

Genome-Wide Association Study of Liver Enzymes in Korean Children

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Liver enzyme elevations, as an indicator of liver function, are widely associated with metabolic diseases. Genome-wide population-based association studies have identified a genetic susceptibility to liver enzyme elevations and their related traits; however, the genetic architecture in childhood remains largely unknown. We performed a genome-wide association study to identify new genetic loci for liver enzyme levels in a Korean childhood cohort (n = 484). We observed three novel loci (rs4949718, rs80311637, and rs596406) that were multiply associated with elevated levels of alanine transaminase and aspartate transaminase. Although there are some limitations, including genetic power, additional replication and functional characterization will support the clarity on the genetic contribution that the *ST6GALNAC3*, *ADAMTS9*, and *CELF2* genes have in childhood liver function.

Keywords: alanine transaminase, aspartate transaminase, childhood liver enzyme, genome-wide association study

Introduction

Plasma liver enzyme tests are broadly applied to predict biochemical liver functions and to diagnose liver diseases. Alanine transaminase (ALT) and aspartate transaminase (AST) are closely associated with hepatocyte damage and steatosis, including non-alcoholic fatty liver disease (NAFLD) [1]. Elevated liver enzyme levels, as prospective risk factors, have been reported to be involved in type 2 diabetes and coronary heart disease [2, 3]. However, alkaline phosphatase and gamma-glutamyltranspeptidase are related with biliary, cholestatic, and alcoholic diseases [4].

Overweightness in childhood or adolescence is persistent in adulthood obesity and is closely associated with adverse health effects, including metabolic diseases [5]. A recent cohort study in Korean adolescents has shown strong

associations of liver enzyme levels with a cluster of metabolic syndrome components [6]. Thus, finding genetic markers of liver enzyme levels in childhood would be important for early prediction of liver function and its related disease in adulthood.

Liver enzyme levels are affected by environmental and genetic factors. The heritabilities of liver enzyme levels range from approximately 35% to 61% [7, 8]. To date, two genome-wide association studies (GWASs) in European ancestry populations have identified genetic variants located in multiple genes associated with levels of ALT (*HSD17B13-MAPK10*, *TRIB1*, *CHUK*, and *SAMM50*), AST (*NBPF3-ALPL*), and both of them (*CPN1* and *PNPLA3*) [9, 10]. In addition, a meta-analysis identified pleiotropic effects between NAFLD and metabolic traits, such as low-density lipoprotein, high-density lipoprotein, triglyceride, fasting glucose, and insulin

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resistance [11].

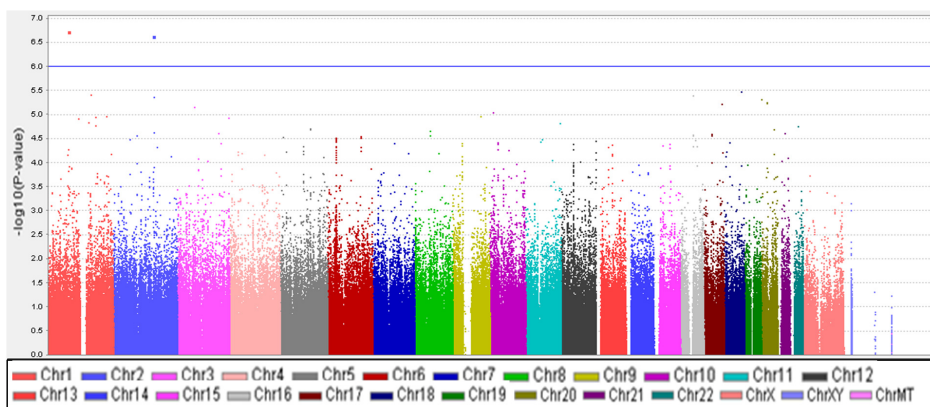
Despite the identification of novel genes or loci associated with liver enzymes in cohort populations, the common variants in childhood are still largely unknown. Here, we present findings from a GWAS in childhood subjects to identify new susceptibility loci for liver enzyme levels.

