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Genetic evidence for a role of adiponutrin in the metabolism of apolipoprotein B-containing lipoproteins

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Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase activity that is regulated by fasting and feeding. Recent genome-wide association studies identified *PNPLA3* to be associated with hepatic fat content and liver function, thus pointing to a possible involvement in the hepatic lipoprotein metabolism. The aim of this study was to examine the association between two common variants in the adiponutrin gene and parameters of lipoprotein metabolism in 23 274 participants from eight independent West-Eurasian study populations including six population-based studies [Bruneck ($n = 800$), KORA S3/F3 ($n = 1644$), KORA S4/F4 ($n = 1814$), CoLaus ($n = 5435$), SHIP ($n = 4012$), Rotterdam ($n = 5967$)], the SAPHIR Study as a healthy working population ($n = 1738$) and the Utah Obesity Case-Control Study including a group of 1037 severely obese individuals (average BMI 46 kg/m^2) and 827 controls from the same geographical region of Utah. We observed a strong additive association of a common non-synonymous variant within adiponutrin (rs738409) with age-, gender-, and alanine-aminotransferase-adjusted lipoprotein concentrations: each copy of the minor allele decreased levels of total cholesterol on average by 2.43 mg/dl ($P = 8.87 \times 10^{-7}$), non-HDL cholesterol levels by 2.35 mg/dl ($P = 2.27 \times 10^{-6}$) and LDL cholesterol levels by 1.48 mg/dl ($P = 7.99 \times 10^{-4}$). These associations remained significant after correction for multiple testing. We did not observe clear evidence for associations with HDL cholesterol or triglyceride concentrations. In conclusion, our study suggests that adiponutrin is involved in the metabolism of apoB-containing lipoproteins.

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INTRODUCTION

Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase activity (1–3). It is upregulated during adipocyte differentiation and in response to feeding and downregulated in fasting state, indicating a potential role in lipid storage in adipose tissue and liver (1–5). An increased energy intake, either acute or long-lasting, leads to an upregulation of adiponutrin (6). *In vitro* adiponutrin gene expression shows features of lipogenic gene expression comparable to fatty acid synthase or adipocyte determination and differentiation of factor-1/sterol regulatory element binding protein-1c (ADD1/SREBP1c) (1). The common non-synonymous variant rs738409 results in an amino acid exchange from isoleucine to methionine in the catalytic patatin domain (see Supplementary Material, Fig. S1 and reference 7). Additionally, rs738409 was reported to activate a putative exonic splicing silencer element and might therefore also affect gene regulation (8). A further SNP, rs2072907, in linkage disequilibrium with rs738409, was reported to be associated with obesity (9).

Recent genome-wide association studies identified the adiponutrin gene to be associated with liver-related phenotypes (7,10). Romeo *et al*. (7) found carriers of the minor allele of rs738409 to have a significantly increased hepatic fat content in a population of Hispanic, African-American and European-American individuals and significantly higher liver enzymes in Hispanics which points to an important role of adiponutrin in the hepatic lipoprotein metabolism. These observations and the experimental findings described above make adiponutrin an interesting candidate gene involved in lipid metabolism.

In the present candidate gene study, we aimed to investigate the association of rs738409 and rs2072907 of the adiponutrin gene with lipid parameters in eight independent populations including 23 274 individuals, representing six population-based studies, a healthy working population and a case–control study of a severely obese population.

RESULTS

Patient and genotype characteristics

Baseline clinical characteristics and laboratory data of the eight 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 ranged from 18.5 to 30.0% for rs738409 and from 16.2 to 24.9% for rs2072907 across the populations (Table 1). Due to these differences in minor allele and genotype frequencies between populations in our and earlier studies (7,10) and due to differences in the lipid levels between populations, the analysis was performed stratified for the eight populations.

