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A common variant in the adiponutrin gene influences liver enzyme levels

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Abstract

Background—Two recent genome-wide association studies identified the liver-expressed transmembrane protein adiponutrin to be associated with liver-related phenotypes such as nonalcoholic fatty liver disease and liver function enzymes. These associations were not uniformly reported for various ethnicities. The aim of this study was to investigate a common nonsynonymous variant within adiponutrin (rs738409, exon 3) with parameters of liver function in three independent West-Eurasian study populations including a total of 4290 participants.

Methods—The study was performed in 1) the population-based Bruneck Study (n=783), 2) the SAPHIR Study from Austria based on a healthy working population (n=1705), and the Utah Obesity Case-Control Study including a group of 1019 severely obese individuals (average BMI 46.0 kg/m²) and 783 controls from the same geographical region of Utah. Liver enzymes measured were alanine-aminotransferase (ALT), aspartate-aminotransferase (AST) and gamma-glutamyl transferase (GGT).

Results and Discussion—We found a strong recessive association of this polymorphism with age- and gender-adjusted ALT and AST levels: being homozygous for the minor allele resulted in a highly significant increase of ALT levels of 3.53 U/L ($p=1.86 \times 10^{-9}$) and of AST levels of 2.07 U/L ($p=9.58 \times 10^{-6}$), respectively. The associations were consistently found in all three study populations. In conclusion, the highly significant associations of this transversion polymorphism within the adiponutrin gene with increased ALT and AST levels support a role for adiponutrin as a susceptibility gene for hepatic dysfunction.

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Competing Interest

None of the authors has a conflict of interest to declare.

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Keywords

PNPLA3; rs738409; genetic association; hepatic dysfunction

Introduction

Environmental as well as genetic factors have an impact on liver enzyme levels. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) are markers of hepatocyte injury and liver fat accumulation, and gamma-glutamyl transferase (GGT) is mainly an indicator of biliary or cholestatic disease and heavy alcohol consumption. Heritability estimates range from 33% for ALT to 61% for GGT [1, 2]. To date, there are only few genes reported that influence liver enzyme levels. Finding such genes might help to elucidate genetic characteristics of individuals who are susceptible for liver dysfunction and related conditions such as metabolic syndrome or nonalcoholic fatty liver disease. Those genes might be helpful for monitoring the course and severity of liver disease and evoke new ways of therapies for these conditions [3].

Two recent genome-wide association studies identified the adiponutrin gene to be associated with liver-related phenotypes such as hepatic fat content and ALT concentrations [3, 4]. The results however, were not entirely consistent and showed inter-ethnic differences.

Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase and transacetylase activity [5–8]. It is upregulated during adipocyte differentiation and in response to fasting and feeding, indicating a role in lipid storage in adipose tissue and liver [5–7, 9, 10]. The common nonsynonymous variant rs738409 is located within the patatin domain and results in an amino acid exchange from isoleucine to methionine involving a putative exonic splicing silencer element. It might therefore be also involved in gene regulation [11]. In the present study we aimed to investigate the association of rs738409 of the adiponutrin gene with liver enzymes ALT, AST, and GGT in three independent populations of 4290 individuals, representing a population-based study, a healthy working population and a severely obese population.

Methods

Study Populations

The investigated populations are of West-Eurasian origin and are described in detail in the Supplementary Material. Briefly, the *Bruneck Study* (n=783) is a prospective population-based gender- and age-stratified random sample of all inhabitants of Bruneck, Italy, designed to investigate the epidemiology and pathogenesis of atherosclerosis [12, 13]. The *Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR)* is an observational study conducted in a healthy working population (n=1705) recruited by health screening programs in large companies in and around the city of Salzburg, Austria [14, 15]. The *Utah Obesity Case-Control Study* (n=1802) is composed of 1019 subjects recruited for severe obesity (BMI between 33 and 92 kg/m²) and a general population sample of 783 persons of the same ethnicity [16–18]. Informed consent was obtained from each participant.

Genotyping

Genotyping of the nonsynonymous SNP rs738409 (a C/G transversion polymorphism) within exon 3 of the gene encoding for adiponutrin was done in our laboratory using a 5' nuclease allelic discrimination (Taqman) assay in all subjects with sufficient amount and

quality of DNA with a genotyping success rate of >97% in all three populations (for details see Supplementary Material).

