Journal of Viral Hepatitis, 2009, **16,** 45–52 doi:10.1111/j.1365-2893.2008.01040.x

A non-synonymous single nucleotide polymorphism in IFNAR1 affects susceptibility to chronic hepatitis B virus infection

J. Zhou,[1] D. K. Smith,[2] L. Lu,[3] V. K. M. Poon,[1] F. Ng,[1] D.-Q. Chen,[1] J.-D. Huang,[2] K.-Y. Yuen,[1] K.-Y. Cao[1,4] and B.-J. Zheng[1] [1]*Department of Microbiology, The University of Hong Kong;* [2]*Department of Biochemistry, The University of Hong Kong;* [3]*Department of Pathology, The University of Hong Kong, Hong Kong; and* [4]*Research Centre for Clinical Laboratory Standard, Zhongshan School of Medicine, Sun Yat-Sen University, Guangdong, China*

Received May 2008; accepted for publication June 2008

SUMMARY. The type I interferon (IFN-α/β) receptor 1 (IFNAR1) mediates the potent antiviral and immuno-regulatory effects of IFN-α/β that are believed to be pivotal to eradicate hepatitis B virus (HBV) infection. IFNAR1 promoter polymorphisms (at −568/−77) have been shown to be associated with susceptibility to chronic HBV infection; however, whether these markers are genetic determinants of HBV infection remains unknown. The functional significance of promoter −568/−77 polymorphisms was assessed by mutagenesis and luciferase assays. Sequencing and restriction fragment length polymorphisms in 328 chronic HBV patients, 130 spontaneous resolvers and 148 healthy blood donors identified other polymorphism at IFNAR1 open reading frame. IFNAR1 expression levels in peripheral blood cells were detected by flow cytometry. We found that the −568/−77 promoter variants were unlikely to affect transcription levels. A C/G single nucleotide polymorphism, in strong linkage disequilibrium with the promoter polymorphisms, was found in the coding sequence of IFNAR1 (nt19158). This resulted in a nonsynonymous substitution in the extracellular region of IFNAR1 protein and correlated with susceptibility to chronic HBV infection. Bioinformatic analysis suggested decreased stability of the IFNAR1 protein. Chronic HBV patients with the 19158C/C genotype (Leu141) exhibited higher IFNAR1 protein expression levels in peripheral blood monocytes than those with the 19158G/G genotype (Val141). In conclusion, IFNAR1 19158C/G polymorphism is primarily associated with susceptibility to chronic HBV infection.

Keywords: HBV infection, IFNAR1, protein stability, SNP.

INTRODUCTION

Hepatitis B virus (HBV) infection results in dichotomous clinical manifestations, viral resolution and viral persistence. The role of host genetic factors in the clinical heterogeneity of HBV infection was first reported in a twin study of disease concordance in Chinese [1]. Mounting evidence has revealed that genetic variations in immuno-relevant genes, i.e. HLA class I and class II [2], tumour necrosis factor-α [3,4], mannose binding protein [5], cytotoxic T-lymphocyte antigen 4 [6] and type I IFN receptor 2 (IFNAR2) [7] contribute to the variable clinical outcomes of HBV infection.

IFN-α/β might be one of the major determinants for the prognosis of HBV infection, as these cytokines function to contain viral replication and modulate host immunity to eradicate virus during the early stage of infection [8,9]. IFN-α/β signalling is elicited through their receptors, IFNAR1 and IFNAR2 [10,11]. Previously we demonstrated that IFNAR1 promoter polymorphisms were correlated with susceptibility to chronic HBV infection [12]. A single nucleotide polymorphism (SNP) at promoter position −568 and linked biallelic repeat instability at position −77 were associated with variable clinical outcomes. However, promoter luciferase assays were unable to show distinguishable variation of gene expression corresponding to the genetic alterations. To ascertain the primary genetic association, we

Abbreviations: CP, chronic (hepatitis B) patients; HBD, healthy blood donors; HBV, hepatitis B virus; IFN, interferon; IFNAR1, type I interferon (IFN-α/β) receptor 1; JLIN, java based LINkage; MFI, mean fluorescence intensity; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SR, spontaneous recovered individuals.

Correspondence: Dr Bo-Jian Zheng, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong, China. E-mail: bzheng@hkucc.hku.hk or Dr Kai-Yuan Cao, Research Centre for Clinical Laboratory Standard, Zhongshan School of Medicine, Sun Yat-Sen University, Zhongshan 2nd Road #74, Guangzhou 510080, China. E-mail: caoky@mail.sysu.edu.cn

Present Address: Jie Zhou, Northern California Institute for Research and Education, San Francisco, CA, USA

subsequently studied the coding sequence of IFNAR1. A C/G SNP was identified at nt19158, which results in a leucine to valine substitution at mature protein position 141. Notably, the 19158 SNP was in strong linkage disequilibrium with the −568/−77 promoter polymorphisms and was shown to correlate with clinical manifestations. Moreover, our experimental evidence and bioinformatic analysis consistently support a functional role for the 19158 SNP.

