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Alexandra Nieters, Nikolaus Becker, Jakob Linseisen

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Alexandra Nieters
Nikolaus Becker
Jakob Linseisen

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prospective investigation into diet and cancer, and b) to investigate potential effects of interactions between dietary fatty acid intake and allelic variants on obesity risk. *Subjects and methods* Within EPIC-Heidelberg, 154 subjects with a body mass index > 35 kg/m^2 and 154 age- and sex-matched normal-weight controls were selected and genotypes determined for 11 candidate genes. Dietary intake was assessed by a validated food frequency questionnaire. Odds ratios (OR) were computed by means of unconditional logistic regression and different adjustment models. Genotyping was performed by PCR-RFLP and allele-specific PCR. *Results* For most of the investigated genes (PPARA, PPARG2, UCP1, UCP2, UCP3, BAR-2, APM1, leptin, SORBS1, HSL, and TNFA) an indication for a minor effect on obesity risk was found. Indication of a risk-increasing effect was strongest for the homozygous form of leptin –2548AA with an adjusted OR of 3.53 ($p < 0.009$). Additionally, for the polymorphic sites of BAR-2 (Arg16Gly and Gln27Glu) a significant effect on obesity risk was seen. Importantly, the results of the analysis of gene-diet interactions suggest that the allelic variants of candidate genes (leptin, TNFA, PPARG2) might strongly affect diet-related obesity risk. *Conclusions* The results support some but not all previous reports about a risk-modulating effect of polymorphisms in genes affecting obesity risk. The most important finding is an indication of substantial interaction between allelic variants of particular genes and fatty acid intake-related obesity risk. These observations suggest that future studies on polymorphisms in obesity genes should take data on dietary habits into account.

Dr. A. Nieters · N. Becker · J. Linseisen
German Cancer Research Center
Division of Clinical Epidemiology
Im Neuenheimer Feld 280
69120 Heidelberg, Germany
Tel.: +49-62 21/42 22 21
Fax: +49-62 21/42 22 03
E-Mail: a.nieters@dkfz.de

J. Linseisen
Unit of Human Nutrition
and Cancer Prevention
Technical University of Munich
Munich, Germany

Introduction

Obesity is a multifactorial condition affected by the combined effects of genes, environment and their interactions [1]. A large number of studies have investigated the role of variants in one or a few candidate genes for obesity, frequently with contradictory results. Many of these studies have not included dietary factors or other lifestyle factors which are expected to modulate body weight phenotype. Thus, the understanding of the mechanisms by which lifestyle factors such as diet and physical activity interact with genetic factors is still in its infancy.

The present study aimed to explore the interplay of several candidate obesity genes with dietary fatty acid intake in a well-characterized study population of individuals with BMI ≥ 35 kg/m^2 and matched normal

weight controls (BMI 18.5 ≤25 kg/m^2). A set of polymorphisms in candidate obesity genes was selected for which an effect of the genetic variants on human obesity risk or associated conditions has been previously reported; however, for this project, key factors of the neuroendocrine regulation system were omitted. The 11 selected candidate genes are the following: 3 genes encoding for uncoupling proteins (UCP1, UCP2, UCP3), beta2 adrenergic receptor (BAR-2), 2 peroxisome proliferator-activated receptor genes (PPARA, PPARG2), hormone sensitive lipase (HSL), tumor necrosis factor alpha (TNFA), leptin, APM1/adiponectin, and the sorbin and SH3-domain-containing-1 gene (SORBS1). For PPARG2 and BAR-2 two different polymorphic sites each have been considered, which sums up to a total of 13 polymorphisms to be analyzed.

Briefly, the candidate genes code for molecules involved in the regulation of body weight (fat mass) and thermogenesis. A key element in the physiological system that regulates body weight is the hormone leptin. It is produced by fat cells and is crucial for the regulation of appetite and energy expenditure. The leptin –2548 promoter polymorphism has been linked with leptin levels in obese girls [2] and overweight [3]. PPARγ influences the storage of fatty acids in the adipose tissue, whereas PPARα operates in the catabolism of fatty acids in the liver [4]. Variants of PPARγ have been repeatedly associated with diabetes and variants of both genes have been implicated in the obese phenotype [5]. Uncoupling proteins belong to a family of proteins which can uncouple ATP-production from mitochondrial respiration, thereby dissipating energy as heat and affecting energy expenditure [6]. UCP1 is a factor in brown adipose tissue and responsible for the regulation of fatty acid combustion. UCP2 and 3 are expressed in muscle tissue and variants of these genes have been associated with obesity related parameters in several investigations [7]. The beta2 adrenergic receptor influences energy homeostasis by its impact on glycogen breakdown and stimulation of lipid mobilization. Two polymorphisms (Arg16Gly and Gln27Glu) in this gene have been linked to BMI and related variables in various studies [8]. A role in obesity is also discussed for the hormone sensitive lipase, an enzyme that catalyzes the rate-limiting step for triglyceride breakdown in fat cells [9]. TNFα is a modulator of gene expression in adipose tissue, impacts on insulin signaling and is an inducer of apoptosis of fat cells. A promoter polymorphism (–307 G/A) with potential functional relevance has repeatedly been shown to be relevant for body fat accumulation [10, 11].

The physiological role of APM1/adiponectin, another adipocyte-specific gene, has not yet been elucidated. However, polymorphisms in this gene have been associated with type 2 diabetes, with lower adiponectin levels in subjects with higher BMI [12] and with obesity risk [13]. The sorbin and SH3-domain-containing-1 gene (SORBS1) is the human homologue of the mouse gene SH3P12, which has been shown to be an important signaling molecule in insulin-stimulated glucose uptake. Recently population association studies revealed that the Ala-variant of a Tyr228Ala polymorphism of SORBS1 is a protective factor for both obesity and diabetes [14].

Material and methods

Subjects

The present study was performed as a case-control study embedded in the Heidelberg part of the European Prospective Investigation into Cancer and nutrition (EPIC). The EPIC-Heidelberg cohort comprises 25,544 participants from the city Heidelberg and its adjacent communities, with an age range from 35–65 years at recruitment in women and 40–65 years in men. At recruitment, detailed information on the participants was obtained by means of questionnaires, face-to-face interviews, and anthropometric measurements. Additionally, all participants were asked for a 24 ml blood sample which was obtained from 95.8 % of the cohort, aliquoted and stored at liquid nitrogen.

