Diabetologia (2006) 49: 1795–1805
DOI 10.1007/s00125-006-0291-7

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N. Siitonen · L. Pulkkinen · U. Mager · J. Lindström · J. G. Eriksson · T. T. Valle · H. Hämäläinen · P. Ilanne-Parikka · S. Keinänen-Kiukaanniemi · J. Tuomilehto · M. Laakso · M. Uusitupa · for the Finnish Diabetes Prevention Study Group

Association of sequence variations in the gene encoding adiponectin receptor 1 (*ADIPOR1*) with body size and insulin levels. The Finnish Diabetes Prevention Study

Received: 2 January 2006 / Accepted: 27 March 2006 / Published online: 25 May 2006

Abstract *Aims/hypothesis:* Adiponectin is a circulating peptide derived from adipose tissue. It mediates its insulin-sensitising and anti-atherogenic effects on target tissues through two known receptors, adiponectin receptors 1 and 2 (ADIPOR1; ADIPOR2), which are encoded by the genes *ADIPOR1* and *ADIPOR2*. Our aim was to study the association of *ADIPOR1* gene variations with body size and risk of type 2 diabetes in subjects with impaired glucose tolerance, who participated in the Finnish Diabetes Prevention Study (DPS). *Subjects and methods:* We selected seven single nucleotide polymorphisms (SNPs) of the *ADIPOR1* gene to perform association studies with anthropometrics and metabolic parameters at baseline, and with the risk of type 2 diabetes during the 3-year follow-up in the DPS study population. Both single SNP analysis and haplotype effects were studied. *Results:* Three out of seven markers studied (rs10920534, rs22757538 and rs1342387) were significantly associated with various body size measurements including weight, height, waist and hip circumference, sagittal diameter and body mass index. Furthermore, three markers (rs10920534, rs12045862 and rs7539542), of which two were different from those associating with body size, were linked to fasting and 2-h insulin levels, particularly in men at baseline. The haplotype analysis with five markers revealed seven major haplotypes in the DPS study population. The haplotype effects on body size measures were in line with those of single SNP analysis. However, none of the markers were associated with the risk of type 2 diabetes. *Conclusions/interpretation:* Our findings suggest that *ADIPOR1* has a putative role in the development of body size, and that traits for central adiposity and insulin resistance may be dissociated from each other.

Electronic Supplementary Material Supplementary Material is available in the online version of this article at http://dx.doi.org/10.1007/s00125-006-0291-7

N. Siitonen · L. Pulkkinen (✉) · U. Mager · M. Uusitupa
Department of Public Health and Clinical Nutrition,
Clinical Nutrition, and Food and Health Research Centre,
University of Kuopio,
Kuopio, Finland
e-mail: Leena.Pulkkinen@uku.fi
Tel.: +358-17-163609
Fax: +358-17-162792

J. Lindström · J. G. Eriksson · T. T. Valle · J. Tuomilehto
Department of Epidemiology and Health Promotion,
Diabetes and Genetic Epidemiology Unit,
National Public Health Institute,
Helsinki, Finland

H. Hämäläinen
Research Department, Social Insurance Institution,
Turku, Finland

P. Ilanne-Parikka
Finnish Diabetes Association
and Tampere University Hospital Research Unit,
Tampere, Finland

S. Keinänen-Kiukaanniemi
Department of Public Health Science and General Practice,
Oulu University Hospital, University of Oulu,
Oulu, Finland

S. Keinänen-Kiukaanniemi
Department of Sport Medicine, Oulu Deaconess Institute,
Oulu, Finland

J. Tuomilehto
Department of Public Health, University of Helsinki,
Helsinki, Finland

J. Tuomilehto
South Ostrobothnia Central Hospital,
Seinäjoki, Finland

M. Laakso
Department of Medicine, University of Kuopio,
Kuopio, Finland

Keywords Adiponectin · Adiponectin receptor · Adiposity · Body size · Genetic variation · Type 2 diabetes

Abbreviations ADIPOR1: adiponectin receptor 1 · ADIPOR2: adiponectin receptor 2 · CEU: CEPH Utah residents with ancestry from northern and western Europe · DPS: Finnish Diabetes Prevention Study · ESM: Electronic supplementary material · SNP: single nucleotide polymorphism

Introduction

Adiponectin is a circulating peptide belonging to the complement 1q family, and it is mostly expressed in and secreted from adipose tissue [1]. There are plenty of data suggesting that adiponectin acts as an insulin-sensitising and anti-atherogenic adipokine [2]. Plasma levels of adiponectin are decreased in individuals with obesity [3], type 2 diabetes [4] and coronary artery disease [5]. On the other hand, weight loss has been shown to result in increased plasma adiponectin levels in obese humans resulting in improved steady-state plasma glucose levels [6]. Furthermore, the role of adiponectin in health and disease is supported by the fact that the adiponectin gene, *ADIPOQ*, is located on chromosome 3q27, where genome-wide scans have mapped a susceptibility locus for type 2 diabetes and the metabolic syndrome [7, 8].

In 2003, Yamauchi et al. characterised two adiponectin receptors, adiponectin receptors 1 and 2 (ADIPOR1; ADIPOR2). These are encoded by the genes *ADIPOR1* and *ADIPOR2*, which are located on chromosomes 1p32.1 and 12p13.33, respectively [2, 9]. These receptors mediate fatty acid oxidation and glucose uptake by adiponectin, resulting in increased AMP kinase and peroxisome proliferator-activated receptor α ligand activities [2, 9]. ADIPOR1 is a receptor for globular adiponectin and is most abundantly expressed in skeletal muscle [9], and previous studies have shown that it is also expressed in bone-forming cells [10], while ADIPOR2 is mostly expressed in the liver, where the full-length adiponectin serves as a ligand [9]. Both receptors have also been shown to be expressed in pancreatic beta cells, where their expression is upregulated by free fatty acids resulting in synthesis of lipoprotein lipase [11]. Furthermore, it has been shown that adiponectin has no effect on insulin secretion in normal islets, but inhibits insulin secretion from islets in insulin-resistant mice [12]. In addition to ADIPOR1 and ADIPOR2, T-cadherin has been shown to bind hexameric and high-molecular-weight species of adiponectin, although the functional pathways are still poorly understood [13].