Association of rs738409 and rs2072907 within adiponutrin and lipid levels

Supplementary Material, Tables S1 and S2 show the age-, gender- and alanine-aminotransferase-adjusted lipid levels using an unconstrained model (not assuming any genetic mode of inheritance) for the three genotypes of the exonic adiponutrin SNP rs738409 and the intronic SNP rs2072907, respectively. Calculation of λ as the quotient of the β-coefficients of heterozygote and homozygote carriers of the minor allele of the unconstrained model revealed an additive mode of inheritance in most of the populations (as an example, see results for rs738409 in Supplementary Material, Table S3). Therefore, the main analyses and meta-regression were based on the additive inheritance assumption. Table 2 shows the β-estimates and *P*-values from linear regression models applying an additive model, once adjusted for age and gender and once additionally adjusted for the liver enzyme alanine-aminotransferase. We observed that the minor allele was associated with lower concentrations of total cholesterol (TC), non-HDL cholesterol (non-HDLC) and LDL cholesterol (LDLC). Since the associations became even stronger when additionally adjusted for alanine-aminotransferase, we assumed that the association of these adiponutrin SNPs with lipid levels is not a secondary effect of liver impairment. Using a fixed effects model, each copy of the minor allele decreased total cholesterol levels by on average of 2.43 mg/dl ($P = 8.87 \times 10^{-7}$), non-HDL cholesterol levels by 2.35 mg/dl ($P = 2.27 \times 10^{-6}$) and LDL cholesterol levels by 1.48 mg/dl ($P = 7.99 \times 10^{-4}$). In addition, we observed a trend for lower triglyceride (TG) levels per copy of the minor allele (estimate on the log scale −0.011, $P = 0.10$) but no association with HDLC concentrations. After correction for multiple testing, the effects on total cholesterol, non-HDLC and LDLC remained statistically significant. When we analyzed the data for triglycerides in the Utah Obesity Case-Control Study, we observed lower triglyceride levels per copy of the minor allele which was more pronounced in the cases than in the controls (estimate −0.073, $P = 0.008$ for cases, and −0.019, $P = 0.54$ for controls). However, this interaction was not significant, and furthermore, after correction for multiple testing, the effect for triglycerides was no longer significant.

Similar associations were observed for rs2072907 (Table 2). Moreover, an additional adjustment for BMI revealed similar estimates for both SNPs. When we repeated the analysis using a random effects model, we observed similar beta estimates as for the fixed effects model.

Bioinformatic analysis

To investigate whether or not adiponutrin interacted directly with cholesterol, we used the BioSapiens DASTY tool, which integrates several tools for protein domain analysis. No direct cholesterol-binding domains could be observed (Supplementary Material, Fig. S1).

Since data mining using STRING 8.0 suggested among others an interaction of adiponutrin with lipoprotein lipase (*LPL*) and hepatic lipase (*LIPC*) (see Supplementary Material, Fig. S2), we investigated a potential co-regulation of the adiponutrin promoter with cholesterol metabolism given that co-regulation and shared transcription factor binding sites often point to a functional interaction (11,12). For this purpose, the adiponutrin promoter was predicted using the Genomatix Promoter Inspector tool and searched for the presence of putative transcription factor binding sites involved in

Table 1. Clinical and laboratory data of participants of the Bruneck Study, KORA S3/F3 Study, KORA S4/F4 Study, CoLaus Study, SHIP, Rotterdam Study, SAPHIR Study and Utah Obesity Case-Control Study with further stratification of the latter in patients with severe obesity and controls

