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A Combination Polymorphism of the Glutathione Synthesis Genes Can Be a Predictive Biomarker for Anti-Tuberculosis Drug-Induced Hepatotoxicity in Japanese Patients with Pulmonary Tuberculosis

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Abstract

Background: To identify certain genes related to anti-tuberculosis drug-induced hepatotoxicity (ATDH) for Japanese patients with pulmonary tuberculosis (TB), we examined an association study of single nucleotide polymorphisms (SNPs) in candidate genes in glutathione synthesis with susceptibility to ATDH.

Method: We studied 100 TB patients treated with anti-TB drugs. The frequencies of alleles and genotypes of 17 tag SNPs in 3 genes between TB patients with and without ATDH were compared by chi-square test or Fisher's exact test in three different inheritance models. A genetic testing was carried out using a single or combination of the associated SNP(s) as a biomarker.

Results: Statistical analyses indicated that a C/C genotype of rs553822 in glutamate cysteine ligase, catalytic subunit (*GCLC*) and an A/T or T/T genotype of rs12140446 in glutamate cysteine ligase, modifier subunit (*GCLM*) independently contributed to susceptibility to ATDH. Genetic testing showed that the TB patients without these polymorphisms of *GCLC* and *GCLM* could safely be treated with anti-TB drugs on the basis of the higher value for the specificity and negative predictive value.

Conclusion: *GCLC* and *GCLM* are ATDH-related genes and may be useful as a new biomarker to predict the high-risk TB patients susceptible to ATDH.

Keywords: Tuberculosis; Anti-tuberculosis drug-induced hepatotoxicity; Single nucleotide polymorphism; Glutathione synthesis; Case-control association study

Introduction

Tuberculosis (TB) is a re-emerging infectious disease that is caused by infection with *Mycobacterium tuberculosis*. TB is widespread in the developing world, thereby leading to the declaration of TB as a global public health emergency by the World Health Organization in 1993 [1]. In addition, TB care involves serious problems, such as complications of acquired immunodeficiency syndrome (~ 1.1 million of the ~ 8.6 million new TB cases in 2012), an increase in multidrug-resistant *M. tuberculosis* (3.6% of newly diagnosed TB cases and 20% of those previously treated for TB in 2012), occurrence of adverse effects of anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin), and a lack of an effective vaccine [1]. In particular, the interruption of TB treatment due to adverse effects of anti-TB drugs can lead to treatment failure, disease relapse, the prevalence of multidrug-resistant *M. tuberculosis*, and eventually a decrease in the quality of life for TB patients because of the long treatment period of 6 to 9 months [1,2].

Hepatotoxicity is the most serious adverse effect of anti-TB drugs [2-4]. Many clinical studies indicate that anti-TB drug-induced hepatotoxicity (ATDH) is related to not only clinical risk factors, *e.g.* advanced age, female gender, malnutrition, alcohol intake, and chronic infection with hepatitis B, hepatitis C, or human immunodeficiency virus [2-6], but also genetic risk factors, e.g. some genes related to metabolizing enzymes, conjugation enzymes, human leukocyte antigen alleles, transporters, immune response and antioxidant response [3-9].

Cytochrome P450 2E1-mediated excessive production of reactive oxygen species (ROS) in hepatocytes is one of the mechanisms of ATDH [10,11]. ROS are rapidly eliminated by antioxidant enzymes such as glutathione *S*-transferases (GSTs), NAD (P) H dehydrogenase quinone 1 and heme oxygenase 1 [12,13]. As our previous study has reported that the antioxidant pathway-related genes are associated with susceptibility to ATDH in Japanese TB patients [9], we next focused on the glutathione (GSH) synthesis pathway as another detoxification mechanism of ROS. GSTs are important phase II

enzymes and play crucial roles in the detoxification of xenobiotics and drug metabolites by conjugation with GSH [14-16]. Although homozygous deletions of *GSTM1* and *GSTT1*, which result in a complete loss of function, are recognized as common polymorphisms [17,18], their frequencies are rare and these deletion polymorphisms were not associated with susceptibility to ATDH in our previous study (data not shown). Therefore, we selected genes related to metabolizing enzymes in the GSH synthesis pathway as candidate genes for a case-control association study.

GSH consists of glutamate, cysteine, and glycine, referred to as γ-glutamylcysteinylglycine. The first step of GSH synthesis is to ligate cysteine to glutamate by the rate-limiting enzyme glutamate cysteine ligase (GCL), thus forming γ-glutamylcysteine. The second step involves the addition of glycine to γ-glutamylcysteine to produce GSH by glutathione synthetase (GSS) [19,20]. Moreover, GCL comprises catalytic and modifier subunits. The former, designated as GCLC, has the active site of the ATP-dependent bond formation between cysteine and glutamate, and can be inhibited by GSH through a feedback mechanism. The latter, designated as GCLM, increases the GCLC efficiency after direct interaction with GCLC by reducing the feedback inhibition by GSH [21].

