, Burini AB, Principato G. SpliceAid 2: A database of human splicing factors expression data and RNA target motifs. Hum Mutat [Internet]. Wiley Online Library; 2012;33:81–5. Available from: https://onlinelibrary.wiley.com/doi/10.1002/humu.21609.
33. Li T, Wernersson R, Hansen RB, Horn H, Mercer J, Slodkowicz G, et al. A scored human protein–protein interaction network to catalyze genomic interpretation. Nat Methods [Internet]. Nature Publishing Group; 2017;14:61–4. Available from: http://www.nature.com/articles/nmeth.4083.
34. Association CM. The guideline of prevention and treatment for chronic hepatitis B (2010 version). Zhonghua Liu Xing Bing Xue Za Zhi. 2011;32:405–15.
35. Hepatitis B. status in Iran until 2020 [Internet]. Available from: https://icdc.behdasht.gov.ir/hepatitis_B_status/- - -B-1400- - .
36. Salehi M, Mohebbi SR, Karkhane M, Kazemian S, Azimzadeh P, Saeedi Niasar M, et al. Lack of genetic association between interleukin-18 gene polymorphism (rs1946518) and chronic hepatitis B infection. J Babol Univ Med Sci. 2018;20:46–52.
37. Zhu SL, Zhao Y, Hu XY, Luo T, Chen ZS, Zhang Y, et al. Genetic polymorphisms – 137 (rs187238) and – 607 (rs1946518) in the interleukin-18 promoter may not be associated with development of hepatocellular carcinoma. Sci Rep. 2016;6:1–12.
38. Maroufi NF, Aghayi E, Garshasbi H, Matin MG, Bedoustani AB, Amoudizaj FF, et al. Association of rs1946518 C/A Polymorphism in Promoter Region of Interleukin 18 Gene and Breast Cancer Risk in Iranian Women: A Case-control Study. Iran J Allergy Asthma Immunol. 2019;18:671–8.
39. Li N, Gao YF, Zhang TC, Chen P, Li X, Su F. Relationship between interleukin 18 polymorphisms and susceptibility to chronic hepatitis B virus infection. World J Hepatol. 2012;4:105–9.
40. Ezzat DA, Morgan DS, Mohamed RA, Mohamed AF. Genetic association of interleukin 18 (-607C/A, rs1946518) single nucleotide polymorphism with asthmatic children, disease severity and total IgE serum level. Cent Eur J Immunol. 2019;44:285–91.
41. Ramazi S, Motovalibashi M, Khazraei H. Association of Interleukin-18)-607A/C) Gene Polymorphism with Allergic Rhinitis in Chaharmahal-va-Bakhtiari Province. J Arak Univ Med Sci. 2014;17:9–16.
42. Zhang S, Yang X, Wang W. Associations of genetic polymorphisms in CTLA-4 and IL-18 with chronic liver diseases: Evidence from a meta-analysis. Genomics [Internet]. United States; 2020;112:1889–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S088875431930607X.
43. Celik SD, Ates O. Genetic analysis of interleukin 18 gene polymorphisms in alopecia areata. J Clin Lab Anal [Internet]. 2018;32:e22386. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jcla.22386.
44. Migita K, Sawakami-Kobayashi K, Maeda Y, Nakao K, Kondoh S, Sugiura M, et al. Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease. Transl Res. 2009;153:91–6.
45. Teixeira AC, Martinelli A, de LC, Donadi. EA. Role of Alleles and Genotypes of Polymorphisms of IL-18 (-607 C/A; and-137 C/G), IFN-γ (+ 874 A/T) and TNF-α (-238 A/G and-308 A/G) and HLA-G Genes in the Susceptibility of Hepatocellular Carcinoma. Hepatocell Carcinoma-Future Outlook. IntechOpen; 2013.
46. Mustarim M, Yanwirasti Y, Jamsari J, Rukmono R, Nindrea RD. Association of gene polymorphism of bactericidal permeability increasing protein rs4358188, cluster of differentiation 14 rs2569190, interleukin 1β rs1143643 and matrix metalloproteinase-16 rs2664349 with neonatal sepsis. Open Access Maced J Med Sci. 2019;7:2728–33.

47. Jia X, Wang B, Yao Q, Li Q, Zhang J. Variations in CD14 gene are associated with autoimmune thyroid diseases in the Chinese population. Front Endocrinol (Lausanne). 2019;10:811.