Since ADIPOR1 and ADIPOR2 mediate the effects of adiponectin on target tissues, they are considered to be strong candidates in the risk of type 2 diabetes [14–17]. Association studies of the *ADIPOR1* and *ADIPOR2* gene variations have revealed contradictory results in different populations. One study demonstrated positive association of both genes with IGT and type 2 diabetes [16], while the results of Wang et al. were negative for *ADIPOR1* [14] and the results of Hara and et al. were negative for both *ADIPOR1* and *ADIPOR2* [15]. In a French population, three *ADIPOR2* single nucleotide polymorphisms (SNPs) showed modest association with type 2 diabetes, while results with *ADIPOR1* SNPs were negative [18]. Furthermore, Stefan et al. reported recently that two SNPs upstream from the translational start codon of the *ADIPOR1* gene might be associated with liver fat content and insulin sensitivity in a German population [17].

The aim of this study was to examine the putative contribution of genetic variations of the *ADIPOR1* gene to body size characteristics and the risk of type 2 diabetes among subjects with IGT participating in the Finnish Diabetes Prevention Study (DPS) [19, 20].

Subjects and methods

The Finnish Diabetes prevention study

The Finnish Diabetes prevention study (DPS) was a randomised, controlled, multi-centre study carried out in Finland between 1993 and 2000. The DPS study design and the methods used have been reported in detail elsewhere [19, 20]. The main inclusion criteria were as follows: BMI >25 kg/m^2, age 40–64 years, IGT based on the mean values of two OGTTs. Altogether 522 individuals with IGT were randomised into either a control group or an intensive, individualised diet and exercise intervention group stratified according to the clinic, sex, and the mean plasma glucose concentration 2 h after an oral glucose load (7.8–9.4 or 9.5–11.0 mmol/l). Their mean BMI was 31.2±4.5 kg/m^2 and mean age was 55.3±7.1 years. The study protocol was approved by the Ethics Committee of the National Public Health Institute in Helsinki, Finland, and all the study participants gave written informed consent. DNA was available for 507 individuals (166 men and 341 women).

Measurements

A medical history was taken and a physical examination performed at baseline and at each annual follow-up visit [19]. In this study, measurements from the baseline to the 3-year examination were used, including height, weight, BMI, waist and hip circumference, WHR, sagittal and horizontal diameter of central body, and 2-h OGTT with glucose and insulin levels before (0 min) and after a 75-g glucose load (120 min) [19]. Plasma glucose was measured at each centre by standard methods. The serum insulin concentration was measured in a central laboratory by a radioimmunoassay method (Pharmacia, Uppsala, Sweden). Serum total cholesterol, HDL cholesterol, and triglycerides were determined using enzymatic assay methods [19]. The diagnosis of type 2 diabetes and other categories of glucose

intolerance were based on the criteria adopted by the World Health Organization in 1985 [21].

SNP analysis

The seven SNPs along the *ADIPOR1* gene were selected for association studies based on earlier studies [14–16] and the HapMap data [22, 23]. Genotyping of the SNPs (rs6666089, rs10920534, rs2275738, rs2275737, rs12045862, rs1342387 and rs7539542) spanning ~19 kb of the *ADIPOR1* gene was performed in 507 DPS subjects whose DNA was available. The SNP analyses were performed using a TaqMan allelic discrimination assay according to instructions provided by the manufacturer and an ABI PRISM 7000 sequence detector (Applied Biosystems, Foster City, CA, USA), except for the SNPs rs2275737 and rs2275738, which were genotyped using RFLP using *Mbo*II and *Msl*I restriction endonucleases, respectively.

Linkage disequilibrium coefficient of polymorphic sites and haplotype analysis

Linkage disequilibrium statistics were analysed by Haploview software [24] and THESIAS programme version 3 was used for haplotype analysis [25]. This programme can be used for haplotype-based association analysis in unrelated individuals and is based on the maximum likelihood model, linked to the SEM algorithm. THESIAS allows the simultaneous estimation of haplotype frequencies and of their associated effects on the phenotypes of interest.

Statistics

The association between the *ADIPOR1* SNPs and the clinical data was analysed using SPSS for Windows, Release 11.0.1. (SPSS, Chicago, IL, USA). Data are presented as means±SD unless otherwise stated. A p value <0.05 was considered significant. Normal distribution was tested with the Kolmogorov–Smirnov (Lilliefors) test, and appropriate transformations were used to achieve normal distribution when needed. The baseline differences in continuous variables between genotypes were evaluated with the univariate ANOVA, general linear model, with adjustments for age, sex, baseline BMI and baseline weight when appropriate. Homogeneity of variances was tested using Levene's test. Kruskal–Wallis test was used instead of ANOVA when homogeneity of variances and normality assumptions were not met even after variable transformations. Longitudinal changes in weight and waist circumference were examined using a general linear model repeated measures procedure with adjustments for age and sex, when appropriate, and the p values were corrected with Bonferroni's correction. Homogeneity of variances and covariances were tested using Box's M-test and Levene's test. The assumptions of the Box's M-test were not met when testing the effect of group on the 3-year development of weight in all subjects (p<0.001). In addition, when testing the genotype effects, in the case of waist circumference in all subjects and in the case of weight in all subjects and in the intervention group, the Box's M-test's assumptions were not met (p<0.001 for all). In the case of both waist circumference and weight in all subjects at all time-points and in the control group at three and two time-points, respectively, the assumptions of Levene's test were not met either (p<0.05 for both). However, we feel that the effect of the above mentioned fact on our results is only minor and in the absence of a corresponding non-parametric test, we present the results of the general linear model repeated measures analyses. The significance of differences in the 3-year incidence of type 2 diabetes between genotypes was analysed by the χ^2 test. Cox regression analysis was used to assess whether the SNPs predicted the development of type 2 diabetes.