PATIENTS AND METHODS

Study subjects and sample collections

The study participants were composed of 328 chronic hepatitis B patients (CP), 130 spontaneously recovered individuals (SR) and 148 healthy blood donors (HBD). Blood samples of the CP group were collected, with informed consent, from Queen Mary Hospital, Queen Elizabeth Hospital (both in Hong Kong SAR), Dong Hu Hospital (Shenzhen, China) and You An Hospital (Beijing, China) after ethical approval was obtained from the Ethics Committee, LKS Faculty of Medicine, The University of Hong Kong. Clinical test information, i.e. HBV e antigen and alanine aminotransferase, were recorded during sample collection.

Two hundred and thirty-eight ethnically matched healthy individuals were obtained from the Red Cross Blood Bank of Hong Kong. These were further classified into the SR (n = 90) and HBD (n = 148) groups based on their antibody status against HBV surface and e antigen, which were detected by ELISA (BIOKIT, Spain) in accordance with the manufacturer's instruction. The other 40 spontaneous resolvers were recruited from You An Hospital (Beijing, China).The demographic and clinical test profiles of study participants are shown in Table 1.

Construction of type I interferon (IFN-α/β) receptor promoter-luciferase reporter vectors and mutagenesis

Luciferase reporter vectors under the control of three naturally occurring haplotypes of IFNAR1 promoter were constructed as described previously [12]. As a result of the genetic linkage in locus −568 and −77, three artificial constructs were generated by site-directed mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene) to dissect the effect of both −568 and −77 allele in isolation. Plasmid constructs, mutated positions, parental plasmids and primers for mutagenesis are described in Table 2. The sequence of each construct was verified by sequencing.

Transient transfection and luciferase assay

Three pairs of pGL3-IFNAR1 promoter vectors and a blank pGL3-Basic plasmid (225 ng/well), together with the internal control pRL-CMV (25 ng/well; Promega, Madison, WI, USA), were transfected, in quadruplicate, to HepG2 cell cultures in 96-well plates using calcium phosphate precipitation methods as previously described [13,14]. The transfected cells were harvested 48 h post-transfection and assayed for firefly and Renilla luciferase using a Dual-Glo luciferase kit (Promega). All experiments were independently repeated three times. Luciferase indices (LI = firefly luminescence/Renilla luminescence) were calculated for each transfectant and compared with that of the unmodified pGL3-Basic plasmid transfectant.

Type I interferon (IFN-α/β) receptor genotyping with restriction fragment length polymorphism

Our primary study of the IFNAR1 coding sequence in 100 study participants revealed that position 649 in the IFNAR1 mRNA (NM_000629) was a polymorphic site with frequency of clinical relevance. However, as a result of limited availability of cDNA samples, genotyping was performed using genomic DNA. Primer pairs (forward: TGAATGA AGGTTTTGGCATTGT, reverse: CATGTAAACTTCAGCAAT ATGG) were designed to amplify a 538 bp fragment of genomic DNA that accommodates the corresponding mutation site, which is situated at locus 19158 (X60459). Polymerase chain reaction products were incubated with Dde I (New England BioLabs, Ipswich, MA, USA) at 37 °C overnight. After digestion with Dde I, all amplicons yielded a 63 bp fragment on an agarose gel, which was used as an

Table 1 Demographic and clinical test profiles of CP and controls

Group	CP		SR		HBD	
No.	231	97	124	6	113	35
Male/female	141/90	52/45	53/71	4/2	47/66	20/15
HBsAg	+	+	−	−	−	−
anti-HBs	−	−	+	−	+	−
HBeAg	+	−	−	−	−	−
anti-HBe	−	+	−	−	−	−
anti-HBc	+	+	+	+	−	−
ALT, normal/elevated	35/196	11/83	NA	NA	NA	NA

CP, chronic hepatitis B patients; SR, spontaneous resolvers; HBD, healthy blood donors; NA, not applicable; ALT, alanine aminotransferase; HBeAg, HBV e antigen.