Methods

Study subjects

Students, aged between 8 and 13 years, were recruited from Gwacheon City and Kyunggi Province from April to June 2010. This study was performed as a part of the Korean Children-Adolescents Study (KoCAS), which has been mo-

(A) ALT



(B) AST

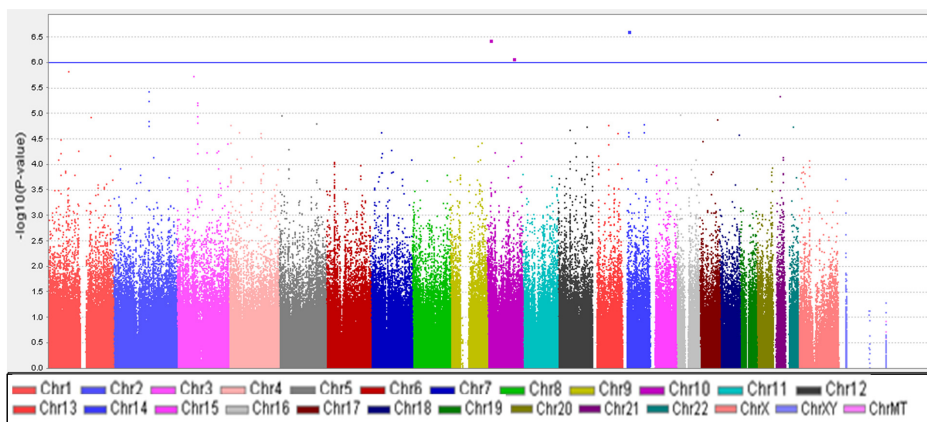
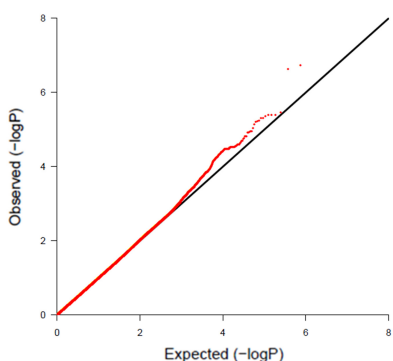


Fig. 1. Manhattan plots of association results for alanine transaminase (ALT) and aspartate transaminase (AST) levels. Manhattan plots of the genome-wide analyses of association with ALT (A) and AST (B) are based on a total of 484 Korean children. Color scale of X-axis represents chromosomes, and negative logarithms of p-values are shown on the Y-axis.

(A) ALT



(B) AST

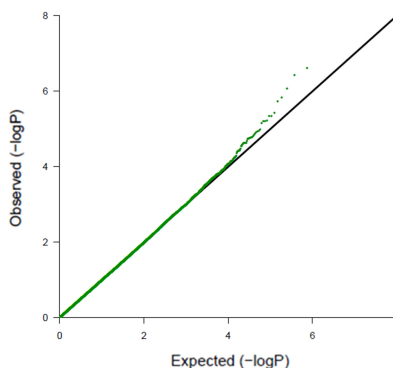


Fig. 2. Quantile-quantile plots of association results for alanine transaminase (ALT) and aspartate transaminase (AST) levels in Korean children for 747,076 single nucleotide polymorphisms (SNPs). Quantile-quantile plots for ALT (A) and AST (B) AST are composed of X-axis and Y-axis indicate the negative log-scale of expected p-values of each SNP and the negative log-scale of the observed p-values, respectively. A straight line indicates the expected results under Hardy-Weinberg equilibrium.

nitored yearly since their entry into elementary school at age 7 in Gwacheon City or fourth grade at age 10 in Seoul and Gyeonggi Province, Korea. Subjects who were enrolled in a specific diet program or were taking any medications known to affect appetite were excluded from the study. AST and ALT levels were measured in the serum of each subject. The study protocol was approved by the institutional review board of Seoul Paik Hospital, Inje University, and the Korea Center for Disease Control and Prevention. Informed consent was obtained from the childrens' parents.

Genotyping and quality control

A total of 532 samples were genotyped using the Illumina HumanOmni1-Quad BeadChip (Illumina Inc., San Diego, CA, USA). Samples that revealed the following properties were excluded: genotyping call < 98%, heterozygosity > 30%, sex inconsistency, cryptic first-degree relative, or duplication. A total of 48 subjects were excluded from the sample QC process. Markers with a high missing call rate (> 5%), minor allele frequency < 0.01, and significant devia-

tion from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$) were also excluded. The remaining 747,076 single nucleotide polymorphisms (SNPs) were used in subsequent analyses for association.

Association analyses

Genome-wide association was analyzed using the PLINK software (<http://pngu.mgh.harvard.edu/~purcell/plink>). The association between genotypes and concentration of liver enzymes in plasma was evaluated with linear regression analysis, controlling for age and body mass index (BMI) as covariates. The Manhattan plots of $-\log_{10}$ (p-value) were made using Haploview v. 4.2. The quantile-quantile plots were generated using R statistics package to assess the overall significance of the genome-wide associations.