Results

Allele frequencies and linkage disequilibrium patterns

All seven SNPs studied (Fig. 1) were in Hardy–Weinberg equilibrium, with the p values ranging between 0.079 and 0.928. The SNP pairs rs10920534/rs6666089 and rs2275737/rs2275738 were in complete linkage disequilibrium, both D' and r^2 values being 1.0 for both pairs (Table 1). Subsequently, the redundant SNPs (rs2275737 and rs6666089) were removed from the further analysis. Furthermore, the SNP rs2275738 was in strong linkage disequilibrium with rs1342387; D' and r^2 were 0.996 and

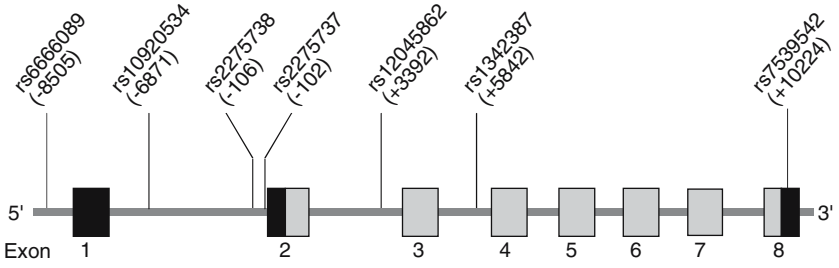

Fig. 1 Schematic presentation of the *ADIPOR1* gene and positions of the SNPs in relation to the translational initiation codon in exon 2. The distances of the SNPs were calculated according to Ensembl Human SNPview website http://www.ensembl.org/Homo_sapiens, accessed 1 March 2006). *Dark shading*, UTR; *light shading*, coding region

0.849, respectively (Table 1). Overall, the allele frequencies and linkage disequilibrium data did not differ much from those reported in the HapMap data for the Centre d'Etude du Polymorphisme Humain (CEPH) Utah residents with ancestry from northern and western Europe (CEU) population (www.hapmap.org, accessed 1 March 2006). The marker rs7539542 was in weak linkage disequilibrium with other SNPs studied; the strongest linkage was with the marker rs12045862 with D' 0.642 and r^2 0.37, while the corresponding values in the CEU population were 0.846 and 0.55, respectively.

Body size measurements and serum lipids

Baseline characteristics according to the SNP rs2275738 are shown in Table 2, and for four other SNPs, rs10920534, rs12045862, rs1342387 and rs7539542 as electronic supplementary material (ESM) (ESM Tables 1, 2, 3, and 4, respectively). In general, the markers rs10920534, rs2275738 and rs1342387 were most often significantly associated with several body size measurements, such as height, weight, waist and hip circumference, WHR, sagittal and horizontal diameter, and BMI either in all individuals or in men and women separately. The general trend seen was that homozygotes for the rs10920534-*T*, rs2275738-*G* and rs1342387-*G* were associated with higher body size measures than other genotypes.

In more detail, subjects homozygous for the rs10920534-*T*, rs2275738-*G*, and rs1342387-*G* alleles and the carriers of the rs12045862-*T* allele were tallest, (p=0.035, p<0.001, p<0.001 and p=0.031, respectively). The differences between genotype groups for the SNPs rs2275738, rs1342387 and rs12045862 were specifically seen in women (p=0.002, p=0.009 and p=0.018, adjusted for age, respectively) (Table 2, ESM Tables 1, 2). Furthermore, in the entire study population, weight differed significantly among the three genotype groups of the SNPs rs10920534, rs2275738 and rs1342387. The subjects with *T/T*, *G/G* and *G/G* genotypes had higher body weight than those with other genotypes (p=0.002, p=0.002 and p=0.008, adjusted for age and sex, respectively), while the BMI differed only for the corresponding genotypes of the first two markers (p=0.039 for both) (Table 2, ESM Tables 2, 3). With respect to fat distribution, subjects homozygous for the rs10920534-*T*, rs2275737-*G* and rs1342387-*G* alleles also had higher values for waist (p=0.003, p=0.012 and p=0.034, respectively), sagittal diameter (p=0.002, p=0.002 and p=0.008, respectively) and horizontal diameter (p=0.002, p=0.004 and p=0.001, respectively) compared to other genotype groups (adjusted for age and sex) (Table 2, ESM Tables 2, 3).

Genotype effects on body size measures of several SNPs were sex-specific. In women, significant differences according to rs10920534, rs2275738 and rs1342387 genotypes were seen in weight (p=0.049, p=0.020 and p=0.040, adjusted for age), hip circumference (p=0.029, p=0.040 and p=0.048) and sagittal diameter (p=0.015, p=0.004 and p=0.028, adjusted for age), respectively, and according to the rs10920534 and rs1342387 genotype a significant difference was seen in horizontal diameter (p=0.029 and p=0.032, respectively, adjusted for age). In men, significant differences according to genotypes of SNPs rs10920534 and rs2275738 were seen in BMI (p=0.030 and p=0.046, adjusted for age) and waist circumference (p=0.029 and p=0.037, adjusted for age), and according to genotypes of SNPs rs2275738 and rs1342387 in horizontal diameter (p=0.027 and p=0.016, respectively, adjusted for age). When adjustments for baseline BMI were made, differences in waist circumference and horizontal diameter were no longer significant (see Table 2, ESM Tables 2 and 3 for details).

There were no significant differences in serum total cholesterol, HDL cholesterol or triglyceride levels according to any studied *ADIPOR1* marker.