Table 2 Denotation of type I interferon (IFN-α/β) receptor 1 promoter-luciferase plasmid constructs and primers for mutagenesis

Parental plasmid		Mutated plasmid			
Denotation	Haplotypes*	Denotation	Mutation locus	Haplotypes*	Primers for mutagenesis
Ia	GCC 15	Ia-M	G(−568)C	CCC 15	F:GCCTCTGCCCCGCTCTCGCT
Ib	GCC 14	Ib-M	G(−568)C	CCC 14	CTGCACACAGCAACGG
					R:CCGTTGCTGTGTGCAGAGC
					GAGAGCGGGGCAGAGGC
II	CCC 5	II-M	C(−568)G	GCC 5	F:GCCTCTGCCCCGCTCTCGGT
					CTGCACACAGCAACGG
					R:CCGTTGCTGTGTGCAGACC
					GAGAGCGGGGCAGAGGC

*Represent tagging alleles at positions −568, −408, −3 and repeats at −77 respectively.

internal control for enzyme digestion. Homozygotes with the wild type C allele were unable to be digested at polymorphic site, which thus yielded two bands, corresponding to 475 bp and 63 bp fragments. Homozygotes with the G allele generate another digestion site, yielding three bands for fragments of 371, 104 and 63 bp and heterozygotes yielded all four bands.

Flow cytometry analysis of type I interferon (IFN-α/β) receptor expression levels

Monocytes in whole blood from a cohort of 46 CP were analyzed by flow cytometry on a fluorescence activated cell sorting calibur (BD Biosciences, Frankin Lakes, NJ, USA) after staining with anti-human IFNAR1 (R&D systems, clone 85228, Minneapolis, MN, USA; and APC-labeled CD14 antibodies (Abcam, Cambridge, MA, USA; clone 61D3). Mean fluorescence intensity (MFI) of AR1 on monocytes, which was recognized by anti-CD14 antibody, was determined using CellQuest software.

Bioinformatic analysis of HuIFNAR1

Java based LINkage disequilibrium plotting programme, was applied for analysis of linkage disequilibrium between polymorphisms within the IFNAR1 gene locus. MU-pro software, available at http://www.ics.uci.edu/~baldig/mutation.html, can accurately predict protein stability changes for single amino acid mutations using only primary sequence [15]. Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) was applied to generate haplotype patterns and used for the association analysis.

A 3D model of the HuIFNAR1 extracellular domain was constructed based on the IFN-gamma receptor 1 structure (IFNGR1, PDB entry 1FYH, chain E) using SWISS-MODEL (http://swissmodel.expasy.org/). Alignment of IFNAR1 sequences was performed by ClustalW.

Statistical analysis

Paired and independent sample *t*-tests were applied for comparison of the luciferase activity of IFNAR1 promoter plasmids and IFNAR1 protein expression levels respectively. A chi-squared test was used to define the distribution of genetic markers in the three study groups. A P-value <0.05 was regarded as significant.

RESULTS

The −568/−77 polymorphisms do not affect promoter activity

Previously, we demonstrated that plasmids with naturally occurring haplotypes, $-568G/-77GT_{15}/-3C$ (pHAP-I) and $-568C/-77GT_{5}/-3C$ (pHAP-II), exhibited comparable luciferase levels in a reporter-driven system [12]. However, we could not exclude the possibility that −568 and −77 locus confer opposite impact to IFNAR1 promoter. To further clarify the individual effect and functional significance of the −568 and −77 polymorphisms, we used pHAP-Ia ($-568G/-77GT_{15}/-3C$), pHAP-Ib ($-568G/-77GT_{14}/-3C$) and pHAP-II ($-568C/-77GT_{5}/-3C$) as parental vectors to generate artificial vectors pHAP-IaM ($-568C/-77GT_{15}/-3C$), pHAP-IbM ($-568C/-77GT_{14}/-3C$) and pHAP-IIM ($-568G/-77GT_{5}/-3C$). These three pairs of vectors, as well as unmodifed pGL3-Basic, were co-transfected with pRL-CMV into HepG2 cells and the transfectants were subjected to a luciferase assay. As shown in Fig. 1, all three pairs of plasmids showed similar luciferase levels, indicating that both the −568G/C polymorphism and the −77GT repeat variants 15, 14 (M) and 5 (L) had no apparent impact on the promoter activity of IFNAR1. This did not explain our earlier findings that carriers of the $-568G/-77GT_{M}$ were less susceptible to chronic HBV infection whereas those with allele

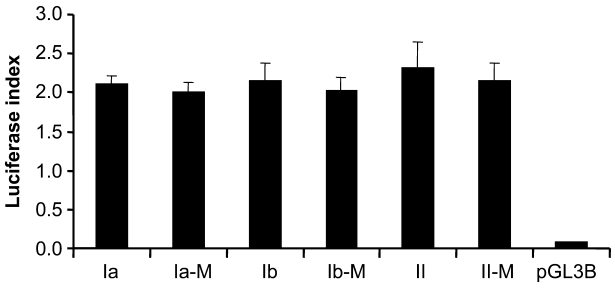

Fig. 1 Luciferase assay of type I interferon (IFN-α/β) receptor 1 (IFNAR1) promoter variants with −568 and −77 polymorphisms. IFNAR1 promoter variants pHAP-Ia (−568G/−77GT$_{15}$/−3C), pHAP-Ib (−568G/−77GT$_{14}$/−3C) and pHAP-II (−568C/−77GT$_5$/−3C), and the mutated derivatives pHAP-IaM (−568C/−77GT$_{15}$/−3C), pHAP-IbM (−568G/−77GT$_{14}$/−3C) and pHAP-IIM (−568G/−77GT$_5$/−3C), as well as unmodified plasmid pGL3-Basic were co-transfected into HepG2 cells with internal control, pRL-CMV. Luciferase indexes represent the pRL-CMV normalized firefly luciferase activities of each plasmid. The luciferase indexes of the three pairs of plasmids were not significantly different. The results are presented as mean and standard deviation of three independent assays.