We herein examined a candidate gene-based association study by selecting three metabolizing enzymes involved in the GST synthesis pathway as candidate ATDH-susceptibility genes by means of investigating whether polymorphisms of candidate genes are associated with susceptibility to ATDH in Japanese TB patients. We further determined whether ATDH-associated polymorphism(s) can be used as a new genetic biomarker for prediction of high-risk Japanese TB patients susceptible to ATDH.

Patients and Methods

Patients

The study subjects were the same patients, populations, and clinical characteristics as reported previously [7,9]. The clinical characteristics and the number of TB patients are shown in Table 1.

Characteristics	Tuberculosis
Number of patients	100
Age range (years)	22-94
Age, mean ± SD (years)	64.0 ± 17.4
Gender (male/female)	56/44
Body mass index, mean ± SD (kg/m^2)	20.3 ± 2.9

Table 1: The clinical characteristics of TB patients in this study. The body mass index was calculated using the following formula: body weight (kg)/height × height (m). Abbreviations: TB: Tuberculosis; SD: Standard Deviation

The study protocol was approved by the Ethics Committee dealing with Human Genome and Gene Analysis at Nagasaki University, as well as the other hospitals, and written informed consent was obtained from each patient.

The definite diagnosis of pulmonary TB was made on the basis of the presence of their symptoms, compatible chest radiographic infiltrate findings, and the presence of acid-fast bacilli on sputum smear and *M. tuberculosis* on sputum culture [22]. Patients with liver cirrhosis, acute and chronic hepatitis, alcoholic liver diseases, or other chronic liver diseases were excluded from the subjects in this study.

Definition of ATDH

ATDH was defined according to the criteria of the International Consensus Meeting [23]. The patients with ATDH showed that the serum alanine aminotransferase (ALT) levels were increased ≥ 2 fold above the upper limit of the normal range (normal ≤ 42 IU/L), or the serum aspartate aminotransferase (AST, normal ≤ 33 IU/L) and total bilirubin (normal ≤ 1.5 mg/dL) levels were increased ≥ 2 fold above the upper limit of the normal range during TB treatment.

Selection of tag single nucleotide polymorphisms in candidate genes

Selected three candidate genes involved in the GSH synthesis pathway are encoding glutamate cysteine ligase, catalytic subunit (GCLC/*GCLC*; OMIM #606857); glutamate cysteine ligase, modifier subunit (GCLM/*GCLM*; OMIM #601176); and glutathione synthetase (GSS/*GSS*; OMIM #601002).

Obtaining information on single nucleotide polymorphisms (SNPs) in the candidate genes, selecting candidate tag SNPs, and determining genotyped tag SNPs were the same methods as reported previously [9,24]. The gene structure and the positions of genotyped tag SNPs in each candidate gene are shown in Figure 1.

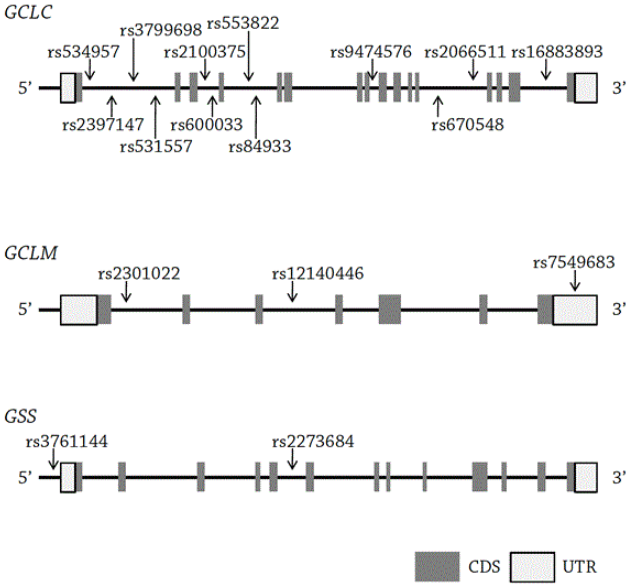

Figure 1: Locations of genotyped tag SNPs in each gene. The horizontal bars indicate the genomic sequences of candidate genes. Full boxes represent exons in each gene, and open boxes show the untranslated regions. The arrows indicate the positions of the genotyped tag SNP sites and their names are presented above each site. Abbreviations: SNPs, Single Nucleotide Polymorphisms; CDS, Coding Sequence; UTR, Untranslated Region

Genotyping of tag SNPs in each gene

Extraction of genomic DNA and genotyping of 17 tag SNPs in 3 genes by PCR-restriction fragment length polymorphism or PCR-direct DNA sequencing were identical with previously reported procedures [9,24]. Information on sequences of primers for PCR and sequencing, annealing temperature, and analytical methods for each SNP is shown in Table 2.