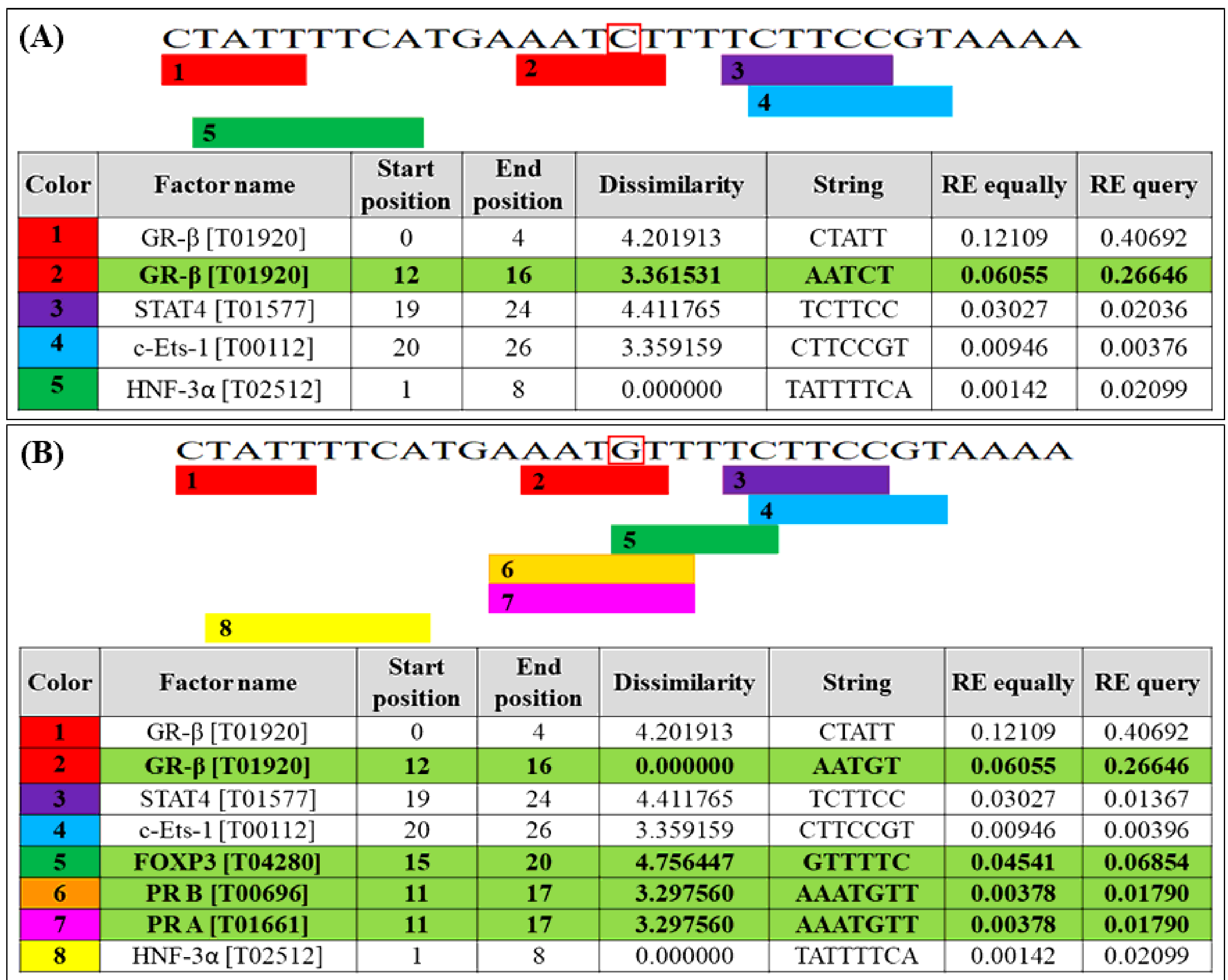
48. Palumbo ML, Prochnik A, Wald MR, Genaro AM. Chronic Stress and Glucocorticoid Receptor Resistance in Asthma. Clin Ther [Internet]. 2020;42:993–1006. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149291820301296.

49. Li K, Wang Z. Splicing factor SRSF2-centric gene regulation. Int J Biol Sci [Internet]. 2021;17:1708–15. Available from: https://www.ijbs.com/v17p1708.htm.

50. Kotlajich MV, Hertel KJ. Death by Splicing: Tumor Suppressor RBM5 Freezes Splice-Site Pairing. Mol Cell [Internet]. United States; 2008;32:162–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276508006904.