	Bruneck Study ($n = 800$)	KORA S3/F3 Study ($n = 1644$)	KORA S4/F4 Study ($n = 1814$)	CoLaus Study ($n = 5435$)	SHIP ($n = 4012$)	Rotterdam Study ($n = 5967$)	SAPHIR Study ($n = 1738$)	Utah Obesity Case-Control Study	
								Severe obesity ($n = 1037$)	Controls ($n = 827$)
Age, years	62.7 ± 11.1	62.5 ± 10.1	60.9 ± 8.9	53.5 ± 10.8	49.7 ± 16.3	69.4 ± 9.1	51.8 ± 6.1	44.3 ± 11.4	52.7 ± 8.5***
Gender: male/female, *n* (%)	398/402 (49.8/50.2)	813/831 (49.4/50.6)	884/930 (48.7/51.3)	2560/2875 (47.1/52.9)	1972/2040 (49.2/50.8)	2359/3465 (40.5/59.5)	1093/645 (62.9/37.1)	194/843 (18.7/81.3)	399/428 (48.2/51.8)***
rs738409: CC/CG/GG *n* (%)	381/343/65 (48.3/43.5/8.2)	941/613/90 (57.2/37.3/5.5)	1193/571/50 (65.7/31.5/2.8)	3074/2037/324 (56.6/37.5/5.9)	2391/1432/189 (59.6/35.7/4.7)	3647/2078/242 (61.1/34.8/4.1)	885/720/105 (51.8/42.1/6.1)	612/366/45 (59.8/35.8/4.4)	468/272/44 (59.7/34.7/5.6)
MAF	30.0	24.1	18.5	24.7	22.8	21.5	27.2	22.3	23.0
Call rate (%)	98.6	100[a]	100[a]	100[a]	100[a]	99.9	98.0	98.6	94.8
rs2072907: CC/CG/GG *n* (%)	464/272/43 (59.6/34.9/5.5)	1104/490/50 (67.2/29.8/3.0)	1015/694/105 (55.9/38.3/5.8)	3641/1624/170 (67.0/29.9/3.1)	2674/1212/126 (66.7/30.2/3.1)	3979/1773/222 (66.6/29.7/3.7)	1086/553/73 (63.4/32.3/4.3)	715/287/22 (69.8/28.0/2.2)	540/219/26 (68.8/27.9/3.3)
MAF	23.0	17.9	24.9	18.1	18.3	18.5	20.4	16.2	17.3
Call rate (%)	97.4	100[a]	100[a]	100[a]	100[a]	100[a]	98.1	98.7	94.9
Body mass index, kg/m^2	25.6 ± 3.8	28.1 ± 4.5	28.2 ± 4.8	25.9 ± 4.6	27.3 ± 4.7	26.3 ± 3.7	26.8 ± 4.1	46.0 ± 7.6	27.6 ± 4.9***
Total cholesterol, mg/dl	230.0 ± 42.6	221.3 ± 40.3	221.8 ± 39.3	224.0 ± 41.3	222.9 ± 47.8	255.1 ± 47.2	228.6 ± 40.1	186.8 ± 36.2	187.1 ± 33.9
Non-HDL cholesterol, mg/dl	171.3 ± 42.9	163.2 ± 40.0	165.3 ± 38.5	153.9 ± 41.0	166.8 ± 49.2	203.2 ± 47.4	169.0 ± 41.8	140.8 ± 35.1	137.0 ± 33.6*
HDL cholesterol, mg/dl	58.7 ± 16.2	58.0 ± 16.9	56.5 ± 14.5	63.6 ± 17.0	56.09 ± 16.6	51.8 ± 14.3	59.6 ± 15.7	46.0 ± 11.0	50.1 ± 15.0***
LDL cholesterol, mg/dl	145.5 ± 37.9	130.1 ± 32.7	139.9 ± 35.0	128.5 ± 35.4	138.1 ± 44.8	145.1 ± 34.2	145.5 ± 36.6	108.3 ± 27.6	105.0 ± 27.5*
Alanine-aminotransferase, (U/L) (25th; 50th; 75th percentile)	23.1 ± 13.1 (15;20;27)	20.4 ± 11.4 (14;18;24)	26.4 ± 16.2 (16;22;30)	27.7 ± 19.5 (17;23;32)	30.0 ± 18.0 (17;23;33)	18.0 ± 11.4 (12;16;20)	17.4 ± 10.6 (11;15;20)	27.9 ± 17.4 (17,24;33)	25.8 ± 13.6* (17;22;30)
Triglycerides, mg/dl (25th; 50th; 75th percentile)	131.7 ± 71.9 (81;111;158)	142.3 ± 123.2[b] (85;115;163)	132.7 ± 94.6[b] (77,110;157)	123.9 ± 104.8 (71;98;142)	–[b]	137.9 ± 67.6[b] (94; 122; 163)	125.6 ± 87.7 (72;101;149)	186.1 ± 105.6 (118;165;220)	156.0 ± 105.0*** (94;133;184)
Diabetes mellitus, *n* (%)	76 (9.5)	181 (11.0)	167 (9.2)	246 (4.5)	142 (3.5)	631 (10.6)	55 (3.2)	224 (21.6)	54 (6.5)***
Use of lipid lowering drugs, *n* (%)	25 (3.1)	238 (14.5)	299 (16.5)	636 (11.7)	317 (7.9)	150 (2.5)	79 (4.6)	122 (11.9)	65 (7.9)**

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

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$—comparison between severe obese subjects and controls in the Utah obesity case–control study.

