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CXCL12 G801A polymorphism is associated with significant liver fibrosis in HIV-infected Thais: a cross-sectional study

Thitiilat Chiraunyanann,[1,4] Khaimuk Changsri,[2] Warisara Sretapunya,[5] Kornanong Yuenyongchaiwat,[3] Chareeporn Akekawatchai[2]

Abstract

Background: Previous studies indicate high prevalence of liver diseases in HIV-infected patients, and their genetic risk factors are still unclear. The chemokine CXCL12 plays important roles in development of chronic liver injury and a single nucleotide polymorphism (SNP) G to A change at position 801 in CXCL12 gene has been demonstrated to affect CXCL12 production levels.

Objective: This study aimed to analyze the association of CXCL12 G801A SNP with liver complication in HIV-infected Thais.

Methods: A cross-sectional study was conducted in 164 patients who were evaluated for transaminitis and significant liver fibrosis, defined by fibrosis-4 (FIB-4) score and AST to platelet ratio index (APRI), and genotyped for the SNP using tetra-primer PCR-SSP.

Results: There were high rates of patients with transaminits (28.0%), and significant liver fibrosis by FIB-4 score (18.9%) and by APRI (14.0%). The CXCL12 G801A AA/GA genotypes were significantly associated with transaminitis (p = 0.014) and significant fibrosis by APRI (p = 0.020). Univariate and multivariate analyses identified the AA/GA genotypes as predictive factors for significant fibrosis (OR 6.8, 95%CI 1.7-28.2, p = 0.008), together with age older than 40 years, $CD4^+$ cell count < 350 cells/μl and hepatitis B and/or C virus coinfection. The significantly higher medians of APRI and FIB-4 score, in patients with AA/GA than those with GG genotypes (p < 0.05) were observed in the ART-naïve, but not ART-experienced groups.

Conclusion: The CXCL12 G801A AA/GA genotypes are significant predictive factors for hepatic fibrosis potentially in the ART-naïve HIV-infected Thais.

Key words: CXCL12 G801A polymorphism, HIV, liver fibrosis, APRI, FIB-4

From:
[1] Graduate Program in Medical Technology,
[2] Department of Medical Technology,
[3] Department of Physical Therapy, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand
[4] Bureau of AIDS, TB and STIs, Department of Disease Control, Ministry of Public Health, Tiwanon Road, Muang District, Nonthaburi, Thailand
[5] Nakorn Nayok Hospital, Nakorn Nayok, Thailand

Corresponding author:
Chareeporn Akekawatchai
Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, 99 Moo 18, Klongluang, Pathumthani, Thailand
E-mail: cakekawatchai@gmail.com, ejareepo@tu.ac.th

Background

Human immunodeficiency virus (HIV) infection continues to be a major heath issue with estimated 36.7 million people living with HIV worldwide at the end of 2015.[1] During the last decade, the advances in management of HIV-infected patients with combined anti-retroviral therapy (ART) have led to a decrease of acquired immunodeficiency syndrome (AIDs)-related morbidity and mortality with an increased evidence of liver-related diseases. Various factors can cause liver damage in HIV-infected patients including HIV replication in the liver, hepatitis B or C virus (HBV or HCV) coinfection, anti-retroviral therapy (ART), immune reconstitution and opportunistic infections.[2,3] Viral hepatitis has been shown to be the most common risk of liver-related illness in HIV patients.[2-4] Published studies in HIV-monoinfected and hepatitis

virus-coinfected patients have also identified multiple risk factors for development and severity of liver disease including HIV RNA levels, CD4+ cell count, increased body mass index, duration and types of ART, severe alcohol use, diabetes mellitus and host genetic polymorphisms.[5-8] Presently, even though studies have revealed some genetic and immunological parameters influencing liver disease progression specifically in HCV/HIV-coinfected patients,[8] host genetic determinants of liver disease in HIV infection are required more investigation.

Chemokine systems play important roles in the recruitment of immune cells to the liver, regulating immune cell infiltration, chronic inflammation and progression of fibrosis.[9] One of the most interesting systems is CXCL12 or stromal cell -derived factor-1 (SDF-1) and its respective receptors CXCR4 and CXCR7. The CXCL12/CXCR4/CXCR7 system is known to regulate liver homeostasis, and their increased expression are involved in acute liver injury, chronic liver damage or fibrosis, and hepatocellular carcinoma (HCC) development and metastasis.[9,10] Apparently, the common polymorphism in CXCL12 gene, a single nucleotide polymorphism (SNP) G to A change at position 801 in the 3'untranslated region (UTR) of an alternatively spliced mRNA transcript, also termed SDF-13'A or rs1801157, has been reported to affect levels of CXCL12 production *in vitro* and *in vivo*.[11-13] Up to date, only few studies have investigated roles of CXCL12 gene polymorphisms in liver diseases. SNPs in CXCL12 gene have been demonstrated to influence occult HBV infection, therapeutic responses in HCV/HIV coinfection, and susceptibility and pathological development of hepatocellular carcinoma (HCC),[14-16] but lack of significant association with death and HCC occurrence in HCV-related and alcoholic cirrhosis.[17,18] CXCL12 is also well known to contribute dramatically to pathogenesis and progression of HIV infection and many previous studies have indicated an impact of CXCL12 gene polymorphisms including the G801A SNP on resistance of HIV infection, progression to AIDs and responses to ART.[11,13,19-22] However, influence of the CXCL12 gene polymorphism on chronic liver diseases developed in HIV-infected patients is still unknown.