Statistical Analysis

Differences of log-transformed liver enzyme levels between the genotype groups of each study population were tested using general linear regression models adjusted for age and gender assuming a recessive genetic model. A pooled effect size was obtained by meta-regression analysis assuming a fixed effects model as well as a random effects model where appropriate. Further details are described in the Supplementary Material.

Bioinformatic Analysis

Expression profiles of adiponutrin were retrieved using the BioGPS tool of the Genomics Institute of the Novartis Research Foundation (biogps.gnf.org). The potential effects of rs734809 on the protein function were evaluated using the tools Polyphen (<http://genetics.bwh.harvard.edu/pph/>) [19] and SIFT (<http://blocks.fhcrc.org/sift/SIFT.html>) [20] as well as by inspecting its position relative to the known protein domains in the BioSapiens DASTY tool (<http://www.ebi.ac.uk/dasty/>) [21].

Results

Patient and Genotype Characteristics

Baseline clinical characteristics and laboratory data of the three study populations are reported in Table 1 and are stratified by case-control status for the Utah Obesity Case-Control Study. The minor allele frequencies for rs734809 ranged from 22–23% in the Utah population to 27.2% and 29.9% in the SAPHIR and Bruneck Study, respectively (Table 1). We observed no statistically significant difference in genotype frequencies between the Bruneck Study and the SAPHIR Study ($p=0.09$) as well as between cases and controls of the Utah Obesity Case-Control Study ($p=0.50$), but between the first two studies and the Utah study population ($p<0.001$). Due to these differences in genotype frequencies between populations in our and earlier studies [4] and due to differences in the liver enzyme levels between populations, the analysis was performed stratified for the three populations.

Association of rs734809 within adiponutrin and liver enzyme levels

Table 2 shows the age- and gender-adjusted linear regression models with a significant recessive association of the exonic adiponutrin SNP rs734809 with higher levels of ALT and AST in all three populations, and a slightly significant association with increased GGT levels. The pooled effect size and combined p-values obtained by meta-regression analysis from all three groups revealed that being homozygous for the minor allele refers to a highly significant increase of ALT levels of 3.53 U/L ($p=1.86\times 10^{-9}$) and of AST levels of 2.07 U/L ($p=9.58\times 10^{-6}$) compared to carriers of the major allele, respectively. For GGT levels we observed a slight increase of 1.88 U/L ($p=0.07$) which was mostly attributable to the more population-based studies of Bruneck and SAPHIR (Table 2).

Sensitivity analysis

Based on the main analysis, we performed three additional sensitivity analysis which resulted in very similar estimates and significant associations between rs734809 and ALT and AST levels: first, we additionally adjusted for potential confounders BMI, type 2 diabetes mellitus and alcohol use (Supplementary Table 1); second, we adjusted for triglyceride and total cholesterol levels (Supplementary Table 2); and third, we excluded in the population-based Bruneck Study 45 participants with established liver disease verified

by hospital chart and/or hepatitis serology to exclude that the results are caused by certain liver diseases (Supplementary Table 3).

Discussion

Our study in three independent populations revealed a strong association of the nonsynonymous polymorphism rs738409 within the adiponutrin gene with liver enzymes ALT and AST. This association was consistently found in all three study populations and followed a recessive mode of inheritance.

Two recent genome-wide association studies identified the adiponutrin gene to be associated with liver-related phenotypes [3, 4]. Looking closer at the phenotypes investigated, however, the results were not entirely consistent. Romeo et al. found rs738409, a common SNP within adiponutrin, to be significantly associated with increased hepatic fat content in a population of Hispanic, African-American and European-American individuals. An association with the liver enzyme ALT was only observed in Hispanics, the group most susceptible to nonalcoholic fatty liver disease but not in the other two populations [4]. In contrast, the study by Yuan et al. performed in European White and Indian Asian populations found strong associations of genetic variants within adiponutrin with ALT concentrations [3]. Therefore, the latter findings together with our results clearly support the association of adiponutrin with liver enzymes ALT and AST not only in Hispanic but also in other ethnicities such as West Eurasians and Indians. Our data moreover point to a clear recessive association which might explain why the association was not uniformly observed in the two other studies which tested an additive model [3, 4].