Additional personnel and technical capacities allowed us to store additional aliquots of blood which have not been used for the mentioned Europe-wide used aliquotation system, for the subgroup of subjects (n = 4000) participating between March 1998 and the end of recruitment (October 1998). Within this subgroup, all subjects with a body mass index (BMI) ≥ 35 kg/m^2, i. e., obesity grade II and III, were selected. Controls with normal weight (BMI 18.5 ≤25 kg/m^2) were identified from the same subgroup and matched by sex and 5-year age group. Thus, 154 obese subjects (96 female, 58 male) and 154 controls were included in the present study. Some basic sample characteristics are given in Table 1. Additionally, descriptive data on variables used in the adjustment models are presented. The daily duration (hours) of watching TV is used as a proxy for physical activity during leisure time.

Anthropometric and dietary assessment

Body weight and height were determined at recruitment by means of standardized procedures [15]. A validated food frequency questionnaire (FFQ) was applied to obtain information on the usual dietary intake over the last year [16, 17]. The completeness of the self-administered FFQ comprising 149 individual food items was checked at the participants' visit at the examination center by trained interviewers. Nutrient intake was calculated by

Table 1 Characteristics (Mean ± SD or %) of a random sample of obese subjects and normal-weight controls from the EPIC-Heidelberg study

Parameter	TotalWomen		Men			
	Cases (n = 154)	Controls (n = 154)	Cases (n = 96)	Controls (n = 96)	Cases (n = 58)	Controls (n = 58)
Age (years)	51.2±8.4	51.3±8.5	49.8±8.8	50.0±9.1	53.7±7.0	53.4±7.0
Weight (kg)	105.6±12.3	65.7±7.7	100.9±10.2	62.0±5.9	113.4±11.6	71.8±6.4
Height (cm)	166.1±8.2	168.9±7.8	161.8±5.5	165.1±6.0	173.2±7.0	175.2±6.2
BMI (kg/m^2)	38.2±2.8	23.0±1.6	38.5±3.0	22.7±1.7	37.7±2.4	23.4±1.2
WHR	0.93±0.11	0.83±0.09	0.87±0.07	0.79±0.06	1.04±0.08	0.91±0.06
Sports activity (%):						
no sports	55.8	30.5	60.4	30.2	48.3	31.0
0–2 h/week	29.9	36.4	25.0	38.5	37.9	32.8
> 2 h/week	14.3	33.1	14.6	31.3	13.8	36.2
Occupational activity (%):						
sedentary	40.9	65.6	35.4	63.5	50.0	69.0
light	48.7	29.9	57.3	34.4	34.5	22.4
moderate and heavy	10.4	4.5	7.3	2.1	15.5	8.6
Watching television: (h/day)	2.3±1.3	1.3±0.9	2.3±1.3	1.3±0.9	2.2±1.4	1.4±0.9
Energy and nutrient intake:						
Energy (kcal/d)	2012±704	1940±631	1802±552	1814±658	2360±791	2149±524
Fat (% energy)	35.3±4.9	33.4±5.3	35.9±4.9	34.3±5.0	34.1±4.7	32.0±5.6
n-6 PUFA[a] (% en.)	5.13±1.32	4.66±1.30	5.3±1.5	4.8±1.4	4.87±1.0	4.40±1.12
n-3 PUFA (% en.)	0.72±0.14	0.68±0.12	0.73±0.13	0.69±0.12	0.69±0.14	0.67±0.13
n-6/n-3 ratio	7.25±1.62	6.82±1.42	7.29±1.67	6.91±1.43	7.17±1.53	6.68±1.39
C18:2 (n-6) (% en.)	5.04±1.32	4.59±1.30	5.20±1.46	4.75±1.38	4.78±1.00	4.33±1.11
C20:4 (n-6) (‰ en.)	0.89±0.30	0.64±0.23	0.89±0.30	0.60±0.23	0.89±0.30	0.69±0.24

[a] Polyunsaturated fatty acids

means of the German food composition table BLS, version II.3 (BgVV, Berlin, Germany).

Genotyping

Genomic DNA from peripheral blood mononuclear cells was extracted using the QiaAmp Blood kit, in accordance with the provider's instructions (Qiagen). Polymerase chain reaction (PCR) amplifications were carried out in a volume of 10 µl in a Mastercycler gradient (Eppendorf). Final concentrations of reagents if not specified otherwise were as follow: 1 x Thermoprime Polymerase Buffer, 200 µM each dNTPs, 1.5 mM $MgCl_2$, 0.25 units Thermoprime^{PLUS} DNA polymerase (all from ABgene), 2.5 µM specific forward primer, 2.5 µM specific reverse primer, (for allele-specific PCRs: 1 µM internal control primer mix), and 20–30 ng genomic DNA. Table 2 summarizes the investigated polymorphisms, the primers, the references of publications from which the genotyping methods were adapted, the PCR conditions, and the methods of analysis for all genotyping methods performed. Allele-specific PCRs and PCR-Restriction Fragment Length Polymorphisms (PCR-RFLPs) were performed as indicated in Table 3. For PCR-RFLPs the PCR products were digested with the restriction enzymes (New England Biolabs) listed in Table 2 and the products were then resolved on agarose gels (Metaphor or Nusieve agarose, FMC BioProducts, Agarose, Life Technology). The gels were stained with ethidium bromide, visualized under UV light and photographed. The underlined bases in the primer sequences of some genes describe mismatches which create a restriction site for the respective restriction enzyme in the presence of one allelic variant.

Statistical analyses

Although for several (but not for all) genes the homozygous mutant variants were rare, a) the presence of any mutant allele as well as the separate effects of b) heterozygous and c) homozygous mutant variants were compared to the homozygous wild-type variant. The allele with the highest frequency was defined as the wild-type variant except for BAR-2. Since in this gene linkage disequilibrium between both selected polymorphic sites has been described, the risk-increasing alleles were defined as mutants. Because no effect of the PPARG2 C/T exon 6 polymorphisms was identified, a combination of both polymorphic sites of the PPARG2 gene was not considered. Dietary intake data were introduced as tertiles in the models.