−568C/−77GT$_L$ were significantly associated with a higher risk of chronic hepatitis B infection [12].

Identification of type I interferon (IFN-α/β) receptor 19158 single nucleotide polymorphism and its strong linkage disequilibrium with the −568/−77 polymorphisms

We therefore proceeded to study whether other genetic alterations in IFNAR1 gene are related to the −568/−77 polymorphisms. If so, these may account for the genetic association of the −568/−77 polymorphisms with the susceptibility to chronic HBV infection. A C/G SNP, which results in a leucine to valine substitution, was identified at IFNAR1 coding sequence (locus 19158 of X60459 or 649 of NM_000629) by sequencing and restriction fragment length polymorphism (RFLP) (Fig. 2). This SNP was found to be in strong linkage disequilibrium with the −568/−77 polymorphisms (Fig. 3). Therefore, it prompted us to postulate that the 19158 SNP is a real genetic determinant associated with viral persistence or clearance.

The 19158 single nucleotide polymorhism associates with susceptibility to chronic hepatitis B virus infection

We assessed the distribution of the 19158C/G SNP in chronic HBV patients, spontaneous resolvers and healthy controls. Table 3 presents the differential distribution of genotypes, alleles and haplotypes in the three study groups. As a result of the strong linkage disequilibrium between positions −568 and 19158, there were two predominant haplotypes that existed in the study participants: −568G/19158C and

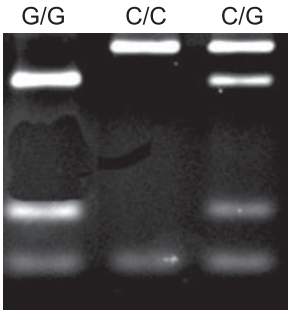

Fig. 2 Identification of type I interferon (IFN-α/β) receptor 1 nt19158 SNP by RFLP. A 538 bp polymerase chain reaction fragment containing the 19158C/G SNP was digested with Dde I. The homozygous wt genotype C/C yielded two bands in sizes of 475 and 63 bp on an agarose gel, whereas mutant genotype G/G yielded three bands, 371, 104 and 63 bp. Heterozygous C/G genotypes yielded all four bands.

−568C/19158G. The frequencies of the 19158C allele and its associated haplotype −568G/19158C were significantly higher in the SR group than in the CP group (P = 0.0067, 0.0034). Conversely, the 19158G allele and its corresponding haplotype −568C/19158G were over-represented in the CP group relative to the SR group (P = 0.0067, 0.0266). The 19158C/C genotype was more often detected in the SR group than in the CP group (P = 0.0086), while the CP group had a higher frequency of genotype G/G with borderline statistical significance (P = 0.0674). The frequencies of these genetic markers in the HBD group lay between those of the CP and SR groups. A significantly biased distribution could also be observed when the HBD group was compared with the CP group.

The 19158 single nucleotide polymorphism correlated to type I interferon (IFN-α/β) receptor expression levels in chronic hepatitis B virus patients

Type I interferon (IFN-α/β) receptor protein expression levels were detected by flow cytometry on monocytes, which were identified by anti-CD14 antibody, from peripheral blood of 46 CP, because monocytes and B cells express the highest levels of IFNAR1 among peripheral hematopoietic cells [16]. As shown in Fig. 4, IFNAR1 levels were significantly higher in genotype 19158C/C carriers (MFI = 1317 ± 804.7) than in their G/G counterparts (MFI = 465 ± 437.5) ($P < 0.05$), while that in heterozygote C/G carriers (MFI = 749 ± 312.7) lay in between.