Changes in weight and waist circumference during a 3-year follow-up

To study further the possible *ADIPOR1* genotype effects on adiposity, repeated measures of weight and waist circumference from the baseline and years 1 to 3 examinations were analysed according to the genotypes of *ADIPOR1* markers. Since significant time–study group interaction was seen in waist circumference and weight (F=14.0,

Table 1 Pairwise linkage disequilibrium between the SNPs of the *ADIPOR1* gene is shown as D' and r^2 values

D'-values (above empty cells), r^2 values (below empty cells)							
SNP	rs6666089	rs10920534	rs2275738	rs2275737	rs12045862	rs1342387	rs7539542
rs6666089 *G/A* (0.252)		1.0	1.0	1.0	1.0	1.0	0.574
rs10920534 *C/T* (0.252)	1.0		1.0	1.0	1.0	1.0	0.574
rs2275738 *G/A* (0.439)	0.264	0.264		1.0	1.0	0.996	0.566
rs2275737 *G/T* (0.439)	0.264	0.264	1.0		1.0	0.996	0.566
rs12045862 *C/T* (0.304)	0.147	0.147	0.342	0.342		0.761	0.642
rs1342387 *G/A* (0.477)	0.308	0.308	0.849	0.849	0.231		0.349
rs7539542 *C/G* (0.328)	0.054	0.054	0.122	0.122	0.37	0.069	

Major/minor alleles and the frequencies of the minor alleles (parenthesis) of the corresponding SNPs are indicated next to each SNP identification number

Table 2 Baseline characteristics of the study population in the Finnish Diabetes Prevention Study divided according to the genotypes of rs2275738 of the *ADIPOR1*gene

rs2275738		A/A	A/G	G/G	*p*	*p*
Sex (male/female)		31/75	71/163	64/103	0.168[g]	
Age (years)		55.8±7.1 (106)	55.1±7.0 (234)	55.3±7.1 (167)	0.770[a]	
Weight (kg)	All	84.7±13.8 (106)	84.5±13.3 (234)	89.6±15.1 (167)	0.002[c]	
	Male	92.5±13.0 (31)	88.8±11.8 (71)	93.3±13.4 (64)	0.050[b]	
	Female	81.5±12.8 (75)	82.5±13.5 (163)	87.3±15.7 (103)	0.020[b]	
Height (cm)	All	164±9 (106)	166±9(234)	168±8(167)	<0.001[a]	
	Male	174±7 (31)	175±7 (71)	175±6 (64)	0.634[b]	0.628[e]
	Female	160±6 (75)	162±6 (163)	163±6 (103)	0.002[b]	0.016[e]
BMI (kg/m^2)	All	31.4±4.4 (106)	30.8±4.4 (234)	31.8±4.8 (167)	0.039[a]	
	Male	30.4±3.6 (31)	29.1±3.1 (71)	30.4±3.7 (64)	0.046[b]	
	Female	31.9±4.6 (75)	31.5±4.6 (163)	32.7±5.2 (103)	0.164[b]	
Waist circumference (cm)	All	101.2±11.3 (105)	99.7±10.2 (234)	103.3±11.6 (166)	0.012[c]	0.511[f]
	Male	106.0±9.8 (31)	101.8±9.1 (71)	105.4±10.0 (64)	0.037[b]	0.582[d]
	Female	99.2±11.3 (74)	98.8±10.5 (163)	102.0±12.3 (102)	0.113[b]	0.580[d]
Hip circumference (cm)	All	110.1±9.1 (106)	108.9±9.7 (234)	111.6±11.3 (167)	0.083[a]	
	Male	106.7±6.6 (31)	103.5±6.8 (71)	106.4±8.2 (64)	0.032[a]	
	Female	111.5±9.6 (75)	111.3±9.8 (163)	114.8±11.8 (103)	0.040[a]	
Waist-to-hip ratio	All	0.92±0.08 (105)	0.92±0.07 (234)	0.93±0.08 (166)	0.776[c]	0.842[f]
	Male	0.99±0.05 (31)	0.98±0.05 (71)	0.99±0.05 (64)	0.539[b]	0.961[d]
	Female	0.89±0.06 (74)	0.89±0.06 (163)	0.89±0.06	0.939[b]	0.857[d]
Sagittal diameter (cm)	All	24.7±3.1 (105)	24.5±3.0 (232)	25.5±3.2 (167)	0.002[c]	0.031[f]
	Male	25.2±3.5 (31)	24.5±3.2 (70)	25.2±2.8 (64)	0.233[b]	0.928[d]
	Female	24.5±2.9 (74)	24.4±2.9 (162)	25.7±3.5 (103)	0.004[b]	0.006[d]
Horizontal diameter (cm)	All	38.3±3.9 (105)	38.1±4.0 (232)	39.1±4.4 (167)	0.004[c]	0.082[f]
	Male	36.3±3.4 (31)	35.7±3.1 (70)	37.2±2.9 (64)	0.027[b]	0.149[d]
	Female	39.2±3.7 (74)	39.1±3.9 (162)	40.3±4.8 (103)	0.087[b]	0.374[d]
Fasting plasma glucose (mmol/l)	All	6.2±0.8 (106)	6.1±0.7 (234)	6.2±0.7 (167)	0.557[c]	0.862[f]
	Male	6.4±0.9 (31)	6.3±0.8 (71)	6.2±0.7 (64)	0.291[a]	
	Female	6.1±0.8 (75)	6.0±0.7 (163)	6.2±0.7 (103)	0.115[a]	
Two-hour plasma glucose (mmol/l)	All	8.7±1.5 (106)	8.9±1.5 (234)	9.0±1.5 (167)	0.267[a]	
	Male	8.3±1.8 (31)	8.9±1.6 71	8.9±1.5 (166)	0.246[a]	
	Female	8.8±1.3 (75)	9.0±1.5 (163)	9.0±1.5 (103)	0.531[a]	
Fasting serum insulin (mU/l)	All	14.5±8.2 (100)	14.3±6.9 (213)	15.6±7.5 (148)	0.179[a]	
	Male	16.82±9.5 (28)	15.9±8.9 (60)	14.9±7.5 (54)	0.654[b]	0.109[d]
	Female	13.6±7.6 (72)	13.7±5.8 (153)	16.0±7.5 (94)	0.037[b]	0.101[d]
Two-hour serum insulin (mU/l)	All	93.3±79.9 (100)	93.2±61.9 (210)	99.2±58.3 (148)	0.250[c]	0.550[f]
	Male	82.7±40.9 (28)	94.7±65.1 (60)	97.9±66.8 (54)	0.840[b]	0.674[d]
	Female	97.5±90.5 (72)	92.6±60.8 (150)	99.9±53.2 (94)	0.249[b]	0.475[d]