Our previous study has demonstrated a high prevalence of transaminitis in Thai HIV-infected patient group with high rates of HBV and/or HCV coinfection and mostly on long -term suppressive ART, suggesting their potential to develop chronic liver injury.[7] The present study therefore aimed to investigate an impact of CXCL12 G801A polymorphisms on hepatic complications of these patients. Frequencies of CXCL12 G801A alleles and genotypes, and their association with liver abnormalities were examined. Risk factors for liver complications in this HIV patient group were also determined using logistic regression analyses.

Materials and Methods

Study population

A cross-sectional study was conducted in 164 HIV patients attending the Antiretroviral Therapy Clinic in Nakorn Nayok Hospital from October 2011 to June 2013. In this study, inclusion criteria were as follows: patients with documented HIV infection, and availability of blood samples and clinical data. Patients who regularly consumed alcohol, herbal medicine, steroid medication, and patients with active opportunistic infections including tuberculosis, were excluded.[2,23] All subjects provided written informed consent and the study protocol was reviewed and approved by the Human Ethics Committees No. 2, Thammasat University, Thailand (Project no. 077/256).

Clinical and laboratory data were obtained as described in the previous study.[7] Briefly, the data collected from patient's medical records and standardized questionnaire were age, gender, duration of antiretroviral therapy (ART), currently used anti-retroviral (ARV) regimens, opportunistic infections, alcohol consumption, herbal medicine and steroid intake. All patients underwent clinical examination and routine blood tests including levels of aspartate and alanine aminotransferases (AST and ALT), platelet count, anti-HCV, HBsAg, and CD4+ cell count. Additionally, ethylene-diamine-tetra-acetic acid (EDTA) blood samples left over from routine testing were subjected to plasma separation within 8 hours after blood collection and stored at -80°C until use.

Genotyping of CXCL12 G801A polymorphism

Genomic DNA was extracted from EDTA blood samples using a Nucleospin® blood extraction kit (Macherey-Nagel GmbH, Duren, Germany) according to the manufacturer's instruction. DNA samples were genotyped for CXCL12 G801A or SDF1-3'A (rs1801157) polymorphism using a polymerase chain reaction-sequence specific primer (PCR-SSP) assay developed in the previous studies.[24,25] Two pairs of specific primer sets were used. Firstly, an A allele-specific primer pair comprises forward inner (5' CATCCACATGGGAGCCA 3') and reverse outer primers (5' ACATTGGTCACAGAGGAGGA 3') producing a 364 bp PCR product. Secondly, a G allele-specific set is composed of forward outer (5' GCTCTGAAACCAGTGTTAGG 3') and reverse inner primers (5' CCAGAAGAGGCAGACCC 3') producing a 495 bp PCR product. The two sets of primers also amplify a PCR product of 826 bp from the two outer primers. DNA samples (25-50 ηg) were amplified using the PCR kit buffer plus 0.25 U of Tag DNA polymerase (NanoHelix, Deajeon, South Korea). PCR conditions were as follows: denaturation at 94°C for 2 min, 35 cycles of 95°C for 20 sec, 64.5°C for 50 sec, and 72°C for 1 min, and elongation at 72°C for 5 min in a T100 Thermal Cycler (Bio-Rad, Hercules, CA). PCR products were then analyzed by electrophoresis in 2% agarose gel with DNA size marker of 100-1,000 bp and visualized using UV fluorescence after staining with ethidium bromide. Positive DNA controls with possible genotypes and negative control with no DNA were included in a panel of genotyping.[24] As shown in **Figure 1**, the GG and AA genotypes yielded 495 and 364 bp PCR products respectively while the GA heterozygous genotype presented both 495 and 364 bp products. Samples of all genotypes exhibited the 826 bp internal control fragments. Thirty four (34) of 164 PCR samples (20.7%) with 8/164 AA, 11/164 GA and 15/164 GG genotypes were subjected to direct DNA sequencing and analyzed by Unipro UGENE v.1.24.2. There was 100% agreement between the results obtained by PCR-SSP and by direct sequencing.