Unconditional logistic regression analyses with

Table 2 Primer pairs, PCR conditions, and detection methods used in gene assays based on polymerase chain reaction

Gene and Polymorphism	Primers	PCR conditions	PCR-type, RE[1]	Product Size	Analysis
PPARA Leu162Val C/G at 484 in exon 5	5′-GACTCAAGCTGGTGTATGACAAGT(f) 5′-CGTTGTGTGACATCCCGACAGAAT _(r) [36]	95 °C 60 s, 30 x (95 °C 30 s, 62 °C 30 s, 72 °C 30 s)	PCR-RFLP *Hinf*I	117 bp	3.5 % Agarose G: 93 bp, 24 bp C: 117 bp
PPARG2 C/T exon 6	5′-GCCCAGGTTTGCTGAATG(f) 5′-TGAAGACTCATGTCTCTC(r) [37]	95 °C 60 s, 30 x (95 °C 30 s, 56 °C 30 s, 72 °C 30 s)	PCR-RFLP *Pm*lI	208 bp	3 % Agarose C: 158 bp, 50 bp T: 208 bp
PPARG2 Pro12Ala C/G	5′-TCTGGGAGATTCTCCTATTGGC(f) 5′-CTGGAAGACAACTACAAGAG(r) [38]	95 °C 60 s, 30 x (95 °C 30 s, 52 °C 30 s, 72 °C 30 s)	PCR-RFLP *Hha*I	154 bp	3 % Agarose Pro12 C: 154 bp Ala12G: 132 bp, 22 bp
UCP1 –3826 A/G	5′-CCAGTGGTGGCTAATGAGAGAA(f) 5′-GCACAAAGAAGAAGCAGAGAGG(r) [39]	95 °C 60 s, 30 x (95 °C 30 s, 60 °C 40 s, 72 °C 30 s)	PCR-RFLP *Bcl*I	279 bp	2 % Agarose A: 157 bp, 122 bp G: 279 bp
UCP2 45 bp exon 8 del/ins*	5′-CAGTGAGGGAAGTGGGAGG(f) 5′-GGGGCAGGACGAAGATTC(r) [40]	95 °C 60 s, 30 x (95 °C 30 s, 65 °C 30 s, 72 °C 40 s)		412 or 457 bp	2 % Agarose ins: 457 bp del: 412 bp
UCP3 –55C/T	5′-GAGCTATATTAAAGCACCCCGGGTCAAGAGGAC(f) 5′-TCTGCTGCTTCTGGCTTGGCACTGGTCTTATACACCC(r) [41]	95 °C 60 s, 30 x (95 °C 30 s, 65 °C 40 s, 72 °C 50 s)	PCR-RFLP *Sma*I	188 bp	3 % NuSieve Agarose C: 132 bp, 37 bp, 19 bp T: 169 bp, 19 bp
BAR-2 Arg16Gly G/A	5′-CTTCTTGCTGGCACGCAAT(f) 5′-CCAGTGAAGTGATGAAGTAGTTGG(r) [20]	95 °C 60 s, 30 x (95 °C 30 s, 56 °C 40 s, 72 °C 40 s)	PCR-RFLP *Bsr*DI	200 bp	3 % Metaphor Arg16: 130 bp, 56 bp, 14 bp Gly16: 108 bp, 56 bp, 22 bp, 14 bp
BAR-2 Gln27Glu C/G	5′-CTTCTTGCTGGCACGCAAT(f) 5′-CCAGTGAAGTGATGAAGTAGTTGG(r) [20]	95 °C 60 s, 30 x (95 °C 30 s, 56 °C 40 s, 72 °C 40 s)	PCR-RFLP *Fnu4H*I	200 bp	2.5 % Agarose Gln27C: 150 bp, 50 bp Glu27G: 200 bp
APM1 Gly15Gly T/G	5′-GAAGTAGACTCTGCTGAGATGG(f) 5′-TATCAGTGTAGGAGGTCTGTGATG(r) [42]	95 °C 60 s, 30 x (95 °C 30 s, 56 °C 40 s, 72 °C 40 s)	PCR-RFLP *Sma*I	372 bp	2 % Agarose T: 372 bp G: 216 bp, 156 bp
SORBS1 Thr228Ala A/G	5′-TACCTCACTGCATGCCCACTCTC(f) 5′-GACTGCTGGGAGGAGACATTCAGAA(r) [14]	95 °C 60 s, 30 x (95 °C 30 s, 65 °C 30 s, 72 °C 40 s)	PCR-RFLP *Kas*I	518 bp	1.5 % Agarose A: 518 bp G: 360 bp, 158 bp
HSL –60 G/C	5′-GAGGGAGGAGGGGCTATGGGTGTGGAC(f) 5′-GGGCGGGGCTGGCCTGCCCAGGGGGTA(r) [43]	95 °C 60 s, 30 x (95 °C 30 s, 61 °C 40 s, 72°C 50 s)	PCR-RFLP, *Nla*IV	209 bp	3 % Metaphor C: 209 bp G: 187 bp, 22 bp
TNFA –307 G/A	5′-ATAGGTTTTGAGGGGCATGG(f) 5′-AATAGGTTTTGAGGGGCATGA(f) 5′-TCTCGGTTTCTTCTCCATCG(r) [44]	95 °C 60 s, 10 x (95 °C 15 s, 65 °C 50 s, 72 °C 40 s) 20 x (95 °C 20 s, 59 °C 50 s, 72 °C 50 s)	Allele-specific	184 bp	2 % Agarose
leptin –2548 G/A	5′-TTTCCTGTAATTTTCCCGTGAG(f) 5′-AAAGCAAAGACAGGCATAAAAA(r) [3]	95 °C 60 s, 30 x (95 °C 30 s, 50 °C 45 s, 72 °C 45 s)	PCR-RFLP *Hha*I	242 bp	2 % Agarose A: 242 bp G: 181 bp, 61 bp