Gene	tag SNP	Major>Minor	Sequence of primer (5' to 3')		Annealing temperature (°C)	Analytical method (restriction enzyme)
			Forward	Reverse		
GCLC	rs534957	G>C	GGGAGACCAAGGTTTCACTG	GCTGCTTTTACCAGCATTTT	54	PCR-direct DNA sequencing
	rs2397147	T>C	CAGAGTCTTACTGAATGTTACTCAGG	CAGTGAATAGGTTTAGCCAGCTT	56	PCR-direct DNA sequencing
	rs3799698	A>G	ACCCAATGTCCCTTCCAGTT	TGAGGCCTATACGCAGATCG	56	PCR-RFLP (HpyCH4 IV)
	rs531557	T>A	TGCCAAACGGATGACATTTA	TGATGTTGCTGCAATTCTGG	52	PCR-RFLP (Mbo I)
	rs2100375	C>T	AGCCACCACAGCTGACAGA	TCTCCTCCGTAGCCATCCTA	58	PCR-RFLP (HpyCH4 IV)
	rs600033	G>C	GCCCAAATCCACCTTGTAAA	AATGAAGCAGTGCCGTAGGT	54	PCR-RFLP (Xsp I)
	rs553822	T>C	CCCAGTAGGCATTTACAGACC	AAACCCAACCCAGTGGAAG	56	PCR-RFLP (Nsi I)
	rs84933	G>A	TGCACATAGCAAACGTGAGG	GGCTTCTTGGTTGTTGATGC	56	PCR-RFLP (Hpy188 I)
	rs9474576	A>G	CAAAGATTTCCATGGGTGGT	TTTGCAGGAGCTGACTTTTG	54	PCR-RFLP (Btg I)
	rs670548	T>C	TCTGCCAGCATGTCTTTCAC	CTGTTGTAAGCCCGTGTGG	56	PCR-RFLP (HpyCH4 IV)
	rs2066511	C>T	AGCAGCTTGTTGCAGCATAA	AAAGCCTGTTCCTCCCACTG	54	PCR-RFLP (Rsa I)
	rs16883893	G>A	TCCATCTGGCAACTGTCATT	TCGCTCAAGGACCCTACAGT	54	PCR-RFLP (EcoR I)
GCLM	rs2301022	G>A	GAGTTTCTGACTGCAAACACAGTA	TGGAGCTCTGGGTGTTACAAA	55	PCR-RFLP (Tas I)
	rs12140446	A>T	CGAAGACCTATAAAAGCAGAAAGAC	ACAATTCATCCCTGGGAAAT	54	PCR-RFLP (Tas I)
	rs7549683	G>T	GCCCGGATGAAATACAAGAG	CCTTAATTCAGGGCCGACAT	56	PCR-RFLP (Mse I)
GSS	rs3761144	G>C	CCATCCCTGATTCCTTCAGA	CAGGGGCTGAGCCTGTATTA	56	PCR-RFLP (Fnu4H I)
	rs2273684	T>G	CCTTACTTGGGTGAGCCGTA	CCAGCTATGATTGTGCTTTCC	56	PCR-RFLP (Xcm I)

Table 2: Information on genotyping of tag SNPs in candidate genes. Abbreviations: SNP, Single Nucleotide Polymorphism; PCR, Polymerase Chain Reaction; RFLP, Restriction Fragment Length Polymorphism

Statistical analysis

Data are indicated as the means ± standard deviations. Differences in the clinical characteristics, baseline laboratory data, and the frequencies of alleles and genotypes between TB patients with and without ATDH, as well as the significance of deviation from the Hardy-Weinberg equilibrium (HWE) in each SNP were analyzed by means of the same methods as reported previously [9,24].

Moreover, a comparison of the genetic factors for susceptibility to ATDH, which showed a significant association with susceptibility to ATDH by univariate analyses, between TB patients with and without ATDH was performed by multivariate logistic regression analysis using the JMP Pro 11 software program (SAS Institute Inc., Tokyo, Japan). A *P* value of less than 0.05 was considered to be statistically significant.

Results

No differences in the clinical characteristics and baseline laboratory data

Eighteen TB patients had ATDH with the ALT levels >3 fold above the upper normal limit during TB treatment among 100 patients enrolled in this study. The TB treatment for these patients was interrupted until improvement of hepatotoxicity. Therefore, 82 patients without ATDH indicated the resistance to hepatotoxicity. Whereas, 18 with ATDH showed sensitivity to hepatotoxicity.