Data are means±SD (*n*). [a]Kruskal–Wallis test; [b]ANOVA for comparison among all three genotype groups, adjusted for age; [c]ANOVA for comparison among all three genotype groups, adjusted with age and sex; [d]ANOVA for comparison among all three genotype groups, adjusted for age and BMI; [e]ANOVA for comparison among all three genotype groups, adjusted for age and weight; [f]ANOVA for comparison among all three genotype groups, adjusted for age, sex and BMI; [g]χ^2 test

df=2.591, 1091.469, $p<0.001$ and $F=27.6$ *df*=2.453, 1054.890, $p<0.001$, respectively) in all subjects, genotype effects were analysed separately in intervention and control groups. In general, the genotype-specific differences at baseline with markers described above remained similar also during the follow-up period. In the entire study population, significant differences were seen again according to the genotypes of rs10920534, rs2275738 and rs1342387 in weight ($p=0.002$, 0.001 and 0.007, respectively) and waist circumference ($p=0.001$, 0.003 and 0.012, respectively) when adjusted for age and sex. An example of allele-specific effects on development of weight and waist circumference during a 3-year follow-up in all subjects and in intervention and control group separately is presented for the marker rs10920534 in Fig. 2. Significant overall differences for waist circumference and weight between all three genotype groups were seen in the control group ($p=0.006$ and $p=0.012$, respectively, adjusted for age and sex), but not in the intervention group. The time–genotype

interaction for both waist circumference and weight was not significant in either study group.

Glucose and insulin metabolism and risk of type 2 diabetes

Since adiponectin mediates its insulin-sensitising effects through the adiponectin receptors, we also determined the effects of different *ADIPOR1* genotypes on glucose and insulin concentrations and the risk of type 2 diabetes in the DPS population. Surprisingly, the two markers that were not associated with body size measures, rs12045862 and rs7539542, were significantly associated with fasting insulin levels at baseline in men ($p<0.001$ and $p=0.001$, adjusted for age and BMI, respectively); homozygotes for the major alleles (*C* for both) showed the highest values (ESM Tables 1 and 4; Fig. 3). These two markers were also in stronger linkage disequilibrium with each other than with any of the other SNPs studied ($D'=0.642$, $r^2=0.37$; Table 1). In addition, rs10920534 and rs12045862 were associated with 2-h insulin levels in men, with the *T*- and *C*-alleles showing the highest levels ($p=0.027$ and $p=0.001$, adjusted for age and BMI, respectively). In women, subjects with an rs12045862 *T*-allele had higher 2-h insulin levels ($p=0.029$, adjusted for age and BMI) than the other genotypes (Fig. 3). Markers rs12045862 and rs7539542 were also associated with fasting plasma glucose levels in men ($p=0.023$ and 0.033, respectively) with subjects carrying the major allele (*C* for both) having the highest levels. None of the markers were associated with the risk of type 2 diabetes in the entire DPS population or in the intervention and control group separately (data not shown).

Haplotypes

To study haplotype effects on body size and insulin levels at baseline in all subjects, we constructed five-marker haplotypes using THESIAS software. The covariate adjusted effects of each haplotype were compared to those of the most common haplotype (encoded by 11111) on quantitative phenotypes, and the haplotype frequencies were compared between subjects who developed type 2 diabetes during the 3-year follow-up and those who did not. This analysis revealed seven major haplotypes with frequencies over 0.03 in the DPS population (Table 3). Significantly higher weight ($p=0.0003$), BMI ($p=0.0008$),