Bioinformatic analysis of 19158 SNP

MU-pro software [15] predicted that the leucine to valine substitution at human IFNAR1 (HuIFNAR1) 141 caused by the 19158 SNP would lead to a decrease in protein stability with a ΔΔG value of −0.8528. ΔΔG stands for the energy change resulting from a single mutation, which is the sign of relative stability change. The positive value

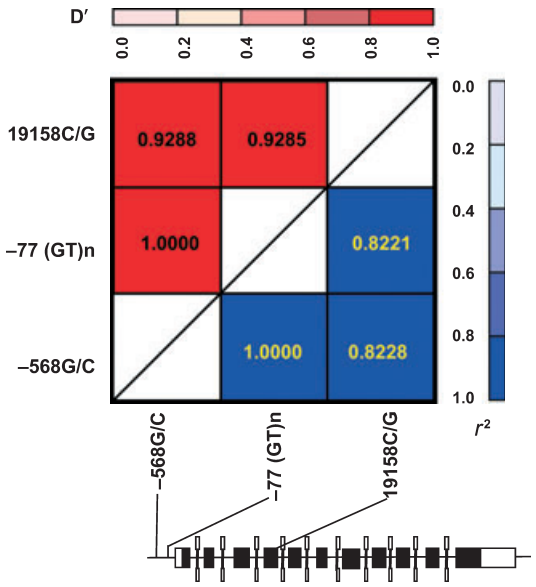

Fig. 3 Linkage disequilibrium (LD) analysis of positions −568, −77 and nt19158 by JLIN (java based LINkage). JLIN displays pairwise LD statistics. Two statistics, D′ and r^2 are presented. For biallelic markers, D′ is equal to 1 if one or more than one haplotype are absent, which mean no recombinant haplotypes are present in the population. Although D′ is a better measurement of defining the pattern of LD, r^2 is more directly relevant to the power of association study since it takes into account of both recombination and allelic frequencies. It represents the statistical correlation between two loci. It is equal to 1 if only two haplotypes are present. The D′ is shown in the top left-hand triangle of the display (red squares). The r^2 is shown in the bottom right hand triangle (blue squares). The scale for the D′, along with numerical increments, is presented at the top, while that for r^2 is presented on the right hand side. The name of each polymorphism is shown along the left and bottom of the display, to present a matrix of pairwise comparisons between loci. At the bottom of the figure is a schematic diagram showing the relative positions of the input markers. Black boxes represent exons. White open boxes are 3′ and 5′ untranslated regions.

means mutation increases stability, negative one represents destabilizing effect. A 3D model of HuIFNAR1 was constructed by SWISS-MODEL based on the IFN-γ receptor and this showed that the substituted residue lies in a cavity on the exterior of the molecule (Fig. 5) where it could affect local packing interactions and thus protein stability. Another possibility is that this cavity may be a binding surface for an unknown accessory protein and the polymorphism could affect the stability of this interaction.

DISCUSSION

An important issue in genetic association studies of disease pathogenesis is linkage disequilibrium, which reveals that a particular allelic combination is presented more often than is expected by chance. This can confound interpretation of genetic association studies because certain polymorphisms may not be causal factors but rather in linkage disequilibrium with the true determinant. Therefore, functional studies are needed to validate the genetic association.

Previously we demonstrated a genetic association between IFNAR1 promoter −568/−77 polymorphisms and susceptibility to chronic HBV infection. However, a functional study of the promoter did not support these two linked variations being the causal determinants of the clinical outcome. In this study, we further confirmed that both the −568 and −77 polymorphisms did not confer a transcriptional difference. Another SNP, detected at nt19158, which is in strong linkage disequilibrium with the −568/−77 polymorphisms, might explain this discrepancy. An association study revealed that the 19158 C/G SNP was correlated with susceptibility to chronic HBV infection. We proceeded to seek supportive functional evidence for this genetic association.

The two subunits of type I IFN receptors, IFNAR1 and IFNAR2, are members of the class II cytokine receptor family, which share conserved fibronectin type III (FnIII) structural domains to form the extracellular ligand-binding region [17,18]. IFNAR1 is the only member of this family to have an extracelluar domain consisting of four FnIII domains, designated SD1-SD4. Each FnIII domain contains seven β-strands connected by loops. The IFN binding site was shown to reside predominantly in SD2 and SD3 [19]. The 19158 C/G polymorphism results in a leucine to valine substitution in amino acid 141 of mature IFNAR1 protein, which is located in the third β-strand of SD2. The genetic association results obtained here raise the question whether the Leu141Val substitution could affect the IFNAR1 protein and the subsequent potency of IFN-α/β signaling.

A major obstacle to the study of human IFNAR1 (HuIFNAR1) is its low intrinsic affinity for IFN-α/β, which has hampered the identification of residues critical for ligand binding. However, the homologous bovine IFNAR1 (BoIFNAR1) binds human IFN-α2 with high affinity, therefore the critical residues in BoIFNAR1 responsible for human IFN binding were identified by site-directed mutagenesis [20]. The knowledge obtained from this BoIFNAR1 study provides an elegant approach to circumvent obstacles in the study of HuIFNAR1. In BoIFNAR1, five aromatic residues in SD2 and SD3 were found to be the foci of the receptor/ligand binding interface. However, these five aromatic residues are conserved between HuIFNAR1 and BoIFNAR1 (Fig. 6). The clusters of residues surrounding these key amino acids modulate ligand binding and account for the substantial difference in binding affinity between BoIFNAR1 and HuIFNAR1 [20].