Evaluation of liver complication

From routine laboratory data, several surrogate markers were used to determine liver abnormality in HIV patients including thrombocytopenia, transaminitis, fibrosis-4 (FIB-4)

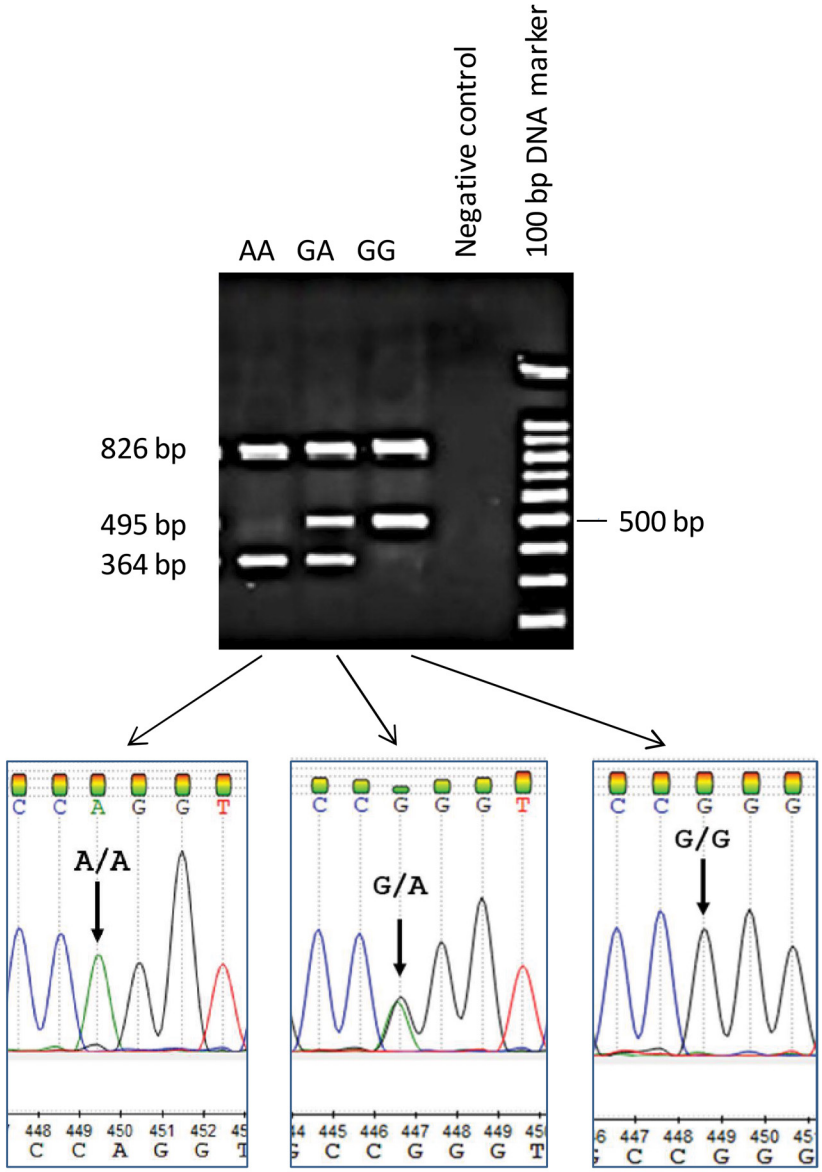

Figure 1. Genotyping of CXCL12 G801A polymorphism by tetra-primer PCR-SSP. A representative gel demonstrating AA in lane 1, GA in lane 2, GG genotypes in lane 3, negative control in lane 4 and 100 bp DNA ladders in lane 5. The genotyping was confirmed by direct sequencing and Unipro UGENE v.1.24.2. analysis.

score and AST-to-platelet ratio index (APRI). Platelet count was utilized to assess thrombocytopenia which was classified into 4 grades. 75,000 to 150,000/μL were defined as grade 1, 50,000 to < 75,000 /μL as grade 2, 25,000 to < 50,000 /μL as grade 3 and < 25,000 /μL as grade 4.[26,27] Transaminitis was defined as an increased level from the normal upper limit (ULN) of either AST or ALT (> 40 U/L).[7] FIB-4 score was calculated and classified into class 1 (< 1.45), class 2 (1.46 to 3.25) and class 3 (> 3.25).[28,29] APRI value was calculated and classified into class 1 (< 0.5), class 2 (0.51 to 1.5) and class 3 (> 1.5).[30]

Statistical Analysis

Genotype and allele frequencies were calculated by direct counting. Hardy-Weinberg equilibrium was assessed using χ^2 test with one degree of freedom from online analysis tools, considering equilibrium when $p > 0.05$.[31] χ^2 test or Fishers's exact test, and odds ratio with 95% confidence interval (CI) were used to determine an association of CXCL12 G801A genotypes with categorical variables. The level of statistical significance was determined at $p < 0.05$. Univariate and multivariate logistic regression were performed to determine risks for the liver complication assessed by transaminitis and APRI > 0.5. Odds ratio (OR) with 95% confidence interval (CI) and *P* value were calculated. All *P* values < 0.05 were considered as statistically significant. The PASW statistics 18 software (SPSS Inc.) was used for statistical analysis.