[a]Genotypes were imputed with very high imputation quality [RSQR for MACH and proper_info for IMPUTE >0.90 (Kora S3/F3 and S4/F4), RSQR for MACH for the Rotterdam Study (>0.99), proper_info for IMPUTE for the CoLaus Study >0.97 and for SHIP >0.96].

[b]Triglycerides are provided only for fasting participants which were 675 in KORA S3/F3, 1789 in KORA S4/F4, 5389 in CoLaus and 2038 in Rotterdam. Participants of SHIP were non-fasting at the time of blood collection.

Table 2. Association between rs738409 (C>G) and rs2072907 (C>G) of the adiponutrin gene and lipid levels gene in the Bruneck Study, KORA S3/F3 and S4/F4 Study, CoLaus Study, SHIP, Rotterdam Study, SAPHIR Study and Utah Obesity Case-Control Study (additive model)

Parameters/study	rs738409 (C>G)				rs2072907 (C>G)			
	Adjusted for age and gender		Adjusted for age, gender and ALT		Adjusted for age and gender		Adjusted for age, gender and ALT	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Total cholesterol, mg/dl								
Bruneck Study	1.49 (2.40)	0.53	1.18 (2.40)	0.62	−1.48 (2.54)	0.56	−1.55 (2.54)	0.54
KORA S3/F3 Study	−1.65 (1.72)	0.34	−2.45 (1.73)	0.16	−1.26 (1.91)	0.51	−2.12 (1.92)	0.27
KORA S4/F4 Study	−2.53 (1.58)	0.11	−3.00 (1.59)	0.06	−3.84 (1.80)	0.03	−4.34 (1.80)	0.02
CoLaus Study	−2.07 (0.96)	0.03	−2.56 (0.96)	0.008	−1.24 (1.07)	0.25	−1.76 (1.07)	0.10
SHIP	−2.04 (1.30)	0.12	−3.18 (1.28)	0.01	−1.61 (1.38)	0.24	−2.44 (1.37)	0.07
Rotterdam Study	0.35 (1.05)	0.74	0.59 (1.24)	0.63	−1.11 (1.09)	0.31	−1.13 (1.27)	0.38
SAPHIR Study	−2.89 (1.62)	0.08	−3.46 (1.62)	0.03	−3.33 (1.72)	0.05	−3.71 (1.72)	0.03
Utah Cases	−4.79 (2.05)	0.02	−5.07 (2.07)	0.01	−4.57 (2.31)	0.05	−4.65 (2.32)	0.05
Utah Controls	−5.67 (2.08)	0.007	−5.62 (2.07)	0.007	−3.04 (2.30)	0.19	−2.97 (2.29)	0.20
Pooled analysis[a]	**−1.84**	**1.24×10^{-4}**	**−2.43**	**8.87×10^{-7}, [b]**	**−1.94**	**1.87×10^{-4}**	**−2.41**	**7.01×10^{-6}, [b]**
Non-HDL cholesterol, mg/dl								
Bruneck Study	2.26 (2.44)	0.35	1.69 (2.42)	0.49	−0.55 (2.59)	0.83	−0.70 (2.57)	0.79
KORA S3/F3 Study	−0.15 (1.73)	0.93	−1.31 (1.74)	0.45	0.16 (1.92)	0.93	−1.17 (1.93)	0.54
KORA S4/F4 Study	−2.44 (1.58)	0.12	−3.06 (1.58)	0.05	−2.94 (1.79)	0.10	−3.58 (1.79)	0.05
CoLaus Study	−1.90 (0.96)	0.05	−2.54 (0.95)	0.007	−1.05 (1.06)	0.32	−1.73 (1.05)	0.01
SHIP	−2.28 (1.33)	0.09	−3.52 (1.31)	0.007	−2.00 (1.41)	0.16	−2.89 (1.40)	0.04
Rotterdam Study	0.10 (1.08)	0.93	0.67 (1.26)	0.60	−0.69 (1.12)	0.53	−0.87 (1.30)	0.50
SAPHIR Study	−2.80 (1.70)	0.10	−3.80 (1.69)	0.03	−2.64 (1.81)	0.14	−3.32 (1.79)	0.06
Utah Cases	−4.92 (2.00)	0.01	−5.25 (2.01)	0.009	−4.56 (2.25)	0.04	−4.65 (2.26)	0.04
Utah Controls	−4.51 (2.06)	0.03	−4.51 (2.04)	0.03	−1.76 (2.27)	0.44	−1.73 (2.25)	0.44
Pooled analysis[a]	**−1.69**	**4.95×10^{-4}**	**−2.35**	**2.27×10^{-6}, [b]**	**−1.52**	**0.004**	**−2.14**	**6.96×10^{-5}, [b]**