Results

A total of 747,076 SNP genotype assays were tested on the DNA samples of 484 Korean childhood subjects. ALT and

Table 1. Genetic loci associated with levels of ALT and AST at $p < 1 \times 10^{-5}$ in the GWAS of Korean children (n = 484)

Trait	rs ID	Chr	Gene (Nearest gene)	Position	Class (AA change)	A1/A2	MAF	β (SE)	p-value
ALT	rs4949718	1	ST6GALNAC3	76672052	Intron	A/G	0.402	0.053 (0.01)	1.87E-07
	rs17801127	2	(MMADHC)	150272730	Intergenic	A/G	0.030	0.154 (0.029)	2.37E-07
	rs1539893	18	CCDC102B	64695735	Intron	G/A	0.188	0.06 (0.013)	3.40E-06
	rs12035879	1	RGS5	161409179	Intron	A/G	0.419	0.048 (0.01)	3.97E-06
	rs9941219	16	(BRD7)	48995029	Intergenic	A/G	0.024	0.154 (0.033)	4.06E-06
	rs731660	16	(BRD7)	48998709	Intergenic	A/C	0.024	0.154 (0.033)	4.06E-06
	rs12621256	2	GALNT13	154942564	Intron	G/A	0.066	0.092 (0.02)	4.36E-06
	rs6035126	20	(SIRPA)	1877542	Intergenic	A/G	0.019	0.176 (0.038)	4.94E-06
	rs13433286	20	(SIRPA)	1879500	Intergenic	G/A	0.019	0.176 (0.038)	4.94E-06
	rs844917	20	(CD93)	23089655	Intergenic	A/G	0.041	0.111 (0.024)	5.64E-06
	rs844914	20	(CD93)	23089445	Intergenic	A/C	0.041	0.111 (0.024)	5.98E-06
	rs903107	17	SLC39A11	68573529	Intron	G/A	0.052	0.104 (0.023)	6.11E-06
	rs80311637	3	ADAMTS9	64511688	CDS (V1597M)	A/G	0.029	0.137 (0.03)	7.18E-06
	rs596406	10	CELF2	11264671	Intron	G/A	0.044	0.11 (0.024)	9.18E-06
AST	rs862946	14	(CYB5AP5)	25558307	Intergenic	A/G	0.112	0.043 (0.008)	2.41E-07
	rs596406	10	CELF2	11264671	Intron	G/A	0.044	0.067 (0.013)	3.69E-07
	rs76850691	10	GOT1	101147428	CDS (Q370E)	C/G	0.019	-0.099 (0.02)	8.55E-07
	rs4949718	1	ST6GALNAC3	76672052	Intron	A/G	0.402	0.026 (0.005)	1.49E-06
	rs80311637	3	ADAMTS9	64511688	CDS (V1597M)	A/G	0.029	0.078 (0.016)	1.85E-06
	rs892877	2	THSD7B	137589817	Intron	A/G	0.180	-0.032 (0.007)	3.75E-06
	rs457603	21	(EIF4A1P1)	27579820	Intergenic	G/A	0.033	-0.07 (0.015)	4.57E-06
	rs452621	21	(EIF4A1P1)	27581267	Intergenic	A/G	0.033	-0.07 (0.015)	4.57E-06
	rs984295	2	THSD7B	137615223	Intron	G/A	0.192	-0.031 (0.007)	5.86E-06
	rs7617400	3	ROBO1	79686754	Intron	A/G	0.161	0.034 (0.007)	6.16E-06
	rs11924965	3	ROBO1	79693315	Intron	A/G	0.161	0.034 (0.007)	6.16E-06
	rs7644918	3	ROBO1	79728847	Intron	C/A	0.166	0.033 (0.007)	6.97E-06

ALT, alanine transaminase; AST, aspartate transaminase; GWAS, genome-wide association study; Chr, chromosome; AA, amino acid; MAF, minor allele frequency; SE, standard error.

AST levels of the subjects according to age and sex are presented in Supplementary Table 1. Linear regression analyses were performed to estimate the p-values for the association between genotype distributions and levels of ALT and AST. Manhattan plots of association analyses of all SNPs with respect to ALT and AST are presented with the chromosomal positions (X-axis) and negative logarithm of p-values (Y-axis) of each SNP (Fig. 1). Quantile-quantile plots comparing the association p-values with those expected for a null distribution are also presented (Fig. 2). The highest-ranked SNPs showing the lowest p-values ($p < 1.0 \times 10^{-5}$) of association with ALT and AST levels are listed in Table 1. In addition, genotype clusters of the SNPs are presented in Supplementary Fig. 1.

Although there was no SNP satisfying the criteria of genome-wide significance ($p < 1.0 \times 10^{-8}$), we identified three loci (rs4949718, rs80311637, and rs596406 in *ST6GALNAC3*, *ADAMTS9*, and *CELF2*, respectively) that were suggestively associated with levels of ALT and AST. Of these SNPs, rs4949718 was found to be more strongly associated with ALT levels ($p = 1.87 \times 10^{-7}$) but less in AST levels ($p = 1.49 \times 10^{-6}$). The missense rs80311637 (V1597M) and intronic rs596406 SNPs were more strongly associated with an increased risk of AST than ALT.