There were no significant differences in the clinical characteristics and baseline laboratory data between TB patients with and without ATDH (Table 3).

Characteristics	ATDH		*P* value*
	Presence	Absence	
Number of patients	18	82	
Age, mean ± SD (years)	60.8 ± 17.7	64.7 ± 17.3	0.39
Gender (male/female)	9-Sep	47/35	0.61
Body mass index (kg/m^2)	19.6 ± 2.3	20.5 ± 3.1	0.27
Lean (BMI<18.5/18.5<BMI)	10-Jun	18/55	0.35
Alcoholism (+/-)	12-May	21/59	0.77
Hepatic diseases (+/-)	16-Feb	Dec-68	1
HBs (+/-)	0/14	Jan-74	1
HCV (+/-)	0/14	Apr-61	1
The number of anti-TB drugs	3.5 ± 0.6	3.6 ± 0.6	0.32
INH (mg/kg)	6.8 ± 1.4	6.3 ± 1.4	0.25
RFP (mg/kg)	9.1 ± 1.6	8.8 ± 1.8	0.54
PZA (+/-)	8-Oct	53/29	0.59
The number of concomitant drugs	3.9 ± 2.0	5.0 ± 2.9	0.14
AST (IU/L)	29.1 ± 26.8	26.8 ± 23.3	0.72
ALT (IU/L)	18.0 ± 10.4	21.1 ± 16.6	0.45
T-bil (mg/dL)	0.48 ± 0.19	0.64 ± 0.43	0.11
Albumin (g/dL)	3.51 ± 0.68	3.64 ± 0.64	0.46
γ-GTP (IU/L)	32.4 ± 22.3	43.2 ± 58.4	0.45
ALP (IU/L)	289.1 ± 103.5	298.7 ± 104.8	0.73
LAP (IU/L)	58.3 ± 9.1	62.7 ± 18.6	0.7
LDH (IU/L)	194.9 ± 58.0	195.2 ± 62.1	0.98
S-creatinine (mg/dL)	0.64 ± 0.13	0.88 ± 1.10	0.35
Eosinophil (/μL)	105.1 ± 120.6	115.5 ± 121.8	0.74
Platelet (× 10^4/μL)	31.8 ± 11.0	27.7 ± 9.3	0.11
Hemoglobin (g/dL)	11.7 ± 2.1	12.6 ± 1.7	0.054

Table 3: Comparison of the clinical characteristics and baseline laboratory data between TB patients with and without ATDH. *Characteristics were statistically compared using the Mann-Whitney U test, chi-square test, or Fisher's exact test. Abbreviations: TB, Tuberculosis; ATDH, Anti-TB Drug-Induced Hepatotoxicity; SD, Standard Deviation; BMI, Body Mass Index; Hbs, Hepatitis B Surface; HCV, Hepatitis C Virus; INH, Isoniazid; RFP, Rifampicin; PZA, Pyrazinamide; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; T-Bil, Total Bilirubin; γ-GTP, γ-Glutamyl Transpeptidase; ALP, Alkaline Phosphatase; LAP, Leucine Aminopeptidase; LDH, Lactate Dehydrogenase; S-Creatinine, Serum Creatinine

Association between tag SNPs and susceptibility to ATDH

A comparison of the distribution of alleles and genotypes of tag SNPs in candidate genes between TB patients with and without ATDH in three different inheritance models: allele, minor allele dominant and minor allele recessive, is shown in Table 4.

With regard to *GCLC*, the chi-square or Fisher's exact test indicated that the frequencies of a T/C or C/C genotype of rs553822 in the minor allele dominant model and a C/C genotype of rs553822 in the minor allele recessive model were significantly increased in TB patients with ATDH as compared to those without ATDH (*P*=0.032, OR=3.125 and *P*=0.019, OR=7.524, respectively; Table 4). These results imply that there were a ~ 3.1 and ~ 7.5 fold increase in susceptibility to ATDH, respectively.