51. Fushimi K, Ray P, Kar A, Wang L, Sutherland LC, Wu JY. Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5. Proc Natl Acad Sci [Internet]. 2008;105:15708–13. Available from: https://pnas.org/doi/full/10.1073/pnas.0805569105.

Figures

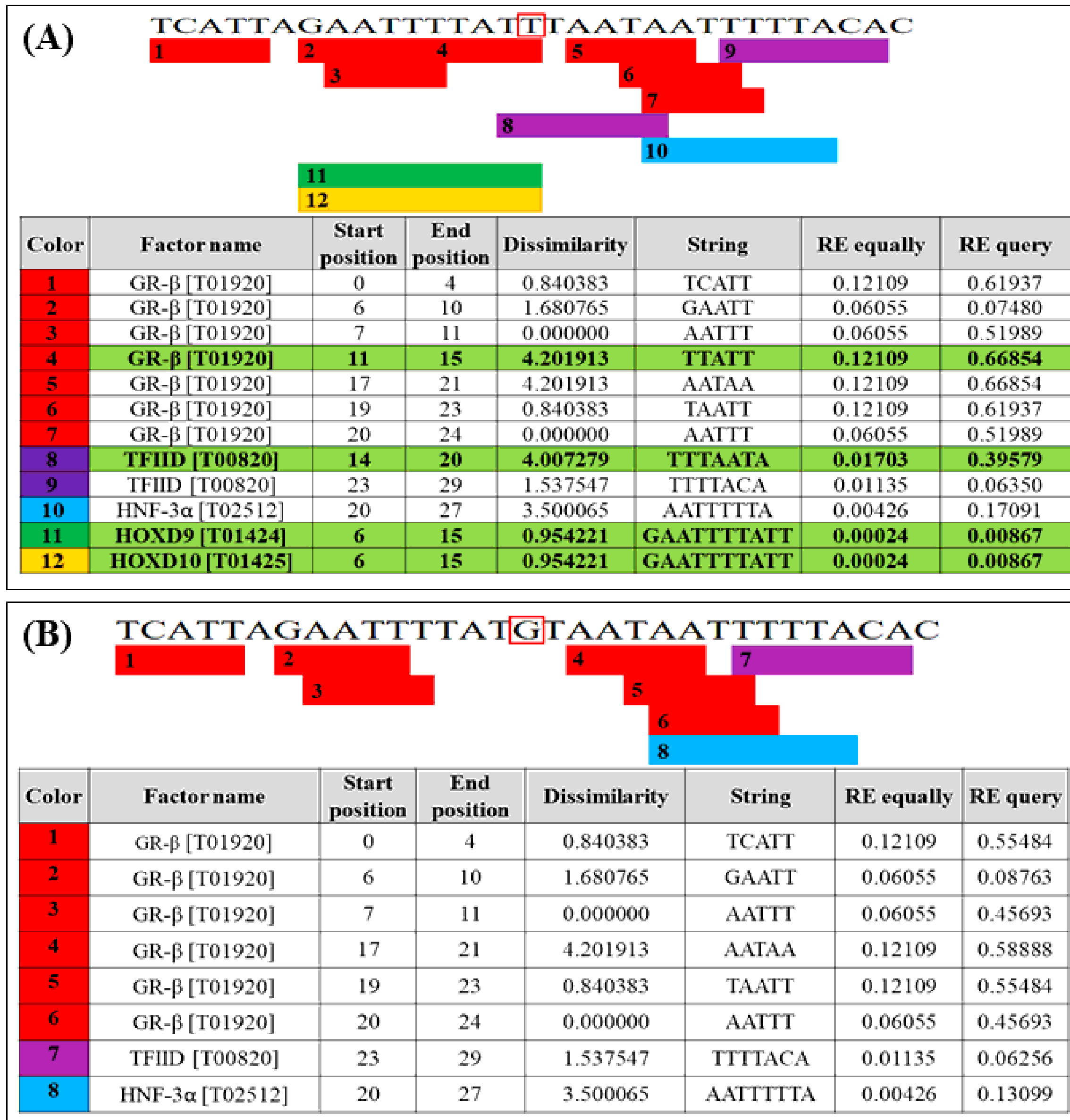


Color	Factor name	Start position	End position	Dissimilarity	String	RE equally	RE query
1	GR-β [T01920]	0	4	4.201913	CTATT	0.12109	0.40692
2	**GR-β [T01920]**	**12**	**16**	**3.361531**	**AATCT**	**0.06055**	**0.26646**
3	STAT4 [T01577]	19	24	4.411765	TCTTCC	0.03027	0.02036
4	c-Ets-1 [T00112]	20	26	3.359159	CTTCCGT	0.00946	0.00376
5	HNF-3α [T02512]	1	8	0.000000	TATTTTCA	0.00142	0.02099