[1] *RE* Restriction endonuclease, *deletion/insertion

dummy variables for categorical terms were fitted to test the null hypotheses of no association between gene polymorphisms and obesity risk with or without adjustment for other predictors of obesity risk. The effects of the different genetic variants and the gene-diet interactions are presented as odds ratios (OR) with corresponding p-values. In principle, two different ways of how to introduce genes in the model were considered: first, each gene was evaluated separately (Table 4, crude), and secondly all genes were treated as independent factors in a common model (Table 4, models 1–3). After an explorative search for genes which might affect obesity risk in which all OR with a corresponding p-value of $p < 0.3$ have been taken into account, a score was gener-

Table 3 Distribution (n, %) of polymorphisms in selected candidate genes of obesity in a random sample of obese subjects and normal-weight controls in EPIC-Heidelberg

Gene, polymorphic site	Genotype	Cases (n = 154)		Controls (n = 154)		
		(n)	(%)	(n)	(%)	p[a]
PPARA Leu162Val	CC	136	88.9	138	92.0	0.459
	CG	16	10.5	12	8.0	
	GG	1	0.7	0	0	
PPARG2 C/T exon 6	CC	107	69.9	104	68.0	0.930
	CT	42	27.5	45	29.4	
	TT	4	2.6	4	2.6	
PPARG2 Pro12Ala C/G	CC	115	74.7	109	70.8	0.733
	CG	36	23.4	42	27.3	
	GG	3	1.9	3	1.9	
UCP1 –3829 A/G	AA	84	54.5	84	54.9	0.998
	AG	64	41.6	63	41.2	
	GG	6	3.9	6	3.9	
UCP2 exon 8 del/ins	del/del	88	57.1	78	51.7	0.197
	del/ins	58	37.7	57	37.7	
	ins/ins	8	5.2	16	10.6	
UCP3–55C/T	CC	82	53.2	91	59.5	0.300
	CT	59	38.3	55	35.9	
	TT	13	8.4	7	4.6	
BAR-2 Arg16GlyA/G1	AA	18	11.7	20	13.2	0.529
	G1A	79	51.3	85	55.9	
	G1G1	57	37.0	47	30.9	
BAR-2 Gln27GluC/G2	CC	43	28.1	53	34.6	0.467
	CG2	83	54.2	75	49.0	
	G2G2	27	17.6	25	16.3	
BAR-2 Arg16GlyG1/A and BAR-2 Gln27GluC/G2 combined	AACC	18	11.8	20	13.2	0.438
	AG1CC or AACG2	20	13.1	27	17.8	
	AAG2G2 or AG1CG2 or G1G1CC	61	39.9	63	41.4	
	G1G1CG2 or AG1G2G2	29	19.0	18	11.8	
	G1G1G2G2	25	16.3	24	15.4	
APM1 T/G exon 2	TT	124	80.5	126	82.4	0.788
	TG	27	17.5	23	15.0	
	GG	3	1.9	4	2.6	
Leptin –2548 G/A	GG	40	26.5	44	28.9	0.037
	GA	69	45.7	84	55.3	
	AA	42	27.8	24	15.8	
SORBS1 Thr228AlaA/G	AA	141	91.6	141	92.8	0.370
	AG	11	7.1	11	7.2	
	GG	2	1.3	0	0	
HSL –60C/G	CC	132	85.7	139	90.8	0.162
	CG	22	14.3	14	9.2	
	GG	0	0	0	0	
TNFA –307 G/A	GG	109	70.8	116	75.8	0.310
	GA	41	26.6	36	23.5	
	AA	4	2.6	1	0.7	

[a] chi^2-test

ated summing up the presence of several risk-increasing or risk-decreasing genetic variants, respectively, on the individual level.

Gene-diet interactions were first analyzed in the adjusted logistic regression model including all genes (≥1 mutant allele) and all possible interactions with fatty acid intake (Table 5). The results were used to select potentially existing interactions. Due to the low number of subjects per cell, logistic regression was re-run for each nutrient, including only identified interaction terms for this nutrient with the results depicted in Figs. 2a and 2b. Departure from Hardy-Weinberg equilibrium was tested by using a Chi-square test.

All analyses were performed with the statistics software SPSS, Version 10.0.1 (SPSS Inc., Chicago, IL, USA).

Table 4 Crude and adjusted[a] odds ratios for the isolated (i. e., each gene considered separately; crude) and the combined effect (models 1–3) of allelic variants of selected genes on obesity risk obtained in a random sample of obese subjects and normal-weight controls in EPIC-Heidelberg (n = 308)