Gene	Tag SNP (Major>Minor)	Genotype	ATDH (%)		Inheritance model*	*P* value	OR	95% CI
			Present n=18	Absent n=82				
GCLC	rs534957	MAF	0.417	0.378	Allele	0.666	1.175	0.564-2.449
	G>C	G/G	5 (27.8)	30 (36.6)				
		G/C	11 (61.1)	42 (51.2)	Dominant	0.478	1.5	0.487-4.621
		C/C	2 (11.1)	10 (12.2)	Recessive	1	0.9	0.180-4.511
	rs2397147	MAF	0.25	0.287	Allele	0.658	0.83	0.363-1.897
	T>C	T/T	11 (61.1)	43 (52.4)				
		T/C	5 (27.8)	31 (37.8)	Dominant	0.504	0.702	0.248-1.989
		C/C	2 (11.1)	8 (9.8)	Recessive	1	1.156	0.224-5.967
	rs3799698	MAF	0.556	0.482	Allele	0.422	1.345	0.651-2.778
	A>G	A/A	5 (27.8)	20 (24.4)				

	A/G	6 (33.3)	45 (54.9)	Dominant	0.769	0.839	0.266-2.644
	G/G	7 (38.9)	17 (20.7)	Recessive	0.129	2.433	0.820-7.220
rs531557	MAF	0.417	0.329	Allele	0.318	1.455	0.695-3.045
T>A	T/T	7 (38.9)	33 (40.2)				
	T/A	7 (38.9)	44 (53.7)	Dominant	0.915	1.058	0.372-3.010
	A/A	4 (22.2)	5 (6.1)	Recessive	0.053	4.4	1.050-18.438
rs2100375	MAF	0.389	0.421	Allele	0.726	0.876	0.419-1.834
C>T	C/C	5 (27.8)	25 (30.5)				
	C/T	12 (66.7)	45 (54.9)	Dominant	0.82	1.14	0.367-3.543
	T/T	1 (5.6)	12 (14.6)	Recessive	0.453	0.343	0.042-2.824
rs600033	MAF	0.417	0.451	Allele	0.718	0.869	0.418-1.804
G>C	G/G	5 (27.8)	23 (28.0)				
	G/C	11 (61.1)	44 (53.7)	Dominant	0.982	1.014	0.325-3.164
	C/C	2 (11.1)	15 (18.3)	Recessive	0.73	0.558	0.116-2.692
rs553822	MAF	0.444	0.213	Allele	0.004	2.949	1.384-6.282
T>C	T/T	6 (33.3)	50 (61.0)				
	T/C	8 (44.4)	29 (35.4)	Dominant	0.032	3.125	1.066-9.163
	C/C	4 (22.2)	3 (3.7)	Recessive	0.019	7.524	1.517-37.312
rs84933	MAF	0.417	0.457	Allele	0.657	0.848	0.408-1.760
G > A	G/G	5 (27.8)	23 (28.0)				
	G/A	11 (61.1)	43 (52.4)	Dominant	0.982	1.014	0.325-3.164
	A/A	2 (11.1)	16 (19.5)	Recessive	0.515	0.516	0.108-2.474
rs9474576	MAF	0.444	0.421	Allele	0.794	1.101	0.532-2.279
A>G	A/A	7 (38.9)	24 (29.3)				
	A/G	6 (33.3)	47 (57.3)	Dominant	0.424	0.65	0.225-1.878
	G/G	5 (27.8)	11 (13.4)	Recessive	0.158	2.483	0.739-8.336
rs670548	MAF	0.361	0.14	Allele	0.002	3.465	1.540-7.794
T>C	T/T	7 (38.9)	60 (73.2)				
	T/C	9 (50.0)	21 (25.6)	Dominant	0.005	4.286	1.476-12.447
	C/C	2 (11.1)	1 (1.2)	Recessive	0.083	10.13	0.865-118.471
rs2066511	MAF	0.139	0.274	Allele	0.089	0.427	0.156-1.165
C>T	C/C	14 (77.8)	45 (54.9)				
	C/T	3 (16.7)	29 (35.4)	Dominant	0.074	0.348	0.105-1.146
	T/T	1 (5.6)	8 (9.8)	Recessive		0.544	0.064-4.646
rs16883893	MAF	0.278	0.189	Allele	0.232	1.65	0.721-3.775
G>A	G/G	9 (50.0)	54 (65.9)				