Color	Factor name	Start position	End position	Dissimilarity	String	RE equally	RE query
1	GR-β [T01920]	0	4	4.201913	CTATT	0.12109	0.40692
2	**GR-β [T01920]**	**12**	**16**	**0.000000**	**AATGT**	**0.06055**	**0.26646**
3	STAT4 [T01577]	19	24	4.411765	TCTTCC	0.03027	0.01367
4	c-Ets-1 [T00112]	20	26	3.359159	CTTCCGT	0.00946	0.00396
5	**FOXP3 [T04280]**	**15**	**20**	**4.756447**	**GTTTTC**	**0.04541**	**0.06854**
6	**PR B [T00696]**	**11**	**17**	**3.297560**	**AAATGTT**	**0.00378**	**0.01790**
7	**PR A [T01661]**	**11**	**17**	**3.297560**	**AAATGTT**	**0.00378**	**0.01790**
8	HNF-3α [T02512]	1	8	0.000000	TATTTTCA	0.00142	0.02099

Figure 1

TFBS prediction at rs187238 C>G in the *IL-18* gene promoter. The red square indicates the SNP position and different transcription factor binding motifs identified for polymorphic alleles are determined in **bold** and green. The Random Expectation (RE) gives the number of expected occurrences of the match in a random sequence of the same length as the query sequence based on the dissimilarity index. Data is achievable through http://factor.genexplain.com/cgi-bin/transfac_factor/search.cgi *via* inserting the transcription factor ID. TFBS, transcription factor binding site; SNP, single-nucleotide polymorphism; rs, reference SNP; *IL-18*, interleukin 18; GR-beta, glucocorticoid receptor beta; FOXP3, forkhead box P3 protein; PR B, progesterone receptor form B; PR A, progesterone receptor form A



(A)

Color	Factor name	Start position	End position	Dissimilarity	String	RE equally	RE query
1	GR-β [T01920]	0	4	0.840383	TCATT	0.12109	0.61937
2	GR-β [T01920]	6	10	1.680765	GAATT	0.06055	0.07480
3	GR-β [T01920]	7	11	0.000000	AATTT	0.06055	0.51989
4	**GR-β [T01920]**	**11**	**15**	**4.201913**	**TTATT**	**0.12109**	**0.66854**
5	GR-β [T01920]	17	21	4.201913	AATAA	0.12109	0.66854
6	GR-β [T01920]	19	23	0.840383	TAATT	0.12109	0.61937
7	GR-β [T01920]	20	24	0.000000	AATTT	0.06055	0.51989
8	**TFIID [T00820]**	**14**	**20**	**4.007279**	**TTTAATA**	**0.01703**	**0.39579**
9	TFIID [T00820]	23	29	1.537547	TTTTACA	0.01135	0.06350
10	HNF-3α [T02512]	20	27	3.500065	AATTTTTA	0.00426	0.17091
11	**HOXD9 [T01424]**	**6**	**15**	**0.954221**	**GAATTTTATT**	**0.00024**	**0.00867**
12	**HOXD10 [T01425]**	**6**	**15**	**0.954221**	**GAATTTTATT**	**0.00024**	**0.00867**

(B)

Color	Factor name	Start position	End position	Dissimilarity	String	RE equally	RE query
1	GR-β [T01920]	0	4	0.840383	TCATT	0.12109	0.55484
2	GR-β [T01920]	6	10	1.680765	GAATT	0.06055	0.08763
3	GR-β [T01920]	7	11	0.000000	AATTT	0.06055	0.45693
4	GR-β [T01920]	17	21	4.201913	AATAA	0.12109	0.58888
5	GR-β [T01920]	19	23	0.840383	TAATT	0.12109	0.55484
6	GR-β [T01920]	20	24	0.000000	AATTT	0.06055	0.45693
7	TFIID [T00820]	23	29	1.537547	TTTTACA	0.01135	0.06256
8	HNF-3α [T02512]	20	27	3.500065	AATTTTTA	0.00426	0.13099

Figure 2

TFBS prediction at rs1946518 T>G in the *IL-18* gene promoter. The red square indicates the SNP position and different transcription factor binding motifs identified for polymorphic alleles are determined in **bold** and green. The Random Expectation (RE) gives the number of expected occurrences of the match in a random sequence of the same length as the query sequence based on the dissimilarity index. Data is achievable through http://factor.genexplain.com/cgi-bin/transfac_factor/search.cgi *via* inserting the transcription factor ID. TFBS, transcription factor binding site; SNP, single-nucleotide polymorphism; rs, reference SNP; *IL-18*, interleukin 18; GR-beta, glucocorticoid receptor beta; TFIID, transcription factor IID; HOXD 9, homeobox D9 protein; HOXD10, homeo box D10 protein