Discussion

The levels of ALT and AST, known as transaminases, are important indicators for determining hepatocyte injury or hepatocellular diseases. However, ALT is known to be found in the highest level in the liver; thus, ALT levels are more liver-specific indicators [4]. Moreover, it has been reported that ALT levels are more significantly associated with a cluster of metabolic syndrome than AST levels in a Korean adolescent cohort study [6].

In this study, we identified three novel genes (*ST6GALNAC3*, *ADAMTS9*, and *CELF2*) that were multiply associated with elevated levels of ALT and AST. Of the three loci, rs4949718 in the intron region of *ST6GALNAC3* yielded the lowest p-value for the association with ALT, compared to AST levels (Table 1). In addition, rs80311637 (*ADAMTS9*) and rs596406 (*CELF2*) were shown to be more strongly associated with AST levels, compared to ALT. These results indicate that the liver-specific feature of ALT was not driven as the secondary association for AST. In recent, a previous GWAS in an Amish population identified two genetic variants (rs17209512 and deletion) in glutamic-oxaloacetic transaminase 1 (*GOT1*) that were associated with serum AST. However, the rs17209512 SNP, which is in incomplete LD with rs76850691, was not replicated in our study (Table 1).

For the three multiply associated loci, we performed conditional analyses to test whether the association with the other trait was simply secondary to the primary association (Supplementary Table 2). In these analyses, the association signals on rs4949718 for AST and rs80311637 and rs596406 for ALT were abolished by adjustment of the ALT and AST, respectively. It is assumed that the multiple effects of these loci on ALT and AST resulted from the secondary effects by physiologically correlated features between ALT and AST [12]. In addition, when comparing with BMI-unadjusted data, the association signals in ALT were relatively weaker than those of the BMI-adjusted data. However, there was no significant difference in p-values before and after adjusting for BMI in AST (data not shown).

ST6GALNAC3 encodes a type of sialyltransferase that plays a role in the transfer of sialic acids from cytidine 5' monophospho-N-acetylneuraminic acid (CMP-NeuAc) to the carbohydrate termini of glycoproteins and glycolipids [13]. Previous GWASs have revealed the associations of *ST6GALNAC3* SNPs with human electroencephalogram and nicotine dependence [14, 15]; however, there has been no report on an association with liver enzyme levels. Although the association with liver enzymes is still unknown, *ST6GALNAC3* has been shown to be associated with subscapular skin-fold thickness, a type of obesity-related trait, in a Korean adult population [16]. Obesity affects elevated liver enzyme levels by inducing inflammatory responses in the liver, accompanied by increases in C-reactive protein [17]. The involvements between obesity and liver enzyme levels have been found in cohort studies [18-20]. Therefore, the association of *ST6GALNAC3* with obesity may influence liver enzyme levels indirectly. In addition, *ST6GALNAC3* protein interacts with $\alpha 2$ -Heremans-Schmid glycoprotein (AHSG), which is also known as fetuin-A, by enzyme-substrate specificity [13]. AHSG is abundantly distributed in serum protein and is produced mainly in the liver, and elevated levels of this protein are associated with the risk of NAFLD [21]. Considering that liver enzyme levels are closely involved indicators in NAFLD [22, 23], the interaction with AHSG might induce an association between *ST6GALNAC3* and liver enzyme levels.

ADAMTS9 is a metalloproteinase that plays a role in cleavage of proteoglycans and is expressed in skeletal muscle and pancreas [24]. *ADAMTS9* has been reported as a type 2 diabetes susceptibility region in a GWAS [24]. This gene has also shown an association with insulin resistance and impaired beta-cell function in the pancreas [25]. Taken together, the association of *ADAMTS9* with liver enzymes might be due to its susceptibility to diabetes and insulin resistance.

CELF2 (also known as *CUGBP2*) induces the skipping of exon 11 of insulin receptor (*IR*). The mRNA expression level

of exon-skipped *IR* is significantly lower in liver than in other organs in mouse models [26]. It has been reported that a decrease in the number of IRs on target cells can cause insulin resistance [27]. Therefore, it is assumed that *CELF2* affects liver enzyme levels by lowering expression of *IR* in liver that induces insulin resistance.

In summary, our data demonstrate new multiple associations with ALT and AST levels in a Korean childhood cohort. Further replication and functional studies remain to be done to clarify the genetic factors of liver dysfunction.

Supplementary materials

Supplementary data including two tables and one figure can be found with this article online at <http://www.genominfo.org/src/sm/gni-11-149-s001.pdf>.

Acknowledgments

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