Gen		Crude		Model 1[a]		Model 2[a]		Model 3[a]	
		OR	p*	OR	p*	OR	p*	OR	p*
PPARA Leu162Val	CC	1 (L/L)		1		1		1	
	CG	1.35	0.45	1.36	0.501	1.77	0.285	1.90	0.276
	GG	–	–	–	–	–	–	–	–
	≥ 1 G allel	*1.45*	*0.359*	*1.31*	*0.515*	*1.66*	*0.301*	*1.83*	*0.260*
PPARG2 C/T exon 6	CC	1		1		1		1	
	CT	0.91	0.702	0.91	0.775	0.84	0.674	0.93	0.866
	TT	0.97	0.969	1.35	0.767	0.79	0.828	0.79	0.850
	≥ 1 T allel	*0.91*	*0.711*	*0.97*	*0.935*	*0.97*	*0.934*	*1.04*	*0.936*
PPARG2 Pro12Ala C/G	CC	1		1		1		1	
	CG	0.81	0.431	0.83	0.614	0.79	0.570	0.62	0.307
	GG	0.95	0.948	0.74	0.791	1.58	0.713	1.53	0.768
	≥ 1 G allel	*0.82*	*0.443*	*0.80*	*0.512*	*0.71*	*0.388*	*0.59*	*0.236*
UCP1 –3826 A/G	AA	1		1		1		1	
	AG	1.02	0.947	0.90	0.697	0.91	0.742	1.01	0.986
	GG	1.00	1.000	0.58	0.402	0.66	0.589	1.13	0.884
	≥ 1 G allel	*1.01*	*0.950*	*0.97*	*0.882*	*0.92*	*0.745*	*1.00*	*0.995*
UCP2 exon 8 del/ins	del/del	1		1		1		1	
	del/ins	0.90	0.671	0.93	0.789	1.11	0.727	0.95	0.878
	ins/ins	0.44	0.077	0.39	0.068	0.62	0.405	0.41	0.194
	≥ 1 ins allel		*0.80*	*0.336*	*0.81*	*0.378*	*0.94*	*0.832*	*0.80* *0.480*
UCP3–55C/T	CC	1		1		1		1	
	CT	1.19	0.470	0.95	0.844	1.07	0.831	1.00	0.996
	TT	2.06	0.142	2.39	0.104	2.84	0.094	2.84	0.125
	≥ 1 T allel		*1.29*	*0.271*	*1.27*	*0.329*	*1.35*	*0.278*	*1.36* *0.329*
BAR-2 Arg16GlyG1/A and BAR-2 Gln27GluC/G2	AACC	1		1		1		1	
	AG1CC or AACG2	0.82	0.657	0.96	0.938	1.14	0.821	1.54	0.496
	AAG2G2 or AG1CG2 or G1G1CC	1.08	0.844	1.18	0.685	1.72	0.278	2.64	0.076
	G1G1CG2 or AG1G2G2	1.79	0.188	2.56	0.052	3.03	0.062	5.16	0.012
	G1G1G2G2	1.16	0.745	1.10	0.841	1.60	0.410	2.87	0.101
	≥ 2 mutant (G1, G2) allels			*1.36*	*0.237*	*1.36*	*0.236*	*1.77*	*0.072* *2.29* *0.019*
APM1 T/G exon 2	TT	1		1		1		1	
	TG	1.19	0.570	1.23	0.560	1.11	0.796	1.29	0.592
	GG	0.76	0.726	0.37	0.263	0.69	0.693	1.63	0.613
	≥ 1 G allel	*1.13*	*0.680*	*1.20*	*0.553*	*1.27*	*0.495*	*1.64*	*0.228*

Results

Results of genotyping the study population are given in Table 3. For most polymorphic sites, the number of homozygous mutant variants is low, except for BAR-2 (both polymorphic sites combined) and leptin. The allelic distributions were in Hardy Weinberg equilibrium for all of the variants investigated except for the BAR-2 Arg16Gly polymorphism (data not shown).

The unadjusted (crude) OR for the mutant alleles of the 11 genes indicate possible, but non-significant effects of polymorphisms of UCP2, UCP3, BAR-2, leptin, HSL and TNFA on obesity risk (Table 4). Considering all genetic variants as independent factors in one model (Table 4, model 1) did not substantially modify risk estimates as compared to the crude results; for all of the above mentioned polymorphic sites the p-values decreased, including the results for the APM1 polymorphism.

Two steps of adjustments were applied. First, parameters of energy intake and energy expenditure, i. e., sports activity, leisure-time activity (watching TV), and occupational activity which have been identified in the total EPIC cohort as significant predictors of relative body weight (BMI) at recruitment, were introduced in the model. Second, dietary fat and fatty acid intake (in % of energy intake) was considered as a modifier of obesity risk. Total fat intake as well as intake of n-6 polyunsaturated fatty acids (when compared to saturated, monounsaturated and n-3 PUFA) provided significantly increased OR (data not shown). However, when linoleic acid (C18:2, n-6) and arachidonic acid (C20:4, n-6) as the main representatives of n-6 PUFA were included into the model, total fat intake was no longer significant. The ratio of n-6 and n-3 PUFA as well as the dietary intake of conjugated linoleic acid (CLA) did not show an association with obesity (data not shown).

With further adjustments (models 2 and 3), the effects

Table 4 *continued*

Gen		Crude		Model 1[a]		Model 2[a]		Model 3[a]	
		OR	p*	OR	p*	OR	p*	OR	p*
Leptin –2548 G/A	GG	1		1		1		1	
	GA	0.90	0.710	0.91	0.746	1.04	0.915	1.12	0.768
	AA	1.93	0.057	2.21	0.034	3.35	0.005	3.53	0.009
	≥ 1 A allel	*1.13*	*0.633*	*1.14*	*0.633*	*1.38*	*0.307*	*1.47*	*0.275*
SORBS1 Thr228AlaA/G	AA	1		1		1		1	
	AG	1.00	1.000	1.29	0.593	2.07	0.169	1.64	0.391
	GG	–	–	–	–	–	–	–	–
	≥ 1 G allel	*1.18*	*0.695*	*1.25*	*0.613*	*1.98*	*0.178*	*1.59*	*0.401*
HSL –60C/G	CC	1		1		1		1	
	CG	1.66	0.165	1.85	0.122	1.75	0.212	1.46	0.420
TNFA –307 G/A	GG	1		1		1		1	
	GA	1.21	0.467	1.13	0.669	1.18	0.615	1.41	0.365
	AA	4.22	0.200	4.67	0.201	3.75	0.333	5.83	0.369
	≥ 1 A allel		*1.29*	*0.319*	*1.32*	*0.308*	*1.360.321*	*1.49*	*0.246*
Sports activity	0 h/week					1		1	
	1–2 h/week					0.38	0.003	0.34	0.004
	> 2 h/week					0.26	0.000	0.22	0.000
Occupational activity	sedentary					1		1	
	> sedentary					2.04	0.013	2.32	0.011
TV watching	0 h/day					1		1	
	h/day, continuous					2.04	0.000	1.78	0.000
Linoleic acid intake (tertiles)	low							1	
	medium							2.19	0.055
	high							2.61	0.014
Arachidonic acid intake (tertiles)	low							1	
	medium							2.10	0.060
	high							9.03	0.000

[a] *Model 1* combined model including all genes/polymorphisms; *model 2* adjusted for sports activity, occupational activity, daily hours of watching TV, and energy intake (not significant); *model 3* in addition to model 2, adjusted for linoleic acid and arachidonic acid intake (tertiles; in % of total energy intake); * Wald-χ^2 statistics.

observed for BAR-2 and leptin became statistically significant. Additionally, p-values for an effect of PPARA, PPARG2 Pro12Ala, and SORBS1 decreased. When model 3 was compared with an equally adjusted model for the isolated allelic variants (data not shown), the OR did not differ substantially from each other, but were more pronounced with all the genes included in one model.