It is accepted that residues involved in ligand binding are most often in the loops and turns of cytokine receptors, rather than in the β-strands [21]. Consistent with this, four of the five critical residues are located in loops within SD2

Table 3 Distribution of genetic markers in patients and controls

Polymorphisms	Frequency			*P*-value	
	CP (n = 328)	SR (n = 130)	HBD (n = 148)	CP *vs* SR	CP *vs* HBD
19158 alleles					
C	37.20%	46.92%	45.27%	**0.0067**	**0.0184**
G	62.80%	53.08%	54.73%		
−568/19158 haplotypes					
GC	34.86%	45.52%	43.17%	**0.0034**	**0.0149**
GG	1.78%	0.82%	1.39%	0.2955	0.6659
CC	2.72%	1.23%	2.07%	0.1865	0.5569
CG	60.64%	52.43%	53.37%	**0.0266**	**0.0365**
19158 genotypes					
C/C	14.33%	24.62%	19.59%	**0.0086**	0.1466
C/G	45.73%	44.62%	51.35%	0.8287	0.2558
G/G	39.94%	30.77%	29.05%	0.0674	**0.0225**

Primary genotype information were input as case/control data and analyzed with Haploview 3.32. Single marker and haplotype association statistics were calculated. Significant associations are in bold. n is the total number of participants in each group. CP, SR and HBD stand for chronic patients, spontaneous resolvers and healthy blood donors, respectively.

and SD3. The only exception is BoIFNAR1 Tyr141, which is conserved in all IFNAR1 and IFNAR2 sequences. In a 3D homology model, BoIFNAR1 Tyr141 and its HuIFNAR1 homologue Tyr138 form the third β-strand of SD2. Mutation of this residue in BoIFNAR1 leads to a modest decrease in cell surface expression levels, and a dramatic decrease in IFN-α binding affinity compared with parental receptor [20]. Therefore, although BoIFNAR1 Tyr141 appears buried in the protein interior and not directly involved in ligand binding, it is likely be required for proper presentation of surrounding key residues and stabilization of the protein structure. It is implicated that its HuIFNAR1 homologue Tyr138 might also play a similar role in human IFNAR1 molecule.

The residue of our interest, HuIFNAR1 Leu141Val, lies in the same β-sheet as Tyr138. Mu-pro, a program designed to evaluate the effect of mutations in proteins [15], predicted that the HuIFNAR1 Leu141Val substitution might introduce instability to HuIFNAR1. In agreement with the bioinformatic analysis, we observed that patients with Leu141 expressed higher levels of IFNAR1 than those with Val141. Based on a homology model of HuIFNAR1, we analyzed

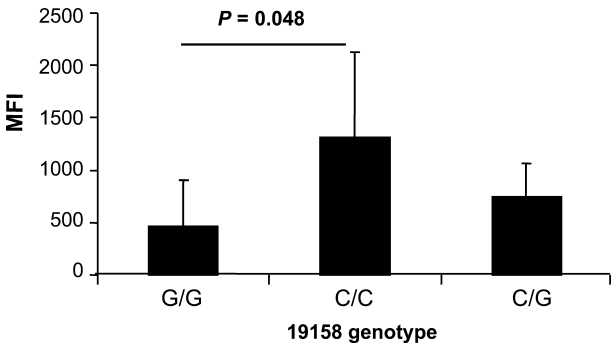

Fig. 4 Type I interferon (IFN-α/β) receptor 1 (IFNAR1) expression on monocytes of chronic hepatitis B patients (CP) is higher in those with the 19158C/C (Leu141) genotype. IFNAR1 expression was detected in monocytes of CP by flow cytometry and is presented as mean florescence intensity with standard deviation. Genotype 19158C/C carriers express significantly higher levels of IFNAR1 than genotype 19158G/G carriers. *P* values between the compared groups are indicated.

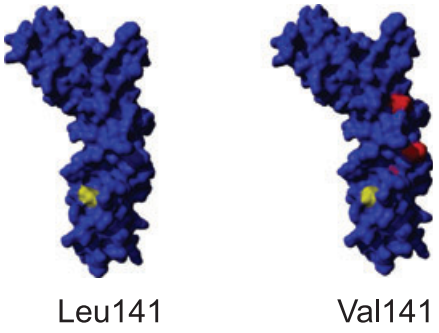

Fig. 5 Surface representations of models of human type I interferon (IFN-α/β) receptor 1 (IFNAR1) domains SD1 and SD2. The location of residue 141 (left Leu, right Val) is shown in yellow in a cavity on the surface of the molecule. The location of the residues identified as responsible for ligand binding in human IFNAR1 or homologous to those in bovine IFNAR1 are shown in red on the surface of the Val141 model.