Results

Characteristics of the study population

Characteristics and clinical features of the subjects are summarized in **Table 1**. A total of 164 patients, 90 male (54.9%) and 74 (45.1%) female with the mean age of 39.8 (±11.3) years, were included in this study. The median $CD4^+$ cell count of these patients was 371 [1-1601] cells/μl. Prevalence of HBV/HIV,

HCV/HIV and HBV/HCV/HIV triple coinfection were 9.1% (12/132), 8.3% (11/132) and 0.8% (1/132), respectively. One hundred and seventeen out of 164 patients (71.3%) were on combined anti-retroviral (ARV) drugs with the median duration of 39 months [range, 16-55] and 104 of the 117 patients (88.9%) were on ART for longer than 6 months. With regards to the 117 ART-experienced patients, 74 (63.2%) were on the hepatotoxic nevirapine-based regimen.

HIV-infected patients were evaluated for liver complication using available noninvasive markers as shown in **Table 1**. In the 147 HIV patients with a mean platelet count of 276 (±105) × 10^9/μL, 10 (6.8%) patients were assessed as having grade 1 or 2 thrombocytopenia. Of the 143 patients, 40 (28.0%) had transaminitis, 20 (14.0%) were assessed as having class 2 or 3 APRI (APRI > 0.5) and 27 (18.9%) were classified as class 2 or 3 (FIB-4 score > 1.45).

Genotypic and allelic distribution of CXCL12 G801A polymorphism and association with liver complication in HIV-infected Thais

As shown in **Table 2**, 93 (56.7%), 58 (35.4%) and 13 (7.9%) patients were detected with GG, GA and AA genotypes and frequencies of G and A alleles were 0.744 (244) and 0.256 (84) respectively. In the dominant model, the percentages of GG and combined GA/AA genotypes were 56.7% (93/164) and 43.3% (71/164) consequently. The CXCL12 genotypes were consistent with the Hardy-Weinberg equilibrium ($p > 0.05$). Statistic analysis indicated that the characteristics of the patients possessing AA, GA and GG genotypes, and A and G alleles, including CD4$^+$ cell count, HBV and HCV coinfection, and FIB-4 score were similar ($p > 0.05$), except for transaminitis ($p = 0.024$, and $p = 0.006$ respectively) and fibrosis assessed by having APRI > 0.05 ($p = 0.007$ and $p = 0.003$ respectively). Genotypes under the dominant model were also significantly associated with transaminitis (OR 2.5, 95%CI 1.2-5.3, $p = 0.014$) and having APRI > 0.5 (OR 3.1, 95%CI 1.2-8.4, $p = 0.020$). Notably, there was no statistically significant difference between the patient groups with different genotypes and alleles regarding ARV regimens currently used, nevirapine experience and duration of ART (p > 0.05) (data not shown).

CXCL12 G801A polymorphism as a predictive factor for liver complication in the ART-naïve patients

To examine possible risk factors for liver abnormalities assessed by transaminitis and APRI in this study population, logistic regression analyses were conducted on variables which were CXCL12 G801A genotypes, gender, age, CD4$^+$ cell count, hepatitis B and/or C virus coinfection, navirapine experience and ART duration as shown in **Table 3**. Univariate analysis of risk factors for transaminitis indicated that being male, coinfection with HBV and/or HCV and the CXCL12 G801A AA/GA genotypes were risk factors for developing transaminitis (OR 3.7, 95%CI 1.6-8.4, $p = 0.002$, OR 3.3, 95%CI 1.3-8.9, $p = 0.015$ and OR 2.5, 95%CI 1.2-5.3, $p = 0.015$, respectively). In subsequent multivariate analysis, only coinfection with HBV and/or HCV was predictive for transaminitis in this study group (OR 3.1, 95%CI 1.1-8.6, $p = 0.035$). In univariate analysis of risk factors for having APRI > 0.5, several factors including being male, age older than 40 years, having CD4$^+$ cell count < 350 cells/μl, coinfection with HBV and/or HCV and the CXCL12 G801A AA/GA genotypes were identified as predictors of having APRI > 0.5 (OR 3.6, 95%CI 1.1-11.2, $p = 0.030$, OR 3.1, 95%CI 1.1-8.6, $p = 0.031$, OR 5.3, 95%CI 1.7-17.0, $p = 0.004$, OR 4.2, 95%CI 1.4-12.7, $p = 0.013$, OR 3.1, 95%CI 1.2-8.4, $p = 0.025$, respectively). Multivariate analysis subsequently indicated that the AA/GA genotypes together with age older than 40 years, CD4$^+$ cell count < 350 cells/μl and HBV

Table 1. Characteristics and clinical features of Thai HIV-infected patients (n = 164).