Based on the results of model 3 (Table 4), for each subject scores for a) obesity risk-increasing polymorphisms (carrier of mutant alleles of BAR-2, leptin, UCP3, PPARA, APM1, TNFA) and b) risk-decreasing polymorphisms (carrier of mutant alleles of PPARG2 Pro12Ala and UCP2) were calculated. The OR for the risk of obesity by the number of genes with mutant alleles increasing or decreasing the risk are shown in Fig. 1. An excess of two or three genes with risk-increasing allelic variants over the risk-decreasing ones resulted in a significantly increased risk of obesity with OR of 4.06 and 7.75 ($p \leq 0.001$), respectively (Fig. 1).

The search for possible effects of interaction between genetic polymorphisms and fatty acid (linoleic acid and arachidonic acid) intake on obesity risk was carried out with the fully adjusted model 3 including all possible interaction terms. The OR for all interactions with $p < 0.20$ for the interaction term and $p < 0.10$ for the trend test are shown in Table 5. The results indicate an effect of interaction particularly for leptin, TNFA and PPARG2. For the mutations of these genes, Figs. 2a and 2b show the OR computed separately for each fatty acid. They suggest a substantial elevation of obesity risk with increasing intake of linoleic acid or arachidonic acid depending upon the presence of one or two mutant alleles. For example, Fig. 2b shows that carriers of genetic variants of PPARG2 and TNFA are at increased risk of obesity with increasing intake of arachidonic acid.

Discussion

It was only recently recognized that the adipose tissue operates as an endocrine organ which releases hormones in response to specific extra- and intracellular stimuli or changes in metabolic status and is more complex than previously thought [18]. Leptin, which acti-

Table 5 Effects of interactions between allelic variants of selected genes[a] and the dietary intake[b] of linoleic acid (C18:2 n-6) or arachidonic acid (C20:4 n-6) on obesity risk as assessed in a logistic regression model including all selected genes, all gene-fatty acid interaction terms, and with further adjustments[c]. Odds ratios (OR, and corresponding p-values) of those interactions are given for which the interaction term was $p < 0.2$ or the trend statistics was $p \leq 0.1$

Gene	p for interaction	Tertile of dietary fatty acid intake 1 OR	2 OR (p)	3 OR (p)	p for trend
		Linoleic acid			
PPARG2 C/t exon6 *(≥ 1T allel)*	0.166	1	18.90 (0.059)	4.91 (0.293)	0.064
APM1 T/G exon 2 *(≥ 1G allel)*	0.183	1	0.064 (0.077)	0.210 (0.262)	0.093
Leptin –2548 G/A *(≥ 1A allel)*	0.045	1	0.217 (0.231)	0.030 (0.014)	0.025
TNFA –307 G/A *(≥ 1A allel)*	0.142	1	2.99 (0.382)	10.04 (0.048)	0.064
		Arachidonic acid			
PPARG2 Pro12Ala C/G *(≥ 1G allel)*	0.080	1	8.66 (0.203)	50.91 (0.026)	0.062
HSL –60C/G *(≥ 1G allel)*	0.095	1	2.64 (0.569)	0.07 (0.075)	0.052
TNFA –307 G/A *(≥ 1A allel)*	0.095	1	7.82 (0.060)	8.97 (0.087)	0.071

[a] homozygous wild-type versus ≥ 1 mutant allele; [b] tertiles; in % of total energy intake; [c] adjusted for sports activity, occupational activity, daily hours of watching TV, and energy intake

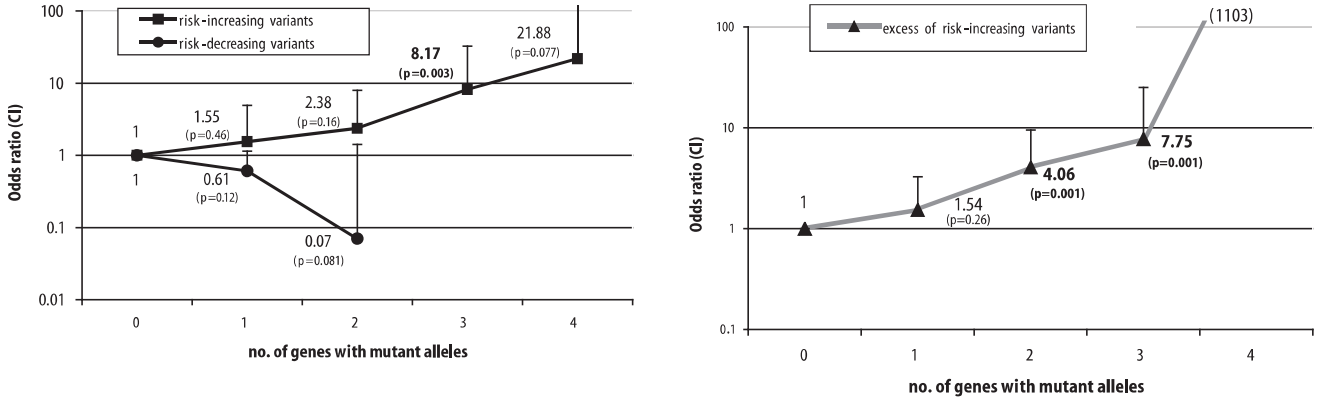

Fig. 1 Odds ratios (95 %-confidence intervals, p-values) for the risk of obesity by the number of genes with mutant alleles increasing the risk (PPARA, UCP3, BAR-2, APM1, leptin, TNFA) or decreasing the risk (PPARG2-Pro12Ala, UCP2) as obtained in a random sample of obese subjects and normal-weight controls in EPIC-Heidelberg (n = 308) (Calculation of the indices: value 1 for homozygous mutants of UCP2, UCP3, Leptin; for all other genes, value 1 is given for ≥ 1 mutant allele. Adjusted for sports activity, occupational activity, TV watching, energy intake, linoleic acid and arachidonic acid intake (tertiles, % of energy).
P for trend: $p < 0.001$ for risk-increasing variants, $p = 0.059$ for risk-decreasing variants, $p < 0.001$ for excess of risk-increasing variants over risk-decreasing variants.)

vates the parts of the hypothalamus and brainstem known to regulate appetite and energy expenditure, may be among the most prominent endocrine hormones secreted by adipocytes. In our analysis homozygosity for the A-allele of the leptin –2548 G/A polymorphism is significantly associated with an increased risk for obesity, which is evident in all applied models. Both leptin mRNA and plasma leptin correlate with BMI in humans and leptin variants have been associated with human obesity [3, 19]. Inconsistencies regarding the magnitude and direction of the association of this polymorphism with obesity may be due to population-specific differences in the degree of linkage disequilibrium of the investigated SNP with functional variants in the highly polymorphic 5'-flanking region [19].