		G/A	8 (44.4)	25 (30.5)	Dominant	0.207	1.929	0.688-5.405
		A/A	1 (5.6)	3 (3.7)	Recessive	0.554	1.549	0.152-15.811
GCLM	rs2301022	MAF	0.333	0.207	Allele	0.104	1.912	0.868-4.210
	G>A	G/G	7 (38.9)	54 (65.9)				
		G/A	10 (55.6)	22 (26.8)	Dominant	0.034	3.031	1.059-8.676
		A/A	1 (5.6)	6 (7.3)	Recessive		0.745	0.084-6.600
	rs12140446	MAF	0.611	0.384	Allele	0.013	2.519	1.201-5.283
	A>T	A/A	2 (11.1)	33 (40.2)				
		A/T	10 (55.6)	35 (42.7)	Dominant	0.019	5.388	1.161-25.002
		T/T	6 (33.3)	14 (17.1)	Recessive	0.189	2.439	0.780-7.566
	rs7549683	MAF	0.278	0.183	Allele	0.198	1.718	0.749-3.940
	G>T	G/G	8 (44.4)	54 (65.9)				
		G/T	10 (55.6)	26 (31.7)	Dominant	0.09	2.411	0.856-6.791
		T/T	0 (0)	2 (2.4)	Recessive	1	0.87	0.040-18.915
GSS	rs3761144	MAF	0.444	0.311	Allele	0.124	1.773	0.849-3.700
	G>C	G/G	5 (27.8)	44 (53.7)				
		G/C	10 (55.6)	25 (30.5)	Dominant	0.054	3.011	0.983-9.218
		C/C	3 (16.7)	13 (15.9)	Recessive	1	1.062	0.269-4.194
	rs2273684	MAF	0.25	0.177	Allele	0.311	1.552	0.660-3.647
	T>G	T/T	10 (55.6)	56 (68.3)				
		T/G	7 (38.9)	23 (28.1)	Dominant	0.302	1.723	0.609-4.873
		G/G	1 (5.6)	3 (3.7)	Recessive	0.554	1.549	0.152-15.811

Table 4: Allele and genotype comparisons in three inheritance models between TB patients with and without ATDH. *Allele, allele model; Dominant, the minor allele dominant model; Recessive, the minor allele recessive model. Abbreviations: TB: Tuberculosis; ATDH: Anti-TB Drug-Induced Hepatotoxicity; SNP: Single Nucleotide Polymorphism; MAF: Minor Allele Frequency; OR: Odds Ratio; CI: Confidence Interval

Moreover, the frequency of a T/C or C/C genotype of rs670548 in the minor allele dominant model was significantly higher in patients with ATDH in comparison to those without ATDH (P=0.005, OR=4.286; Table 4).

With regard to *GCLM*, the frequencies of both a G/A or A/A genotype of rs2301022 and an A/T or T/T genotype of rs12140446 in the minor allele dominant model were significantly increased in TB patients with ATDH as compared to those without ATDH (P=0.034, OR=3.031 and P=0.019, OR=5.388, respectively; Table 4), thereby indicating that these genotypes are associated with a ~ 3.0 and ~ 5.4 fold susceptibility to ATDH, respectively.

There were no significant differences in the frequencies of the other alleles and genotypes between TB patients with and without ATDH.

The gene-gene interactions for susceptibility to ATDH

Out of the associated four SNPs (rs553822 and rs670548 in *GCLC* as well as rs2301022 and rs12140446 in *GCLM*) with susceptibility to ATDH by univariate analyses, we selected two SNPs, rs553822 in *GCLC* and rs12140446 in *GCLM* as genetic risk factors because they indicated higher OR than another associated SNP in each gene. Therefore, we subsequently carried out the multivariate logistic regression analysis of the influence of the gene-gene interaction of two genetic risk factors (the C/C genotype of rs553822 in *GCLC* and the A/T or T/T genotype of rs12140446 in *GCLM*) with susceptibility to ATDH. This analysis revealed that two genetic risk factors independently contributed to susceptibility to ATDH (P=0.029, OR=6.334 and P=0.022, OR=4.847, respectively; Table 5).

Factor	Factor comparison*	
	OR (95% CI)	*P* value
C/C genotype of rs553822 in *GCLC*	6.334 (1.213-37.350)	0.029
A/T or T/T genotype of rs12140446 in *GCLM*	4.847 (1.227-32.462)	0.022

Table 5: The gene-gene interaction for susceptibility to ATDH. *The factors were statistically analyzed using the multivariate logistic regression analysis. Abbreviations: ATDH: Anti-TB Drug-Induced Hepatotoxicity; TB: Tuberculosis; OR: Odds Ratio; CI: Confidence Interval

Verification of genetic testing to predict susceptibility to ATDH

To better predict susceptibility to ATDH against TB patients, we performed a genetic testing using a combination of the two independent genetic risk factors (*GCLC* and *GCLM* genotypes) as a biomarker, thereby indicating that both the C/C genotype at rs553822 in *GCLC* and the A/T or T/T genotype of rs12140446 in *GCLM* were strongly associated with susceptibility to ATDH (*P*=0.0091, OR=11.43; Table 6). In addition, the values of the sensitivity, specificity, positive predictive value, and negative predictive value in this genetic testing were estimated at 22.2%, 97.6%, 66.7%, and 85.1%, respectively.