LDL cholesterol, mg/dl								
Bruneck Study	1.91 (2.14)	0.37	1.68 (2.14)	0.43	−0.68 (2.26)	0.77	−0.73 (2.26)	0.75
KORA S3/F3 Study	−0.33 (1.41)	0.81	−0.77 (1.42)	0.59	−0.12 (1.57)	0.94	−0.61 (1.58)	0.70
KORA S4/F4 Study	−1.32 (1.43)	0.36	−1.75 (1.44)	0.22	−2.03 (1.63)	0.21	−2.51 (1.63)	0.12
CoLaus Study	−1.40 (0.82)	0.09	−1.68 (0.83)	0.04	−0.60 (0.91)	0.51	−0.92 (0.92)	0.32
SHIP	−1.39 (1.21)	0.25	−2.15 (1.21)	0.07	−1.01 (1.29)	0.43	−1.56 (1.29)	0.23
Rotterdam Study	−0.54 (1.22)	0.66	−0.34 (1.27)	0.79	−1.00 (1.26)	0.43	−0.40 (1.31)	0.76
SAPHIR Study	−1.73 (1.49)	0.25	−2.10 (1.50)	0.16	−2.03 (1.59)	0.20	−2.27 (1.59)	0.15
Utah Cases	−1.16 (1.57)	0.46	−1.42 (1.58)	0.37	−0.29 (1.77)	0.87	−0.40 (1.78)	0.82
Utah Controls	−3.37 (1.72)	0.05	−3.31 (1.72)	0.05	−1.95 (1.90)	0.30	−1.88 (1.90)	0.32
Pooled analysis[a]	**−1.18**	**0.007**	**−1.48**	**7.99×10^{-4}, [b]**	**−0.99**	**0.04**	**−1.19**	**0.01[b]**
HDL Cholesterol, mg/dl								
Bruneck Study	−0.89 (0.89)	0.32	−0.65 (0.89)	0.47	−0.98 (0.95)	0.30	−0.94 (0.95)	0.32
KORA S3/F3 Study	−1.55 (0.65)	0.02	−1.14 (0.65)	0.08	−1.39 (0.72)	0.05	−0.91 (0.72)	0.20
KORA S4/F4 Study	−0.21 (0.53)	0.69	−0.05 (0.53)	0.93	−0.96 (0.59)	0.11	−0.78 (0.59)	0.19
CoLaus Study	−0.33 (0.35)	0.30	−0.15 (0.35)	0.66	−0.40 (0.39)	0.35	−0.19 (0.39)	0.62
SHIP	0.44 (0.43)	0.91	0.15 (0.43)	0.73	0.21 (0.45)	0.64	0.28 (0.45)	0.53
Rotterdam Study	0.29 (0.31)	0.35	−0.02 (0.37)	0.96	−0.49 (0.32)	0.13	−0.34 (0.38)	0.37
SAPHIR Study	−0.06 (0.58)	0.92	0.38 (0.57)	0.51	−0.70 (0.62)	0.26	−0.40 (0.61)	0.52
Utah Cases	−0.27 (0.56)	0.63	−0.20 (0.57)	0.72	−0.16 (0.64)	0.81	−0.13 (0.64)	0.84
Utah Controls	−0.89 (0.80)	0.26	−0.83 (0.79)	0.29	−0.94 (0.89)	0.29	−0.86 (0.88)	0.33
Pooled analysis[a]	**−0.14**	**0.40**	**−0.14**	**0.40**	**−0.49**	**0.005**	**−0.32**	**0.08**
Triglycerides, mg/dl[c]								
Bruneck Study	0.039 (0.028)	0.16	0.029 (0.027)	0.28	0.026 (0.030)	0.38	0.024 (0.029)	0.41
KORA S3/F3 Study	0.028 (0.034)	0.40	0.004 (0.034)	0.91	0.027 (0.039)	0.48	0.005 (0.039)	0.90
KORA S4/F4 Study	0.002 (0.021)	0.92	−0.014 (0.020)	0.50	0.001 (0.023)	0.98	−0.015 (0.023)	0.52
CoLaus Study	−0.002 (0.01)	0.86	−0.01(0.01)	0.20	−0.0007 (0.01)	0.95	−0.01 (0.01)	0.28
SHIP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rotterdam Study	0.001 (0.015)	0.95	0.009 (0.016)	0.06	−0.018 (0.016)	0.26	−0.023 (0.017)	0.17
SAPHIR Study	−0.012 (0.022)	0.59	−0.036 (0.021)	0.09	−0.013 (0.023)	0.57	−0.030 (0.023)	0.19
Utah Cases	−0.074 (0.027)	0.007	−0.073 (0.028)	0.008	−0.067 (0.031)	0.03	−0.065 (0.031)	0.04
Utah Controls	−0.017 (0.032)	0.59	−0.019 (0.031)	0.54	−0.017 (0.035)	0.62	−0.019 (0.035)	0.59
Pooled analysis[a]	**−0.003**	**0.60**	**−0.011**	**0.10**	**−0.006**	**0.35**	**−0.016**	**0.03**