An association with obesity was also evident for BAR-2, an important regulator of lipolysis in adipose tissue. The Glu27 and Gly16 alleles have been shown to be associated with obesity and altered lipolysis [8]. Our results, in which a higher proportion of obese are carriers of the Glu27 allele and Gly16 allele, are in concordance with several other studies in the literature [20–22], however, they are the opposite of a French study [23]. Striking divergence in the ethnic distribution of different

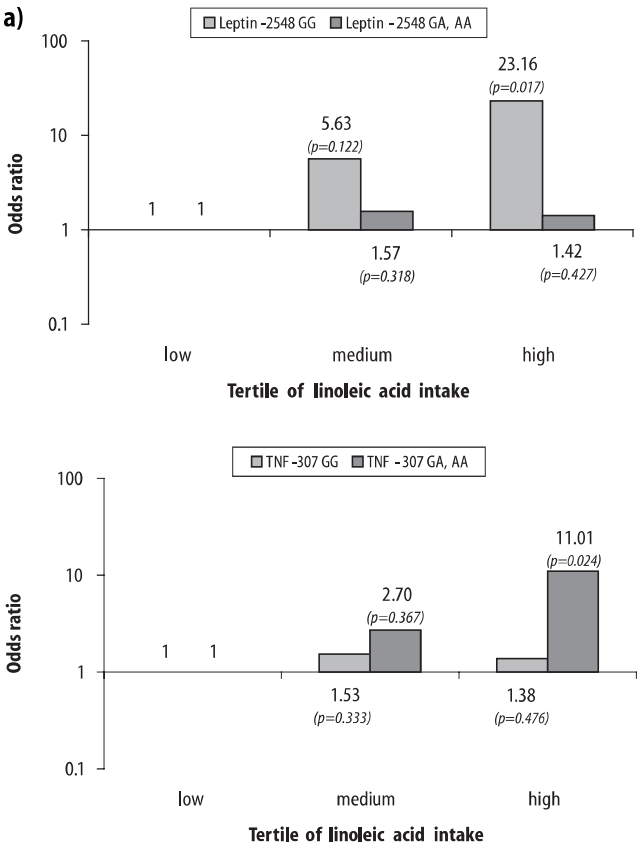

Fig. 2a Odds ratio[a] for the association of the dietary intake of linoleic acid[b] and obesity risk in a random sample of obese subjects and normal-weight controls in EPIC-Heidelberg (n = 308), differentiated into subgroups according to genotypes of (**a**, upper) the leptin polymorphism[c] –2548 and (**a**, lower) the TNFA polymorphism[c] –307 ([a] adjusted for all genes, sports activity, occupational activity, daily hours of watching TV, energy intake, arachidonic acid intake; [b] tertiles; in % of total energy intake; [c] homozygous wild-type versus ≥ 1 mutant allele)

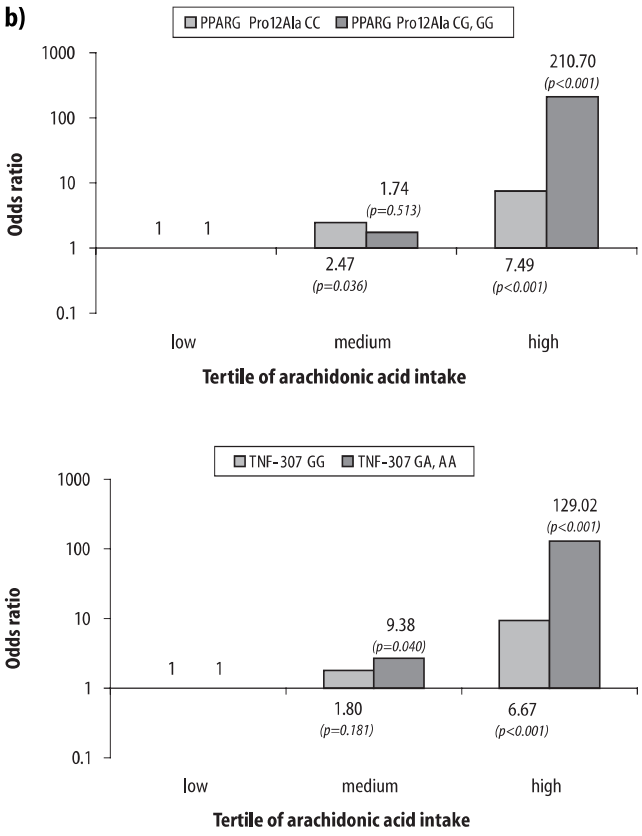

Fig. 2b Odds ratio[a] for the association of the dietary intake of arachidonic acid[b] and obesity risk in a random sample of obese subjects and normal-weight controls in EPIC-Heidelberg (n = 308), differentiated into subgroups according to genotypes of (**b**, upper) the PPARG2 polymorphism[c] Pro12 Ala and (**b**, lower) the TNFA polymorphism[c] –307 ([a] adjusted for all genes, sports activity, occupational activity, daily hours of watching TV, energy intake, arachidonic acid intake; [b] tertiles; in % of total energy intake; [c] homozygous wild-type versus ≥ 1 mutant allele)

BAR-2 haplotypes in four population groups as identified by Drysdale et al. [24] may explain the inconsistent results concerning the association with obesity, if linkage disequilibrium between the investigated polymorphisms and phenotypically associated SNPs varies across populations.