Our study included three different study populations which varied considerably in study design, recruitment procedures and liver enzyme levels: a population-based sample (Bruneck Study), a healthy working population (SAPHIR Study) and a case-control sample recruited for obesity (Utah Case-Control Study). The Utah Obesity Case-Control Study represents on the one hand a population expected to include more subjects with an increased hepatic fat content due to the high frequency of severely obese individuals and individuals showing components of the metabolic syndrome and on the other hand a generally decreased influence of alcohol consumption on liver function enzymes caused by the religious habits in the region of Utah. The results point to a biologically stable effect of the investigated genetic variant on liver function parameters ALT and AST since it was found in all three populations despite pronounced differences in the study design. Despite the differences in absolute overall liver enzyme levels between the populations, the relative differences between the homozygote carriers of the minor allele and the other two genotype groups are similar for each population.

It is unclear at the moment whether the variants in adiponutrin result in hepatic lipid storage defects with a secondary increase in liver enzymes or whether adiponutrin has other effects on the hepatocellular function which result in subtle hepatic dysfunction and inflammatory processes. The effect of this variant on liver enzymes, however, is independent from dyslipidemia and hepatic diseases since an adjustment of the association for total cholesterol and triglyceride levels as well as the exclusion of participants with hepatic disease did not have an influence on the results. The remarkably high expression levels of adiponutrin in the liver, which are among the highest of all human tissues (for details see Supplementary Material), point towards a yet unknown role of adiponutrin in the hepatic metabolism. Since the expression is upregulated during adipocyte differentiation and differentially regulated in response to fasting and feeding, a role in facilitating both energy mobilization and lipid storage in adipose tissue and liver is likely [5–8]. The bioinformatic prediction of potential effects of rs738409 revealed that the SNP is quite consistently reported to affect the protein

function. Assuming that adiponutrin has indeed a lipogenic transacetylase activity [8], it may be conceivable that the modification of the conserved residue I148 within the catalytic patatin domain acts as a kind of "gain of function" mutation enhancing the accumulation of lipids in the liver cell. Since ALT is the most specific and sensitive liver function parameter it may therefore be an indicator of subtle hepatocyte injury related to the resulting increased hepatic fat accumulation. Therefore the association of adiponutrin with liver enzymes opens possible new avenues for therapeutic influences and promotes adiponutrin to an interesting drug target.

It remains to be determined whether genetic variation within adiponutrin is important not only for liver enzymes as intermediate phenotypes but also for various endpoints connected to the metabolic syndrome and diabetes mellitus. Although the investigated variant increased ALT and AST levels by 10–20%, the association was only observed in subjects homozygous for the minor allele. Therefore this variant explains only about 0.4–0.7% of the liver enzyme levels of ALT and AST on a population level. It will therefore require a large number of patients and controls to find an association of this variant with those endpoints.

In summary, our results together with results from recent GWA studies clearly support the association of adiponutrin with liver enzymes ALT and AST not only in various ethnicities but also in studies recruited from the general population, healthy working populations and severely obese populations. Adiponutrin might therefore be involved in hepatic dysfunction and possibly inflammatory processes related to fat accumulation in the liver.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

- We aimed to investigate a common nonsynonymous variant within adiponutrin (rs738409) with parameters of liver function in three independent West-Eurasian study populations including a total of 4290 participants.
- These studies were performed in a population-based study, in a healthy working population and an obesity case-control study.
- We found a strong and consistent recessive association of this polymorphism with age- and gender-adjusted alanine-aminotransferase ($p=1.86 \times 10^{-9}$) and aspartate-aminotransferase ($p=9.58 \times 10^{-6}$) levels.
- This highly significant associations of this polymorphism within the adiponutrin gene with liver enzymes support a role for adiponutrin as a susceptibility gene for hepatic dysfunction.

Clinical and laboratory data of participants of the Bruneck Study (n=783), SAPHIR Study (n=1705) and Utah Obesity Case-Control Study (n=1802) with further stratification of the latter in patients with severe obesity and controls.