Characteristics	Patients N (%)
Patients	164 (100%)
Age[a] (years)	39.8 (±11.3)
Gender (male)	90 (54.9%)
CD4$^+$ cell count (cells/μl) (n = 141)	371 [1-1601]
HBV or HCV coinfection (n = 132)	
HIV monoinfection	108 (81.8%)
HIV/HBV coinfection	12 (9.1%)
HIV/HCV coinfection	11 (8.3%)
HIV/HBV/HCV triple-infection	1 (0.8%)
Current ARV regimens	
Naive to ARV treatment	47 (28.7%)
Lamivudine/Stavudine/Nevirapine	11 (6.7%)
Lamivudine/Zidovudine/Nevirapine	57 (34.8%)
Lopinavir/Ritonavir or Atazanavir	10 (6.1%)
Others	39 (23.8%)
Nevirapine experience (n = 117)	
Nevirapine-based regimens	74 (63.2%)
Duration of ARV treatment (n = 117)[b]	39 [16-55]
Duration of ARV treatment (n = 117)	
≤ 6 months	13 (11.1%)
> 6 months	104 (88.9%)
Platelet count[a] (n = 147)	
Platelet count (× 10^9/L)	276 (±105)
Thrombocytopenia (n = 147)	
Grade 1 or 2 (75,000-150,000 or 50,000-<75,000/μl)	10 (6.8%)
Transaminitis (n = 143)	
AST and/or ALT > ULN (40 U/L)	40 (28.0%)
Significant liver fibrosis (n = 143)	
FIB-4 score > 1.45	27 (18.9%)
APRI > 0.5	20 (14.0%)

[a] and [b] Data shown as mean value ± S.D and, median and interquartile range [IQR] respectively. Some variables had missing data and n is given in parentheses. AST: aspartate aminotransferase, ALT: alanine aminotransferase, FIB-4: fibrosis-4 score, APRI: AST to platelet ratio index

Table 2. Genotypic and allelic distribution of CXCL12 G801A polymorphism, and association with severity of HIV infection and liver abnormalities in Thai HIV patients (n = 164).

Characteristics	Genotypes N (%)			*P*	Alleles N (%)		*P*	Dominant model genotypes N (%)		*P*	OR (95%CI)
	GG	GA	AA		G	A		GG	GA/AA		
Patients	93 (56.7)	58 (35.4)	13 (7.9)	-	244 (74.4)	84 (25.6)	-	93 (56.7)	71 (43.3)	-	-
$CD4^+$ cell count (n = 141)				0.652			0.372				1.3 (0.6-2.5)
≥ 350 cells/μl	45 (54.9)	25 (51.0)	4 (40.0)		115 (54.0)	33 (47.8)		45 (54.9)	29 (49.2)		
< 350 cells/μl	37 (45.1)	24 (49.0)	6 (60.0)		98 (46.0)	36 (52.2)		37 (45.1)	30 (50.8)	0.502	
Hepatitis B or C coinfection (n = 132)				0.565			0.336			0.508	1.4 (0.6-3.3)
HBV- HCV-	62 (83.8)	39 (81.2)	7 (70.0)		163 (83.2)	53 (77.9)		62 (83.8)	12 (50.0)		
HBV+ and/or HCV+	12 (16.2)	9 (18.8)	3 (30.0)		33 (16.8)	15 (22.1)		12 (16.2)	12 (50.0)		
Transaminitis (n = 143)				0.024*			0.006*			0.014*	2.5 (1.2-5.3)
AST and/or ALT ≤ ULN (40 U/L)	67 (79.8)	32 (64.0)	4 (44.4)		166 (76.1)	40 (58.8)		67 (79.8)	36 (61.0)		
AST and/or ALT > ULN (40 U/L)	17 (20.2)	18 (36.0)	5 (55.6)		52 (23.9)	28 (41.2)		17 (20.2)	23 (39.0)		
FIB-4 score (n = 143)				0.127			0.140			0.419	1.4 (0.6-3.3)
≤ 1.45	70 (83.3)	41 (82.0)	5 (55.6)		181 (83.0)	51 (75.0)		70 (83.3)	46 (78.0)		
> 1.45	14 (16.7)	9 (18.0)	4 (44.4)		37 (16.2)	17 (25.0)		14 (16.7)	13 (22.0)		
APRI (n = 143)				0.007*			0.003*			0.020*	3.1 (1.2-8.4)
≤ 0.5	77 (91.7)	41 (82.0)	5 (55.6)		195 (89.4)	51 (75.0)		77 (91.7)	46 (78.0)		
> 0.5	7 (8.3)	9 (18.0)	4 (44.4)		23 (10.6)	17 (25.0)		7 (8.3)	13 (22.0)		

P values and OR with 95%CI were calculated by Chi-square test. Some variables had missing data and n is given in parentheses.
* Data shown as *P* value < 0.05, OR: Odds ratio, CI: Confidence interval, FIB-4: fibrosis-4 score, APRI: AST to platelet ratio index

Table 3. Univariate and multivariate regression analysis of risks for liver complication assessed by transaminitis and having APRI > 0.5 in Thai HIV-infected patients (n = 164).