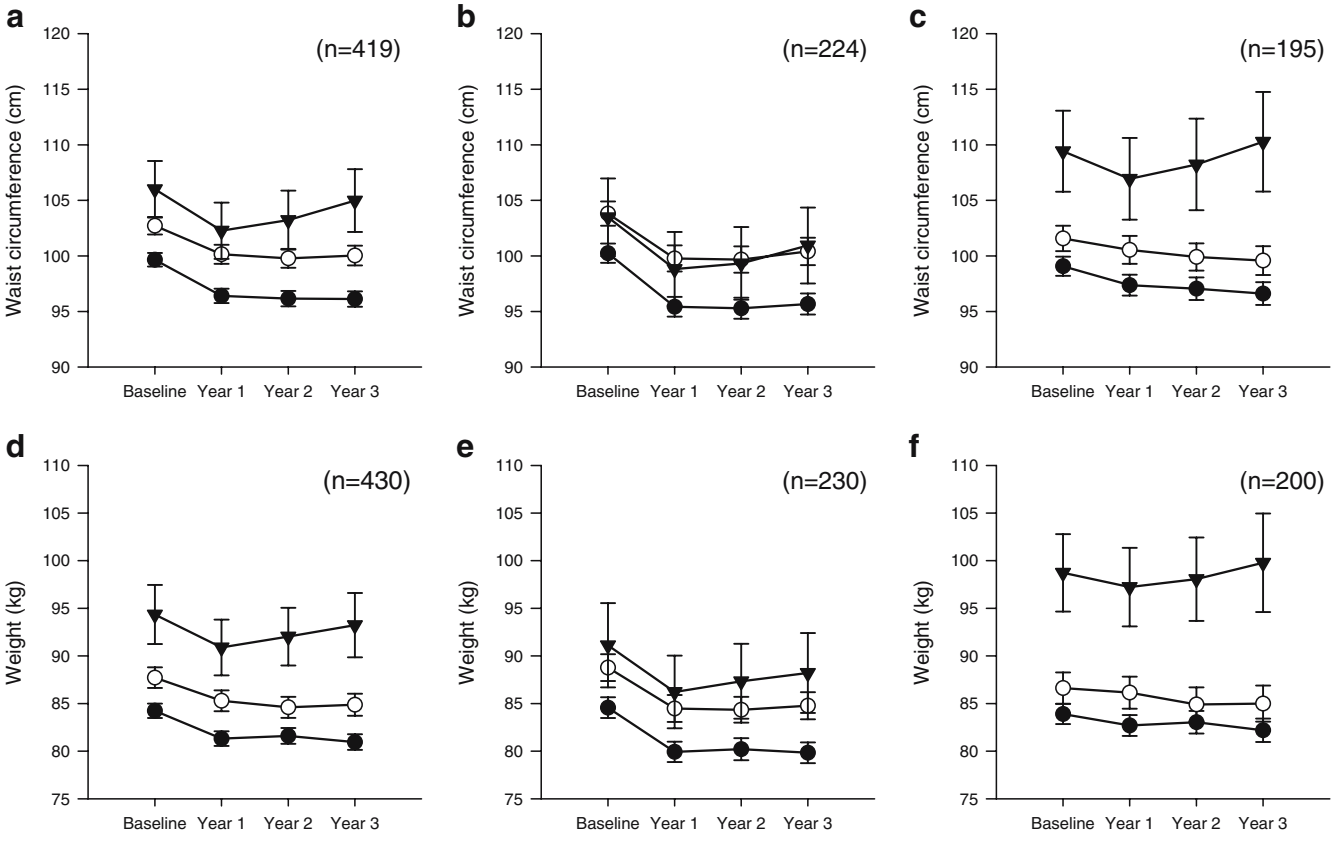

Fig. 2 Repeated measurements of waist circumference (**a**–**c**) and weight (**d**–**f**) according to the SNP rs10950452 in all subjects participating in the Finnish DPS (**a**, **d**), and in the intervention (**b**, **e**) and control croups (**c**, **f**). Data are means±SEM. $n=419$, $n=224$, $n=195$, $n=430$, $n=230$, $n=200$ (**a**–**f**, respectively). **a** $p=0.001$ for trend, $p=0.012$ C/C vs T/T, $p=0.002$ C/C vs C/T; **b** $p=0.082$ for trend, $p=0.048$ C/C vs C/T; **c** $p=0.006$ for trend, $p=0.008$ C/C vs T/T; **d** $p=0.002$ for trend, $p=0.004$ C/C vs T/T, $p=0.049$ C/T vs T/T, $p=0.050$ C/C vs C/T; **e** $p=0.172$ for trend; **f** $p=0.012$ for trend, $p=0.004$ C/C vs T/T, $p=0.018$ C/T vs T/T. All *p* values adjusted for age and sex. *Filled circles*, C/C; *open circles*, C/T; *inverted triangles*, T/T