Table 1

		Utah Obesity Case-Control Study			
		Bruneck Study (n=783)	SAPHIR Study (n=1705)	Severe obesity (n=1019)	Controls (n=783)
Age, yrs		62.7±11.1	51.8±6.0	44.4±11.5	52.7±8.5 ^c
Gender: male/female, n (%)		389/394 (49.7/50.3)	1074/631 (63.0/37.0)	190/829 (18.7/81.3)	387/396 (49.4/50.6) ^c
rs738409: CC/CG/GG	n	379/340/64	884/716/105	611/363/45	467/272/44
	(%)	(48.4/43.4/8.2)	(52.4/2/6)	(60.3/6/4)	(59.6/34.7/5.7)
MAF		29.9	27.2	22.2	23.0
Body mass index, kg/m ²		25.6±3.8	26.8±4.1	46.0±7.6	27.6±4.9 ^c
Total cholesterol, mg/dL		230.03±42.8	228.9±40.1	186.4±36.0	186.8±34.0
Triglycerides, mg/dL		131.4±79.9	125.7±87.8	185.9±105.7	155.5±105.2 ^c
(25th, 50th, 75th percentile)		[81;111;157]	(72;101;150)	(93;132;184)	(118;165;218)
Alanine-aminotransferase (ALT), U/L		23.1±13.1	17.4±10.7	28.0±17.4	26.0±13.8 ^a
Aspartate-aminotransferase (AST), U/L		23.8±9.4	12.3±5.2	25.2±12.6	23.8±8.4 ^a
Gamma-glutamyl transferase (GGT), U/L		36.6±42.3	22.1±23.0	36.8±32.0	26.9±24.4 ^c
Alcohol use, n(%) ^d		406 (52)	726 (71)	193 (19)	144 (40) ^c
Diabetes mellitus, n (%)		68 (8.7)	55 (3)	222 (22)	51 (7) ^c

Values are provided as mean and standard deviation if not indicated otherwise.

^a p<0.05

^b p<0.005

^c p<0.001 - comparison between severe obese subjects and controls above.

^d Data on alcohol use were available in all participants of the Bruneck Study, in 1022 participants of the SAPHIR Study and in 1011 and 358 individuals of the severe obesity group and the controls in Utah, respectively.

Association between rs738409 (C>G) of the adiponutrin gene and liver enzymes in the Bruneck Study, SAPHIR Study and Utah Obesity Case-Control Study

Table 2

Liver enzyme / Study	Genotypes				P value rec
	CC	CG	GG	CC+CG	
Alanine-aminotransferase (=ALT) ^e					
Bruneck Study ^a	20.7±1.02	20.0±1.02	24.8±1.05	20.4±1.02	4.41
SAPHIR Study ^a	14.9±1.02	15.3±1.02	18.2±1.05	15.0±1.01	3.18
Utah Obesity Case-Control Study ^b	23.3±1.01	24.1±1.02	27.2±1.05	23.6±1.01	3.56
Pooled beta estimate and combined p value ^c					3.53
Aspartate- aminotransferase (=AST) ^e					
Bruneck Study ^a	22.7±1.02	22.0±1.02	25.7±1.04	22.4±1.01	3.31
SAPHIR Study ^a	11.4±1.01	11.6±1.01	13.1±1.03	11.5±1.01	1.62
Utah Obesity Case-Control Study ^b	22.7±1.01	23.4±1.01	25.1±1.03	23.0±1.01	2.10
Pooled beta estimate and combined p value ^d					2.07
Gamma-glutamyl transferase (=GGT) ^e					
Bruneck Study ^a	27.7±1.03	26.0±1.03	31.8±1.08	26.9±1.02	4.92
SAPHIR Study ^a	16.8±1.02	16.8±1.02	18.7±1.06	16.8±1.02	1.91
Utah Obesity Case-Control Study ^b	26.0±1.02	25.9±1.02	26.2±1.06	26.0±1.01	0.26
Pooled beta estimate and combined p value ^d					1.88
					0.07

Values are provided as adjusted marginal mean and standard error. Means and estimates are calculated based on log-transformed regression and were re-transformed appropriately.

^a Adjusted for age, gender;

^b Adjusted for age, gender, group status;

^c Pooled effect size and p-value derived from meta-regression using a fixed effects model.

^d Due to heterogeneity between the study populations pooled effect size and p-value were derived from meta-regression using a random effects model.