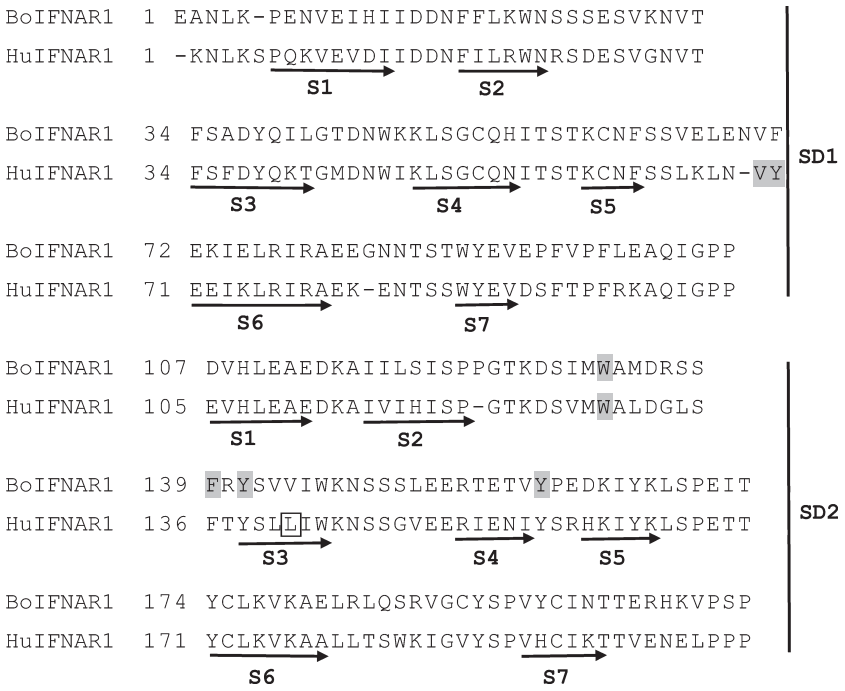

Fig. 6 Alignment of the sequences of the SD1 and SD2 domains of the extracellular region of human (HuIFNAR1) and bovine (BoIFNAR1) type I interferon (IFN-α/β) receptor 1. Putative β-strands, predicted by the IFNGR1 3D structure, are indicated by underlying arrows and designated S1-S7. HuIFNAR1 Leu141, the residue discussed in this work, is boxed. Residues (BoIFNAR1 Trp132, Phe139, Tyr141, Tyr160 and HuIFNAR1 Val69, Tyr70 and Trp129) which are implicated from experimental evidence as important for IFN signaling are shaded gray.

possible conformational changes derived from this substitution. This residue lies in a cavity on the exterior of the molecule. The larger leucine residue appears to provide better packing in this region, which may cause Leu141 HuIFNAR1 to be in a more stable conformation and so be more likely to be recognized by the IFNAR1 antibody used in flow cytometry. This *in silico* analysis and measurement of protein levels are actually consistent with findings in the mutagenesis analysis of BoIFNAR1 Tyr141, which is associated with diminished surface expression levels and subsequent IFN-α binding affinity. As Leu141Val is on the opposite side of the molecule to the previously mentioned aromatic residues (Fig. 5), it will not be directly involved in ligand binding. Apart from the Val substitution decreasing local stability and through that the conformation of the ligand binding surface, it is possible that the cavity surrounding residue 141 could be a binding site for an as yet unknown accessory protein. Valine may alter the conformation of the cavity making it less suitable as a potential protein interaction site. Furthermore, we also cannot rule out the possibility that there is a transcription enhancer at nt19158 in the coding region of the gene.

A recent study by genome scan demonstrated that class II cytokine receptor cluster, which accommodates IFNAR1, IFNAR2 and other cytokine receptors, is a major locus for HBV persistence in Gambian families [7]. A SNP in IFNAR2, which causes an amino acid substitution in the signal peptide, was associated with viral persistence. However, this linkage was not duplicated in a population in Italy. Herein, it is suggested that type I IFN receptors and their signal effects are critical determinants for virus clearance in HBV infection, which is in agreement with our findings. It is also suggested that, in association studies of a certain disease, the identified susceptible marker may differ among various ethnic populations. This incongruity might be related the different genetic background of study participants. To the best of our knowledge, it is unlikely that the reported IFNAR2 is in linkage disequilibrium with our SNP of interest and affects the disease phenotypes in our study subjects.