Factor	ATDH (%)		Factor comparison*	
	Present	Absent	OR (95% CI)	*P* value
Both the C/C genotype of rs553822 in *GCLC* and the A/T or T/T genotype of rs12140446 in *GCLM*	4 (22.2)	2 (2.4)	11.43 (1.908-60.47)	0.0091
Others	14 (77.8)	80 (97.6)		
Total number of patients	18	82		

Table 6: Evaluation of a genetic testing for susceptibility to ATDH. *The factor comparison was analyzed using the chi-square test. Abbreviations: ATDH: Anti-TB Drug-Induced Hepatotoxicity; TB: Tuberculosis; OR: Odds Ratio; CI: Confidence Interval.

Discussion

We have shown that two genetic risk factors, *GCLC* and *GCLM* polymorphisms, independently contributed to susceptibility to ATDH in Japanese TB patients. In particular, possessing both the C/C genotype of rs553822 in *GCLC* and the A/T or T/T genotype of rs12140446 in *GCLM* conferred susceptibility to ATDH at higher OR of 11.43. Our results suggest that *GCLC* and *GCLM* are genetic determinants of predisposition to the onset and/or development of ATDH in Japanese TB patients. However, it remains to be confirmed whether this association is reproducible in a large number of Japanese TB patients and other ethnic populations because the number of patients in this study was very small.

GSH plays multiple roles in detoxification, antioxidant defense, maintenance of the thiol status of proteins, and modulation of cell proliferation. In particular, a major function of GSH is detoxification of xenobiotics and drug metabolites, which is catalyzed by GSTs through conjugation with GSH [14-16], eventually resulting in the irreversible consumption of intracellular GSH. Therefore, oxidative stress as well as depletion of the intracellular levels of GSH in cells can induce the expression of GSH synthetic enzymes, including GCL and GSS [25-27]. In addition, there are three distinct findings:

- The rate-limiting enzyme GCL consists of GCLC and GCLM and is regulated by these two subunits
- GCLC can be inhibited by GSH with the feedback mechanism
- GCLM can increase the GCLC efficiency by reducing the feedback inhibition by GSH [19-21].

From a pathophysiological perspective, we speculate that both the C/C genotype of rs553822 in *GCLC* and the A/T or T/T genotype of rs12140446 in *GCLM* may diminish the function of the enzymatic activity and/or the expression of both genes, resulting in the suppression of GCL enzyme activity and consequently leading to the diminution of GSH synthesis, a decrease in intracellular levels of GSH, and a delayed recovery of depletion of GSH in hepatocytes (Figure 2). Increased ROS due to the diminution of GSH in hepatocytes may affect the dysfunction of mitochondria and DNA repair, disturbance of proteins and inner membranes, resulting in a decrease in the protection of hepatocytes from oxidative stress and drug metabolites, thereby leading to hepatocyte apoptosis, inflammation, and subsequent hepatotoxicity as shown in Figure 2 [28]. Our hypothesis could be supported that acetaminophen-induced hepatotoxicity induces the reduction of intracellular levels of GSH in hepatocytes in humans and mice [29,30].

The previously reported association studies have indicated that the polymorphisms of *GCLC* are associated with susceptibility to cystic fibrosis with a mild trans membrane conductance regulator (CFTR) genotype [31], schizophrenia [32], cardiovascular events [33], nonalcoholic steatohepatitis [34] and chronic obstructive pulmonary disease [35]. In addition, some association studies between the polymorphisms of *GCLM* and susceptibility to myocardial infarction [36], chronic beryllium disease [37], schizophrenia [38], and bronchial asthma [39] have been investigated. Nearly all the associated SNPs as described above are located in the promoter region of these genes, *e.g.* a GAG trinucleotide repeat polymorphism in the 5'-untranslated region of *GCLC*, a C-129T SNP (rs17883901) in the promoter region of *GCLC*, and a C-588T SNP (rs41303970) in the 5'-franking region of *GCLM*. These polymorphisms decrease the expression of the two genes and the enzymatic activities, thereby resulting in lower GSH levels of each cell in their tissue. However, these SNPs were not investigated in the present study because they were not selected by the Haploview 4.1 software program. The two associated polymorphisms of *GCLC* and *GCLM* evaluated in this study are located in intron 4 and 3, respectively. Therefore, we speculate that these intron SNPs in *GCLC* and *GCLM* may activate the repressors and/or non-coding RNAs, or may inactivate the enhancers, resulting in the diminution of function of both genes. However, since function analyses of the polymorphisms of *GCLC* and *GCLM* were not performed in this study, further studies are needed to elucidate these association mechanisms.