Characteristics	Enrolled patients	Patients with Transaminitis N (%)	Transaminitis				Patients with APRI > 0.5 N (%)	APRI > 0.5			
			Crude OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*		Crude OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*
Gender				0.002*		0.085			0.030*		0.224
Female	74	9 (22.0)	1		1		4 (20.0)	1		1	
Male	90	31 (78.0)	3.7 (1.6-8.4)		2.3 (0.9-6.2)		16 (80.0)	3.6 (1.1-11.2)		2.5 (0.6-11.3)	
Ages (years)				0.639		0.365			0.031*		0.009*
≤ 40	92	20 (50.0)	1		1		6 (30.0)	1		1	
> 40	72	20 (50.0)	1.2 (0.6-2.5)		1.5 (0.6-3.7)		14 (70.0)	3.1 (1.1-8.6)		7.5 (1.7-34.4)	
$CD4^+$ cell count (cells/μl)				0.225		0.920			0.004*		0.012*
≥ 350	71	17 (43.6)	1		1		4 (20.0)	1		1	
< 350	66	22 (56.4)	1.6 (0.8-3.4)		1.0 (0.4-2.6)		16 (80.0)	5.3 (1.7-17.0)		7.7 (1.5-38.5)	
HBV and HCV coinfection				0.015*		0.035*			0.013*		0.010*
HIV monoinfection	108	23 (67.6)	1		1		10 (58.8)	1		1	
HBV and/or HCV coinfection	23	11 (32.4)	3.3 (1.3-8.9)		3.1 (1.1-8.6)		7 (41.2)	4.2 (1.4-12.7)		6.5 (1.5-27.4)	
CXCL12 G801A genotypes				0.015*		0.233			0.025*		0.008*
GG	93	17 (42.5)	1		1		7 (35.0)	1		1	
AA/GA	71	23 (57.5)	2.5 (1.2-5.3)		1.7 (0.7-4.0)		13 (65.0)	3.1 (1.2-8.4)		6.8 (1.7-28.2)	
Nevirapine experience											
Naive to ARV treatment	47	7 (17.5)	1				6 (30.0)	1			
Nevirapine-based regimens	74	20 (50)	1.3 (0.5-3.5)	0.583			9 (45.0)	0.6 (0.2-1.8)	0.369		
Non nevirapine-based regimens	43	13 (32.5)	1.7 (0.6-4.8)	0.359			5 (25.0)	0.6 (0.2-2.2)	0.432		
Duration of ARV treatment											
Naive to ARV treatment	47	7 (17.5)	1				6 (30.0)	1			
≤ 6 months	13	3 (7.5)	1.1 (0.2-5.4)	0.866			1 (5.0)	0.4 (0.04-3.5)	0.394		
> 6 months	104	30 (75)	1.5 (0.6-3.8)	0.424			13 (65.0)	0.6 (0.2-1.8)	0.383		

In multivariate analysis, data were adjusted for gender, age, $CD4^+$ cell count, HBV and/or HCV coinfection, and CXCL12 G801A genotypes in the model.
* Data shown as *p* value < 0.05, OR: Odds ratio, CI: Confidence interval, APRI: AST to platelet ratio index

Table 4. Comparison of clinical features regarding liver function and severity of HIV infection between the patients with GG and with AA/GA genotypes in all HIV infected (n = 164), ART-naïve (n = 47) and ART-experienced (n = 117) patients.

	All HIV infected patients		*P*	ART-naïve patients		*P*	ART-experienced patients		*P*
	AA/GA	GG		AA/GA	GG		AA/GA	GG	
CD4⁺ cell count (cells/ml)[a]	322 (158-505)	394 (256-566)	0.096	121 (77-335)	379 (135-489)	0.062	377 (231-528)	398 (295-586)	0.447
AST (U/L)[a]	29 (23-42)	23 (19-34)	0.018*	35 (29-56)	23 (17-31)	0.003*	27 (22-42)	23 (20-35)	0.267
ALT (U/L)[a]	25 (20-42)	22 (16-31)	0.062	25 (18-44)	20 (12-24)	0.123	25 (21-43)	22.5 (17-33)	0.219
Platelet count (10^9/L)[a]	259 (216-313)	271 (220-315)	0.413	236 (146-287)	270 (229-308)	0.151	263 (234-313)	272 (220-326)	0.877
APRI[a]	0.30 (0.19-0.44)	0.23 (0.15-0.35)	0.072	0.44 (0.26-0.75)	0.24 (0.15-0.37)	0.018*	0.27 (0.18-0.38)	0.22 (0.15-0.35)	0.399
FIB-4 score[a]	0.82 (0.52-1.39)	0.73 (0.50-1.22)	0.350	1.71 (0.98-2.85)	0.71 (0.52-1.37)	0.022*	0.71 (0.50-1.18)	0.73 (0.49-1.22)	0.841
Duration of ART (months)[a]	13 (0-46)	29 (0-53)	0.172	-	-	-	35 (13-53)	40 (19-60)	0.294