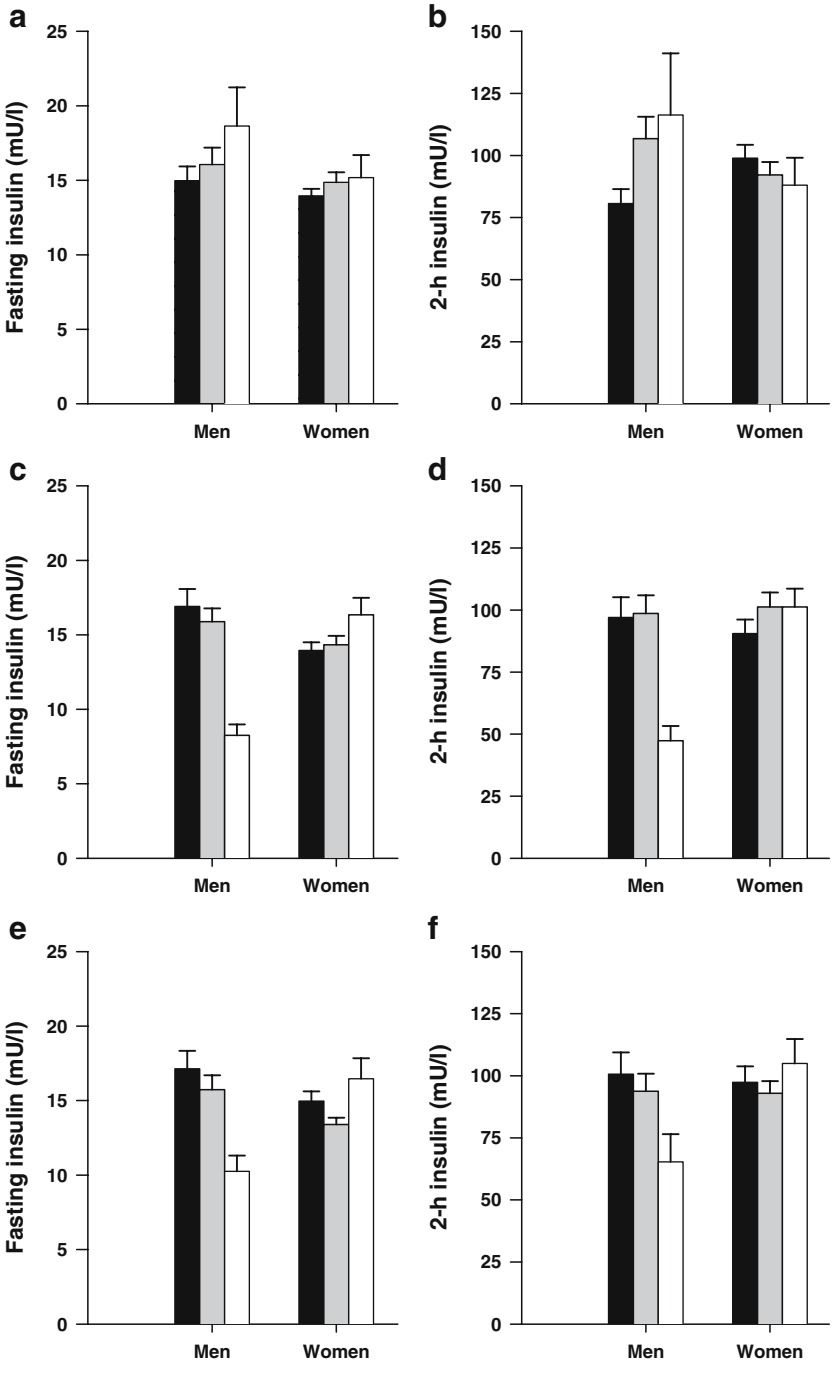

Fig. 3 Genotype effects of the markers rs10920534 (**a**, **b**), rs12045862 (**c**, **d**) and rs7539542 (**e**, **f**) on the fasting and 2-h insulin levels in men and women at baseline of the Finnish Diabetes Prevention Study. Data are means±SEM. Differences of genotype groups were analysed by ANOVA, adjusted for age and BMI (p=0.027 [Men] **[b]**, p<0.001 [Men] **[c]**, p=0.001 [Men] and 0.029 [Women] **[d]**, p=0.001 [Men] and 0.043 [Women] **[e]**) or for age and waist circumference (p=0.035 [Men] **[b]**, p<0.001 [Men] **[c]**, p=0.003 [Men] and 0.035 [Women] **[d]**, p=0.001 [Men] **[e]**) . *Filled bars* **(a–f)**, C/C; *shaded bars* **(a–d)**, C/T; *open bars* **(a–d)**, T/T; *shaded bars* **(e, f)**, C/G; *open bars* **(e, f)** G/G

horizontal diameter (p=0.0031) and hip circumference (p=0.0111) were seen in subjects harbouring haplotype 2 compared with those with haplotype 1. Furthermore, height was significantly greater in individuals harboring haplotype 3 (p=0.01147), and sagittal diameter and WHR were higher in those with haplotype 7 (p=0.0372 and 0.0240, respectively). In addition, lower WHR was seen in subjects with haplotype 5 (p=0.0455).

Regarding insulin levels, haplotype analysis was performed for all individuals instead of groups divided by sex. A trend for lower fasting insulin levels was seen in subjects with haplotype 5 when compared with those with haplotype 1. By using single SNP analysis, only the marker rs1342387 demonstrated a significant difference between genotypes when all subjects were analysed together, the lowest values being also among those who had the *A/A* genotype, indicating that the trend is the same for these two methods. None of the haplotypes were associated with type 2 diabetes in the entire DPS study population or in the intervention and control groups (data not shown).

Table 3 Association of the *ADIPOR1* haplotypes with body size measures and insulin levels at baseline in the Finnish Diabetes Prevention study

Number	Haplotypes	SNP					Frequency	Differences of body size measures and insulin levels according to the haplotypes									
		1	2	3	4	5		Height[a] (cm)	Weight[b] (kg)	BMI[a] (kg/m^2)	Waist[c] (cm)	Sagittal diameter[c] (cm)	Horizontal diameter[c] (cm)	Hip[c] (cm)	WHR[c]	Fasting insulin[c] (mU/l)	2-hour insulin[c] (mU/l)
1	11111	C	A	C	A	C	0.376										
2	22121	T	G	C	G	C	0.218	0.9153	3.3571	1.9806	0.2245	0.2316	0.7037	1.0196	0.0065	0.0667	1.9081
								0.0895	0.0003*	0.0008*	0.6664	0.1626	0.0031*	0.0111*	0.1780	0.9032	0.7349
3	12222	C	G	T	G	G	0.195	1.3335	−0.0696	−0.0289	−0.3253	0.1374	0.3810	0.6992	0.0080	0.0037	3.5118
								0.0114*	0.9496	0.9419	0.5449	0.4390	0.0900	0.0971	0.1023	0.9952	0.5703
4	12221	C	G	T	G	C	0.073	1.3413	−0.6374	−0.1583	−0.1278	0.0759	0.7176	1.3098	−0.0112	0.3000	1.2521
								0.0923	0.7017	0.7974	0.8505	0.7578	0.0541[d]	0.0133*	0.1445	0.7560	0.9146
5	11112	C	A	C	A	G	0.062	−0.3086	−0.1550	0.0709	−1.2762	0.4655	0.5460	0.9565	−0.0177	−2.4096	−8.8833
								0.7375	0.9436	0.9276	0.1353	0.1957	0.2481	0.2247	0.0455*	0.0654[d]	0.5150
6	12212	C	G	T	A	G	0.037	1.5659	0.7196	−0.2826	0.7291	0.4982	0.6265	−0.3340	0.0102	−1.6940	−8.2570
								0.1226	0.7401	0.7276	0.4300	0.1524	0.1536	0.6842	0.2534	0.3899	0.6174
7	22122	T	G	C	G	G	0.034	2.3264	−0.9706	−0.4055	2.0105	0.6908	−0.4244	−0.4735	0.0222	−0.7887	−15.588
								0.1180	0.7199	0.6939	0.1326	0.0372*	0.4401	0.5974	0.0240*	0.6197	0.5861

SNP1, rs10920534; SNP2, rs2275738; SNP3, rs12045862; SNP4, rs1342387; SNP5, rs7539542

p-values are presented below the value indicating the difference between the major haplotype (no. 1) and other haplotypes, *$p<0.05$. Covariates: [a]age and sex; [b]height, age and sex; [c]age, sex and BMI; [d] values with a trend for significance

Discussion

Both *ADIPOR1* and *ADIPOR2* are considered promising candidate genes for type 2 diabetes and metabolic syndrome. In this study, we examined the association of *ADIPOR1* variations with body size measures and the risk of type 2 diabetes in the Finnish DPS population, comprising subjects with IGT who are at high risk of developing type 2 diabetes. We found an association of *ADIPOR1* variation with various body size measurements and insulin levels, but none of the SNPs studied were associated with the risk of type 2 diabetes. This study is one in a series of studies to explore the genetic background and gene–lifestyle interactions in the development of type 2 diabetes and metabolic syndrome in a unique study population of DPS [26]. We chose to study *ADIPOR1* because of its ubiquitous expression and the contradictory results of previous studies: for example, Staiger et al. reported that expression of ADIPOR1, but not *ADIPOR2*, correlated with insulin secretion [27]. Tan et al. demonstrated regulation of *ADIPOR1*, but not *ADIPOR2*, by rosiglitazone in adipose tissue and skeletal muscle of diabetic subjects [28]. Genetic variation in *ADIPOR1* has been associated with insulin resistance and high liver fat, while results with *ADIPOR2* were negative [17]. In other genetic studies, however, variation in both *ADIPOR1* and *ADIPOR2* was associated with IGT and type 2 diabetes [16] or modest association with type 2 diabetes was shown for ADIPOR2 only [18].