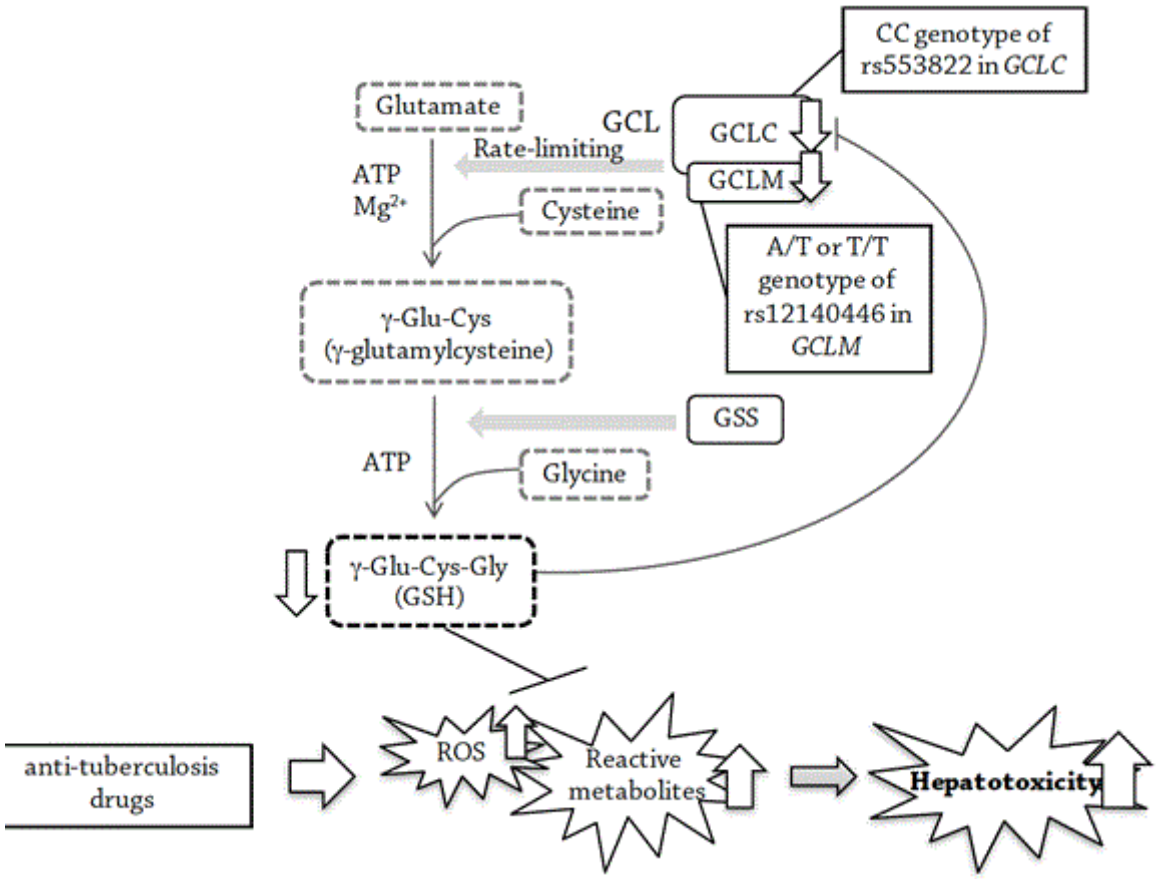

Figure 2: A putative mechanism of ATDH in the glutathione synthesis pathway. Schematic representation indicates the glutathione synthesis pathway including metabolizing enzymes and detoxification of ROS and reactive metabolites in hepatocytes. Decrease in GSH and increase in ROS due to *GCLC* and *GCLM* polymorphisms may induce hepatotoxicity. Abbreviations: ATDH, Anti-Tuberculosis Drug-Induced Hepatotoxicity; ATP, Adenosine Triphosphate; GCL, Glutamate Cysteine Ligase; GCLC, Glutamate Cysteine Ligase Catalytic Subunit; GCLM, Glutamate Cysteine Ligase Modifier Subunit; GSS, Glutathione Synthetase; GSH, Glutathione; ROS, Reactive Oxygen Species

The present genetic testing using the two associated SNPs (*GCLC* and *GCLM* genotypes) as a biomarker to predict susceptibility to ATDH showed the higher specificity of 97.6%, negative predictive value of 85.1%, and OR of 11.43 for ATDH with significant differences. Our data suggest that the TB patients without these polymorphisms of *GCLC* and *GCLM* may safely be treated with anti-TB drugs.

Conclusion

GCLC and *GCLM* are genetic determinants of predisposition to the onset and/or development of ATDH in Japanese TB patients. Moreover, a combination of the polymorphisms of these genes in GSH synthesis, which is crucial for the protection against ROS and drug metabolites in hepatocytes, may be useful as a new biomarker to predict the high-risk TB patients susceptible to ATDH.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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