[a] Data shown as median and interquartile range [IQR], * Data shown as $p < 0.05$, The medians of some variables were not calculated from all subjects due to missing values. AST: aspartate aminotransferase, ALT: alanine aminotransferase, FIB-4: fibrosis-4 score, APRI: AST to platelet ratio index

and/or HCV coinfection remained predictive factors of having APRI > 0.5 in this study population (OR 6.8, 95%CI 1.7-28.2, $p = 0.008$, OR 7.5, 95%CI 1.7-34.4, $p = 0.009$, OR 7.7, 95%CI 1.5-38.5, $p = 0.012$, OR 6.5, 95%CI 1.5-27.4, $p = 0.010$, respectively). The analysis did not show significant association of both transaminits and having APRI > 0.5 with nevirapine experience and ART duration ($p > 0.05$).

To further analyze a possible influence of ART on the genetic effect of CXCL12 G801A polymorphism observed, clinical data indicating hepatic function and HIV disease severity including CD4⁺ cell count, levels of AST and ALT, platelet count, APRI and FIB-4 score in the HIV patients possessing AA/GA were compared with those having GG genotypes, in all HIV -infected patients, ART-naïve and ART-experienced groups, as demonstrated in **Table 4**. In all 164 HIV patients, the patients with AA/GA had significantly higher levels of AST than those with GG genotypes ($p = 0.018$) and there were no significant differences in other markers ($p > 0.05$). Data analysis in ART-naïve patients indicated that the medians of AST levels, APRI and FIB-4 score were significantly higher in the patients having AA/GA than those possessing GG genotypes ($p = 0.003$, $p = 0.018$ and $p = 0.022$ respectively). When compared with those having GG, the patients with AA/GA had relatively lower median of CD4⁺ cell count, even though the difference was not significant ($p = 0.062$). In contrast, there were no significant differences in all serum markers observed in the ART-experienced group ($p > 0.05$). The data indicated that CXCL12 G801A genotypes had an effect on liver complication only in the ART-naïve patients, suggesting influence of ART on the predictive ability of the G801A genotypes in this study group.

Discussion

This cross-sectional study was conducted in 164 HIV patients maintaining high CD4⁺ cell count. There were high prevalence rates of HBV and HCV coinfection and high percentages of patients with long suppressive ART especially hepatotoxic nevirapine-containing regimens. Evaluation of liver abnormalities by non-invasive laboratory test available in this study indicated that there were high rates of patients with thrombocytopenia (6.8%), transaminitis (28.0%), significant fibrosis as shown by FIB-4 score > 1.45 (18.9%) and by APRI > 0.5 (14.0%) consistent with previous studies reporting prevalence of liver fibrosis in HIV-monoinfected and HCV-coinfected patients.[6,32] Our analysis indicated a high prevalence of HIV patients with chronic hepatic injury, which possibly develop to more severe fibrosis and cirrhosis as observed in the studies in HIV patients who were coinfected with HCV and on ART for longer duration.[32,33]

CXCL12 G801A polymorphism has been well characterized in different ethnic groups and established to have an impact on susceptibility to HIV infection, HIV disease progression and ART response[11,13,19-22] whereas few studies indicate its role in liver disease caused by hepatitis viruses and hepatocellular carcinoma.[14,16] In this study, frequency of the A allele of CXCL12 G801A polymorphism was observed as 0.256 while previous studies in Thai HIV-seropositive patients and blood donors reported the frequencies ranging from 0.199-0.43.[20,21] The CXCL12 G801A genotypes were in Hardy-Weinberg equilibrium ($p > 0.05$), indicating that genetic background of the