Values are provided as effect size and standard error.
ALT, alanine-aminotransferase; n.d., not determined.
[a]Pooled effect sizes and *P*-values derived from meta-regression using a fixed effects model.
[b]Still significant after correction for multiple testing, using Bonferroni–Holm procedure assuming 10 independent tests.
[c]Values for triglycerides are calculated based on log-transformed regression and provided only for fasting participants not taking fibrates which were 675 in KORA S3/F3, 1789 in KORA S4/F4, 5389 in CoLaus, 2038 in Rotterdam. Participants of SHIP were non-fasting at the time of blood collection.

cholesterol metabolism. We found a definite combination of binding sites for SREBP factors and the CCAAT binding factor NFY, a known interactor of SREBP factors (13). This combination of binding sites (14) could also be detected in the promoter regions of LPL and LIPC (for details, see Supplementary Material, Fig. S3) and was previously reported to mediate the sterol response in the promoter of the glycerol-3-phosphate acyltransferase during the differentiation of preadipocytes to adipocytes (15). More generally, SREBP factors are known to play a prominent role in the regulation of cholesterol metabolism (16–18).

Finally, expression profiles were retrieved in BioGPS (https://biogps.gnf.org/) and showed a strong expression of adiponutrin in the liver and the adrenal cortex (see Supplementary Material, Fig. S4). Since adrenocortical hormones are synthesized from cholesterol, this finding may further underline a possible yet unknown role of adiponutrin in cholesterol metabolism.

DISCUSSION

Our study revealed a significant association of genetic variants within the adiponutrin gene with total cholesterol, non-HDLC and LDLC levels which remained significant after correction for multiple testing. The findings were consistent in six of the eight study populations for the rs738409 SNP and the six studies represented 72% of the investigated individuals. For the intronic rs2072907, SNP results were consistent across all eight study populations. The populations varied considerably in study design and recruitment procedures which points to a stable and biologically important interconnection.