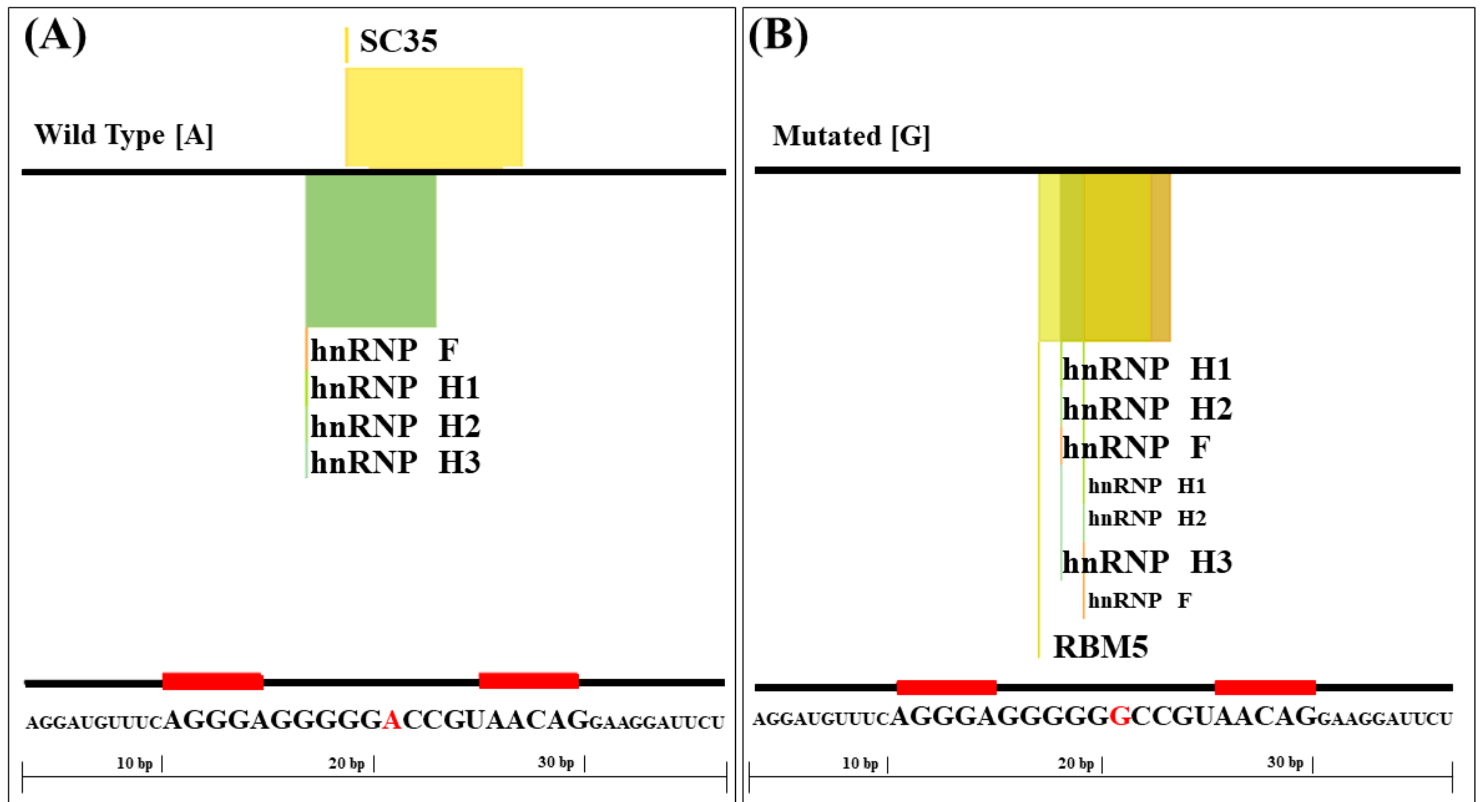


Figure 3

Bioinformatics analyses of a 20-nucleotide flanking region containing *CD14*–rs2569190 A>G polymorphism. *CD14*, cluster of differentiation antigen 14; rs, reference single-nucleotide polymorphism

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.png
- SupplementaryInformation.docx