Tumor necrosis factor-alpha is expressed in adipocytes, and elevated levels of this cytokine have been linked to obesity and insulin resistance. In several studies the A-allele of the –307 promoter polymorphism has been found more frequently in obese than in non-obese individuals [10, 11, 25], which was not supported in another study [26]. We found no evidence for a strong effect of this variant on obesity, but some indication of a risk increasing effect of the A-allele which is stronger in women than in men (data not shown) as was also observed in other studies [10, 25].

PPARα and PPARγ are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors and prominent factors in fatty acid and lipid metabolism [4]. A polymorphism in PPARG2, Pro12Ala, was repeatedly associated with BMI, insulin sensitivity and other obesity related parameters, however, with inconsistent results regarding the amount and direction of the association [27]. In our study population the PPARA and PPARG2 variants had no prominent effect on obesity risk.

We found interesting evidence for an interaction of PPARG2 with dietary intake of PUFAs (Table 5). Individuals who were carrier of the PPARG2 Ala-variant and who consumed high amounts of arachidonic acid had a significantly higher risk of obesity than the carriers of the wildtype allele. A similar effect was seen for carriers of the T-allele for the second PPARG2 polymorphism and intake of linoleic acid.

The mechanisms that underlie the various gene-nutrient interactions we observed may include dietary influences on membrane fatty acid composition, transcriptional regulation and post-transcriptional processes.

PUFAs are known to be important direct regulators of gene expression, especially of genes involved in lipogenesis, triglyceride synthesis and fatty acid oxidation [28]. PUFAs of the n-6 and n-3 families coordinate an upregulation of lipid oxidation and down-regulation of lipid synthesis via their function as ligand activators of PPAR transcription factors and via their effect on sterol regulatory element binding protein-1 (SREBP1), respectively. If the sensible balance between lipogenesis and lipolysis is disturbed due to a combined occurrence of high intake of PUFAs and modified expression or activity of major transcription factors such as PPAR due to genetic polymorphisms, interactive effects on obesity risk may be the consequence. In fact, indications for gene-nutrient interactions at the PPARG2 locus were also recently reported by Luan et al. [29]. They found evidence for an interaction of the dietary polyunsaturated fat to saturated fat ratio and BMI in carriers of the Ala-allele. In longitudinal studies, carriers of the Ala-allele gained significantly more weight than Pro-homozygotes [30] which may argue for a greater sensitivity of mutant allele carriers to weight gain.

Also for leptin a statistically significant interaction with linoleic acid intake could be inferred from our data. Although biological interactions can not be concluded from statistical interaction, a combined effect of dietary fatty acids and variation in the leptin 5'flanking region may be plausible. This is supported by Reseland et al. [31] who reported a direct effect of PUFAs on leptin promoter activity. Moreover, interaction of arachidonic acid and linoleic acid intake and TNFA-mutant carrier status appears to modulate obesity risk. Emerging evidence on the interactions of arachidonic acid and cytokine metabolism and regulation of TNFα by fatty acids provide some biological rationale of the finding [32].

Due to the analysis here presented, interactions may also exist between linoleic acid intake and APM1 as well as arachidonic acid intake and HSL. No indication for interactions with intake of n-3 fatty acids was seen. This may be due to a generally low intake of n-3 PUFA in the whole study sample (Table 1) which does not allow a valid distinction between high and low intake levels. The same is true for dietary conjugated linoleic acid intake (data not shown).

HSL catalyzes the rate-limiting step in the intracellular hydrolysis of triglycerides and is an important regulator of free fatty acid release. For the potentially functional –C60G polymorphism an important role for insulin resistance and some association with higher BMI in women [33] was reported. In our study population this HSL variant appears not to be an important determinant of obesity risk. The observed interaction with dietary intake of arachidonic acid may be worth exploring in further projects.

In a comprehensive review, Dalgaard and Pedersen [7] recently summarized the role of common polymorphisms in the UCP genes in the pathogenesis of obesity as modest, which is consistent with the results of our analysis. The tendency, however, for a protective effect of the UCP2 ins/ins mutant and a risk increasing effect of the UCP3–55TT genotype is in line with recent publications [34, 35].

SORBS1 plays a key role in adipogenesis and the Thr228Ala polymorphism was shown to have a protective effect on the development of obesity in Taiwanese [14]. In Caucasians we found a much lower frequency of the mutant allele (0.12) and no association with obesity. No significant effect on obesity risk was seen for the APM1 polymorphism. The tendency of a higher BMI in G-allele carriers is supported by Stumvoll et al. [13] who reported a mild increase in obesity risk in G-allele carriers without family history of diabetes.

Besides some rare genetic mutations causal for human obesity, it is generally believed that multiple genes each with modest effects rather than one or a few major genes may underlie the common obesity phenotype. In one attempt to take into account the polygenic nature of obesity, the combined effect of risk-increasing and risk-decreasing allelic variants on obesity risk was explored. Although this approach may be criticized for adding up several small risks to a larger and significant risk, this may be a first step towards a model which takes the multigenic basis of common obesity into account. A significantly elevated risk of obesity was not only seen for the sum of risk-increasing variants, but even stronger effects were observed when the excess of risk-increasing over risk-decreasing variants was calculated (Fig. 1).

In summary, we found a statistically significant association of AA homozygosity of the leptin –2548 polymorphism and the G-allele carrier status of the BAR2 codon 16 and 27 variants with obesity in a subgroup of the Heidelberg EPIC population and some indications for an elevated risk due to the combined occurrence of variants which show only minor effects separately. Moreover, evidence for substantial interaction of genetic polymorphisms in PPARG2, leptin, TNFA (and possibly APM1, HSL) and dietary intake of n-6 fatty acids was presented which needs to be confirmed in a larger, independent population. The small number of cases and controls and consequently the limited statistical power, however, strongly argues for a careful interpretation of the results and repetition in a second, independent population. This also holds true for the observed gene-nutrient interactions, which were identified in an explorative approach rather than based on pre-defined hypotheses.

In general, we recommend to include dietary as well as other lifestyle information in association studies involving obesity relevant genes to enhance research on nutrient-gene and gene-environment interactions for a better understanding of the underlying factors regulating BMI.

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