Two recent genome-wide association studies identified the adiponutrin gene to be associated with liver-related phenotypes (7,10). Yuan *et al*. (10) observed a strong association of genetic variants within adiponutrin with alanine-aminotransferase concentrations in European and Asian Indian populations. Romeo *et al*. (7) found rs738409, the common SNP investigated also in our populations, to be significantly associated with increased hepatic fat content in a population of Hispanic, African-American and European-American individuals and with liver enzymes in Hispanics. Despite the observed association with hepatic fat content and the strong involvement of the liver in lipid metabolism, they surprisingly did not find an association with parameters of lipid metabolism in their primary analysis sample. However, in a corresponding analysis in the ARIC study, the authors obviously observed an additive effect for total cholesterol in about 11 000 European-Americans (overall P-value = 0.014) for the rs738409 (7), which is in support of our findings. One might ask whether the observed association between adiponutrin variants and lipoprotein levels are simply a consequence of an impaired liver function as recent studies observed these variants to be associated with liver enzyme levels (7,10). This can clearly be excluded for our investigation, since an adjustment of lipoprotein parameters for enzymes such as alanine-aminotransferase did not attenuate but strengthened the association. This suggests that liver enzymes are not in the main causal pathway for the association between these variants and lipoprotein metabolism in a sense that an impaired liver function originating from genetic variation of adiponutrin mocks the association with cholesterol levels (19).

Several important observations were made in our and other studies which point to a function of adiponutrin in lipid metabolism. The mechanisms underlying this genetic association, however, remain to be elucidated. The accumulation of fat in the liver may be due to impaired release of triglyceride-rich lipoproteins by the hepatocyte into the blood stream. Our study provides evidence that adiponutrin is involved in the metabolism of apoB-containing lipoproteins, possibly by participating in the post-prandial packaging of these lipoproteins in the liver. In contrast, this variant was not associated with HDLC levels. The involvement in the metabolism of apoB-containing lipoproteins is supported by our bioinformatic analysis. Adiponutrin is possibly co-regulated with fatty acid synthase and controlled by sterol regulatory element-binding proteins (SREBPs). SREBPs play a prominent role in the regulation of cholesterol and fatty acid metabolism (16–18). In the nucleus, SREBP-1c transcriptionally activates most genes required for fatty acid synthesis and lipogenesis, whereas SREBP-2 preferentially activates cholesterol synthesis (16–18,20). Indeed, the adiponutrin gene exhibits features of lipogenic genes. In addition to SREBPs, PPAR γ and the carbohydrate responsive element binding protein (ChREBP), a new transcription factor mediating glucose action in liver, were reported to regulate adiponutrin (1,21).

From the data available about adiponutrin in other studies (5,7,22), it was hypothesized that adiponutrin might mostly be involved in triglyceride metabolism. We indeed observed a slight association between our investigated variants and plasma triglyceride levels which was most pronounced in the Utah cases of severe obesity ($P = 0.008$). From this data, we carefully speculate that per copy of the minor allele G the functional activity of the enzyme is higher which shifts more triglycerides into the hepatic cells especially in severely obese persons who are usually exposed to a higher nutritional intake. This is in line with the higher hepatic triglyceride content in carriers of the minor allele of rs734809 observed by Romeo *et al*. (7). Whether the triglyceride association holds true in severely obese individuals as indicated in the Utah Obesity Case-Control Study needs confirmation in other large studies.

The investigated variants decreased apoB-containing lipoprotein fractions by $\sim$3% and the association was observed per copy of the minor allele (7). The variants explained less than 1% of the lipoprotein concentrations on a population level. It will therefore require a large number of patients and controls to find an association of these variants with endpoints such as cardiovascular disease, diabetes mellitus or the metabolic syndrome. Because the adiponutrin variant is associated with lower levels of atherogenic lipoproteins, one may wonder whether this enzyme may represent an attractive drug target for prevention of cardiovascular disease. However, one may keep in mind that pharmacological modulation of this enzyme, if feasible, may be associated with liver steatosis as it was reported for microsomal transport protein inhibitors (23).

In summary, our results clearly support the association of adiponutrin with apoB-containing lipoprotein fractions in West-Eurasian populations not only in studies recruited from

general or healthy working populations but also from severely obese populations. Our and previous studies suggest that adiponutrin might mainly be involved in processes related to disturbed lipid and energy metabolism and fat accumulation especially in the liver.