Our results showed that SNPs of the *ADIPOR1* gene were not associated with type 2 diabetes in the DPS population although we found an association of two SNPs with fasting and post-challenge insulin levels in men. Our results are in accordance with the results of Wang and Hara and their co-workers [14, 15], which failed to demonstrate an association of *ADIPOR1* SNPs with type 2 diabetes in Caucasian families or in a Japanese population, respectively. Damcott et al. found an association between the two intronic *ADIPOR1* SNPs (rs2275737 and rs1342387) and type 2 diabetes in the Amish Family Diabetes Study [16]. However, they failed to associate any of the SNPs with body size. This controversy can be explained by differences in allele frequencies and linkage disequilibrium of *ADIPOR1* SNPs in different populations. In addition, our study population comprised overweight subjects with IGT and we studied the transition from IGT to type 2 diabetes during an average 3-year period, while Damcott et al. cross-sectionally compared two groups (type 2 diabetes +IGT vs normal glucose tolerant) separately for haplotype effects [16].

Height, like BMI, is a complex trait with high heritability [29, 30]. Environmental factors, such as improved nutrition, have led to a progressive increase in height, but the genetic contribution to height is still remarkable, and variations in a number of genes have been associated with height: *DRD2*, which encodes the dopamine D2 receptor [31]; *VDR*, which encodes the vitamin D receptor [32]; *COL1A1*, which encodes the α1-chain of type I collagen [33]; *ESR1*, which encodes the α-chain of the oestrogen receptor [34]; and *LHB*, which encodes the luteinizing hormone beta polypeptide [35].

In this study we show that several SNPs in *ADIPOR1* are associated with height in women with IGT. Since the difference in height according to *ADIPOR1* SNPs rs2275738, rs7539542 and rs12045862 in women remained significant after adjustment for age, it is likely that height achieved at puberty is affected rather than age-related decrease in height at older age (see Table 2, ESM Tables 1 and 4). Sex-specific differences in height could be because serum adiponectin levels are higher in women than in men, and these differences develop during the progression of puberty [36].

Recently, Berner et al. demonstrated transcription, translation, and secretion of adiponectin from primary human osteoblasts and showed that certain fatty acids enhanced adiponectin expression in bone cells. Both adiponectin receptors are also expressed in human osteoblasts [10], which may indicate paracrine or autocrine effects of adiponectin on bone cells. A functional role for adiponectin in bone remodelling was suggested by enhanced proliferation of a mouse osteoblastic cell line upon administration of recombinant adiponectin [10]. Expression of adiponectin and its receptors in bone-forming cells may provide a link between the *ADIPOR1* gene and variations in height seen in women participating in the DPS.

Interestingly, in the present study, several *ADIPOR1* SNPs were strongly and consistently associated with several body size characteristics; the most significant findings were seen in height, weight, BMI, WHR, waist circumference and sagittal diameter. The differences in waist circumference and weight between genotypes persisted throughout the 3-year study period as indicated for the marker rs10920534 in Fig. 2. The differences were more clearly seen in subjects who did not change their lifestyles. The time–genotype interaction for both waist circumference and weight was not significant in either study group. This indicates that the *ADIPOR1* genotype did not affect the outcome of the lifestyle intervention, but rather the differences between genotypes remained constant during the follow-up period. Results from haplotype analyses support the results of single SNP associations. One characteristic that was common to all haplotypes associated with greater body size measurements was that they contain *G*-alleles in the markers rs2275738 and rs1342387, indicating that results from single SNP analyses are in line with those of haplotype analysis. On the other hand, small body size seemed to be associated with haplotypes containing *A*-alleles in the corresponding positions, such as WHR with haplotype 5.

In the DPS population, the *G/G* genotype of rs2275738 and the *G/G* genotype of rs1342387 of the *ADIPOR1* gene were also associated with the indicators of central obesity, which are often linked to insulin resistance and metabolic syndrome irrespective of height or BMI [37]. In addition to the *T/T* genotype of rs10920534, which was associated with elevated 2-h insulin levels, the two other markers that did not associate with body size (rs12045862 and 7539542) were associated with fasting and 2-h insulin levels in men.

Stefan et al. [17] showed that the rs6666089 *A/A* genotype was associated with lower insulin sensitivity and higher liver fat. In our study, the *T*-allele of rs10920534 that was associated with higher 2-h insulin levels in men was in complete linkage disequilibrium with the *A*-allele of rs6666089, indicating that our results were in line with those of Stefan et al. [17]. Elevated fasting and 2-h insulin levels also reflect insulin resistance in persons with IGT [38]. Thus, these results suggest that the genetic background for central obesity (body size) and insulin sensitivity as measured by insulin levels may differ from each other. ADIPOR1 may mediate its effects on adiposity and insulin sensitivity separately through different adiponectin isoforms (full-length/globular) or an additional novel ligand for ADIPOR1 might exist. Furthermore, our results suggest strong sex-related differences in the effects of genetic variation on body size and insulin resistance, but this needs confirmation by larger studies.

In conclusion, these results, based on a carefully examined study population, demonstrate that several polymorphisms separately or representing specific haplotypes of the *ADIPOR1* gene are strong determinants of body size measures and adiposity in persons with IGT. We also suggest that body size (central obesity) and insulin resistance, as measured by insulin values, may be dissociated from each other according to genetic variation in the *ADIPOR1* gene.

Acknowledgements We thank M. Kiuttu, K. Kettunen and T. Onnukka for skillful laboratory assistance. This study was financially supported by grants from the Finnish Academy (40758 to M. Uusitupa; 38387 and 46558 to J. Tuomilehto), the EVO-fund of the Kuopio University Hospital (5106 and 5198 to M. Uusitupa), the Ministry of Education, the Yrjö Jahnsson Foundation, the Juho Vainio Foundation, the Finnish Diabetes Research Foundation and the Finnish Cultural Foundation of Northern Savo.

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