Taken together, we have revealed a contributing factor to chronic HBV infection. Previously, we found that the −568/−77 promoter polymorphisms were correlated with chronic HBV infection. This genetic association is actually ascribed to their strong linkage disequilibrium with another SNP located in IFNAR1 exon 4, which results in a Leu/Val substitution at position 141 of the mature IFNAR1 protein. Bioinformatic analysis suggests that Leu141 might be superior to Val141 in stabilizing the structure of HuIFNAR1 and so may provide a better presentation of the critical residues that are indispensable for ligand binding. By assessing the IFNAR1 protein levels in a cohort of 46 CP, we demonstrated that the Leu141 (19158C/C genotype) carriers exhibited higher protein levels on blood monocytes than Val141 (19158G/G) carriers. This lends support to the

computational prediction and analysis of HuIFNAR1. The combination of wet lab data and dry lab analysis provides enough evidence to explain why the IFNAR1 19158 C/G SNP, which is in linkage disequilibrium with the −568/−77 polymorphisms, affects the clinical outcome of HBV infection.

ACKNOWLEDGEMENT

This work was supported in part by the University Development Fund of the University of Hong Kong.

REFERENCES

1 Lin TM, Chen CJ, Wu MM *et al.* Hepatitis B virus markers in Chinese twins. *Anticancer Res* 1989; 9(3): 737–741.
2 Thio CL, Thomas DL, Karacki P *et al.* Comprehensive analysis of class I and class II HLA antigens and chronic hepatitis B virus infection. *J Virol* 2003; 77(22): 12083–12087.
3 Hohler T, Kruger A, Gerken G, Schneider PM, Meyer zum Buschenefelde KH, Rittner C. A tumor necrosis factor-alpha (TNF-alpha) promoter polymorphism is associated with chronic hepatitis B infection. *Clin Exp Immunol* 1998; 111(3): 579–582.
4 Kim YJ, Lee HS, Yoon JH *et al.* Association of TNF-alpha promoter polymorphisms with the clearance of hepatitis B virus infection. *Hum Mol Genet* 2003; 12(19): 2541–2546.
5 Thio CL, Mosbruger T, Astemborski J *et al.* Mannose binding lectin genotypes influence recovery from hepatitis B virus infection. *J Virol* 2005; 79(14): 9192–9196.
6 Atabani SF, Thio CL, Divanovic S *et al.* Association of CTLA4 polymorphism with regulatory T cell frequency. *Eur J Immunol* 2005; 35(7): 2157–2162.
7 Frodsham AJ, Zhang L, Dumpis U *et al.* Class II cytokine receptor gene cluster is a major locus for hepatitis B persistence. *Proc Natl Acad Sci USA* 2006; 103(24): 9148–9153.
8 Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; 13: 29–60.
9 Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; 19: 65–91.
10 Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev* 2004; 202: 8–32.
11 Brierley MM, Fish EN. Review: IFN-alpha/beta receptor interactions to biologic outcomes: understanding the circuitry. *J Interferon Cytokine Res* 2002; 22(8): 835–845.
12 Zhou J, Lu L, Yuen MF *et al.* Polymorphisms of type I interferon receptor 1 promoter and their effects on chronic hepatitis B virus infection. *J Hepatol* 2007; 46(2): 198–205.
13 Zheng B, Graham FL, Johnson DC, Hanke T, McDermott MR, Prevec L. Immunogenicity in mice of tandem repeats of an epitope from herpes simplex gD protein when expressed by recombinant adenovirus vectors. *Vaccine* 1993; 11(12): 1191–1198.
14 Zheng BJ, Graham FL, Prevec L. Transcription units of E1a, E1b and pIX regions of bovine adenovirus type 3. *J Gen Virol* 1999; 7: 1735–1742.
15 Cheng J, Randall A, Baldi P. Prediction of protein stability changes for single-site mutations using support vector machines. *Proteins* 2006; 62(4): 1125–1132.
16 Pogue SL, Preston BT, Stalder J, Bebbington CR, Cardarelli PM. The receptor for type I IFNs is highly expressed on peripheral blood B cells and monocytes and mediates a distinct profile of differentiation and activation of these cells. *J Interferon Cytokine Res* 2004; 24(2): 131–139.
17 Domanski P, Colamonici OR. The type-I interferon receptor. The long and short of it. *Cytokine Growth Factor Rev* 1996; 7(2): 143–151.
18 Mogensen KE, Lewerenz M, Reboul J, Lutfalla G, Uze G. The type I interferon receptor: structure, function, and evolution of a family business. *J Interferon Cytokine Res* 1999; 19(10): 1069–1098.
19 Lu J, Chuntharapai A, Beck J *et al.* Structure-function study of the extracellular domain of the human IFN-alpha receptor (hIFNAR1) using blocking monoclonal antibodies: the role of domains 1 and 2. *J Immunol* 1998; 160(4): 1782–1788.
20 Cutrone EC, Langer JA. Identification of critical residues in bovine IFNAR-1 responsible for interferon binding. *J biol chem* 2001; 276(20): 17140–17148.
21 Clackson T, Wells JA. A hot spot of binding energy in a hormone-receptor interface. *Science* 1995; 267(5196): 383–386.