study population remains constant. Our data indicated significant associations of the CXCL12 G801A SNP with transaminitis and significant hepatic fibrosis assessed by APRI > 0.5. While univariate analysis suggested the G801A AA/AG genotypes as risk factors for transaminitis, subsequent multivatiate analysis indicated no longer significant risk of the genotypes. However, the analyses have identified the G801A AA/GA genotypes as risk factors for significant liver fibrosis by APRI and it is apparent that the patients with AA/GA carries 6.8 times higher risk than those with GG genotypes. Interestingly, additional analysis demonstrated the significant higher medians of AST, APRI and FIB-4 score, with the relatively lower $CD4^+$ cell count, in patients possessing the AA/GA than those having GG genotypes observed only in the ART-naïve, but not in ART-experienced HIV patient groups. Notably, distribution of the G801A AA/GA and GG genotypes in the ART-naïve and in ART-experienced patients are similar ($p > 0.05$) (data not shown). Collectively, the results suggest a strongly significant effect of the G801A AA/GA genotypes on liver abnormalities during HIV infection, and this effect can possibly be lessened by ARV treatment. Mechanisms for the neutralization of this genetic effect by ART are unclear and remain to be elucidated. To the best of our knowledge, this is the first evidence implicating the CXCL12 G801A polymorphism in the development of liver fibrosis in HIV-infected patients.

Functional roles of the CXCL12 G801A SNP have been studied in several diseases including HIV infection, but have not been clearly described in liver diseases. The G801A SNP is known to be located in a segment of the 3'UTR of β isoform transcript that may serve as a target for cis-acting factors promoting synthesis of CXCL12.[11] The *in vitro* study demonstrated that the 3'A genotype leads to an enhanced CXCL12 mRNA stability and therefore increases levels of the CXCL12 mRNA production.[12] The *in vivo* effect of the G801A SNP on CXCL12 production has also been demonstrated in HIV infection,[13,20] even though its mechanistic roles in susceptibility and disease progression are still controversial and not completely clear.[11,13,19] In liver microenvironment, CXCL12 is constitutively produced mainly by biliary epithelial cells and apparently upregulated during fibrotic injury, leading to an elevation of plasma CXCL12 levels in the patients with chronic liver inflammation and fibrosis whereas over 50% of liver infiltrating cells are CXCR4 positive.[34] In chronic liver injury, CXCL12 is increasingly expressed and activates CXCR4 on liver sinusoidal endothelial cells, triggering a profibrotic response, and induces hepatic stellate cells (HSC) proliferation and activation, leading to α-smooth muscle actin and collagen production. CXCL12 also attracts mesenchymal stem cells from the bone marrow that can promote liver fibrosis.[10] Accordingly, production of CXCL12 particularly in the liver area, which is possibly released into circulation, may influence hepatic fibrogenesis and therefore affecting progression of chronic liver diseases. Thus, an influence of the CXCL12 G801A genotypes on significant fibrosis observed in our HIV-infected patients may be due to their effect on the CXCL12 expression and function during chronic liver injury.

As there were multiple factors possibly associated with liver abnormalities in this study group, logistic regression analysis for risks of liver complication was adjusted for characteristics of patients including gender, age, $CD4^+$ cell count, HBV and/or HCV coinfection, CXCL12 G801A genotypes, navirapine experience and ART duration in the model. Consistent with the findings in our previous study,[7] HBV and/or HCV coinfection remains a risk factor for transaminitis in this study group. Apart from the G801A AA/GA genotypes, other predictive factors for significant liver fibrosis in this study group including age older than 40 years, $CD4^+$ cell count < 350 cells/μl and HBV and/or HBV coinfection were also identified. These risk factors are probably different from many previous studies due to differences in the study design, characteristics of study population, outcome variables, ARV drug regimens and duration of ART.[2,3,5,6,35] However, it is important to note limitations of this study. Firstly, a cross-sectional study design is usually unable to control confounding factors although the multivariate regression analysis was applied to this association study. Besides, this study design may not allow determining the precise effect of particular ARV drugs on hepatic abnormalities because the patients possibly have been switched from other regimens. Secondly, this study contains a limited number of subjects and missing values in some variables, which may limit statistical significance for the variables tested. Thirdly, there is no functional assessment of the G801A genotypes in this study and further evaluation of CXCL12 activities both in circulation and hepatic area is suggested. Fourthly, haplotype analysis of the rs1801157, G801A SNP, along with other SNPs in the 3' UTR of CXCL12 gene such as rs1029153 should be performed to confirm its significance in the liver complication in HIV infection. Lastly, this effect of the G801A SNP on liver complication was analyzed only in Thai patients and it would be interesting to extend the analysis to different ethnic groups.

Conclusion

This cross-sectional SNP analysis provides the evidence that the presence of CXCL12 G801A SNP is associated with development of liver complication probably in the ART-naïve HIV-infected Thais. Specifically, the patients having the unfavorable G801A AA/GA had a significantly higher risk for significant liver fibrosis than those carrying GG genotypes. This finding might provide a new molecular genetic mechanism for liver disease in HIV patients. A further prospective study in a larger number of patients is required to verify our finding.

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Authors' disclosures of potential conflicts of interest

No conflict of interest is declared.

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