MATERIALS AND METHODS

Study populations

The investigated populations are of West-Eurasian origin and are described in detail in the Supplementary Material. Briefly, the *Bruneck Study* ($n = 800$) 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 (24,25). The KORA cohorts (Cooperative Health Research in the Region of Augsburg, KOoperative Gesundheitsforschung in der Region Augsburg) are several cohorts representative of the general population in Augsburg, Germany, and two surrounding counties that were initiated as part of the WHO MONICA Study. For the present analyses, we chose 1644 subjects from the KORA S3/F3 survey, which were part of the recent genome-wide association study (26,27), as well as 1814 subjects from the KORA S4/F4 survey. The *Caucasian Cohorte Lausannoise (CoLaus Study*, $n = 5435$) is a non-stratified random sample of the population registry of the city of Lausanne, Switzerland and investigates the epidemiology and genetic determinants of cardiovascular risk factors and the metabolic syndrome (28). The Study of Health in Pomerania (*SHIP)* ($n = 4012$) is a population sample aged 20–79 years selected using population registries (29). The *Rotterdam Study* is a prospective cohort study that started in 1990 in Ommoord, a suburb of Rotterdam, among 10 994 men and women aged 55 and over (30). The *Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR)* is an observational study conducted in a healthy working population ($n = 1738$) recruited by health screening programs in large companies in and around the city of Salzburg, Austria (31). The *Utah Obesity Case-Control Study* ($n = 1864$) is composed of 1037 subjects recruited for severe obesity (BMI between 33 and 92 kg/m^2) and a general population sample of 827 persons of the same ethnicity (32–34). Informed consent was obtained from each participant.

For the analyses of total, LDL and non-HDL cholesterol, we excluded all study participants receiving statins as lipid-lowering drugs. Individuals receiving fibrates were excluded for the analysis of triglyceride levels.

Genotyping and phenotyping

Genotyping of the non-synonymous SNP rs734809 (a C/G transversion polymorphism) within exon 3 and of the C/G transversion polymorphism rs2072907, a non-coding variant located in intron 5, within the gene encoding for adiponutrin was done using a 5′ nuclease allelic discrimination (Taqman) assay in all subjects with sufficient amount and quality of DNA (for details, see Supplementary Material). For KORA S3/F3 and S4/F4, the CoLaus Study and SHIP genotypes were derived from an imputed SNP data set derived from genotyping with Affymetrix chips with a high imputation quality. In the Rotterdam Study, rs734809 was genotyped within an Illumina HumanHap 550 k chip and rs2072907 was imputed. Methods on the measurement of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and alanine-aminotransferase are provided in the Supplementary Material. Non-HDLC levels were calculated by subtracting HDLC from total cholesterol. Participants of the Bruneck, KORA S4/F4, CoLaus, SAPHIR and Utah Study were fasting at the time of blood collection with the exception of a few individuals. In KORA S3/F3, 675 of the 1644 subjects were fasting. In the SHIP Study, blood collection was performed in non-fasting state. In the Rotterdam Study, TC and HDLC levels used in this analysis were measured in non-fasting people (baseline study), while TG was measured in fasting people (third follow-up examination). Non-fasting individuals of each study were excluded from the analysis of triglyceride levels.

Statistical analysis

Differences of lipid parameters between the genotype groups of each study population were tested using general linear regression models adjusted for age, gender and alanine-aminotransferase using an unconstrained genetic model as well as an additive genetic model. A pooled effect size was obtained by meta-regression analysis using a fixed effects model and compared to a random effects model (35–37). Further details are described in the Supplementary Material.

Bioinformatic analysis

We examined the protein domains of adiponutrin that could potentially influence the cholesterol metabolism by using the BioSapiens DASTY tool (http://www.biosapiens.info/page.php?page=dasty) and we investigated the effects of the amino acid exchange caused by a transversion at rs738409 on the protein function using Polyphen (38) and SIFT (39). Known and predicted protein interactions were analyzed by STRING 8.0 (http://string.embl.de/) (11). The potential regulation of the adiponutrin gene promoter by cholesterol-related transcription factors was investigated using the Genomatix Suite (Genomatix GmbH, Munich, Germany; www.genomatix.de). Expression profiles were retrieved by BioGPS (https://biogps.gnf.org/).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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