**LPIN2 is associated with type 2 diabetes, glucose metabolism and body composition**

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ABSTRACT

Objective: To identify type 2 diabetes (T2D) gene located at the chromosome 18p11.

Research design and methods: We investigated the region in a young genetically isolated population by genotyping 34 single-nucleotide polymorphisms (SNPs) in 78 cases and 101 controls. Two SNPs were selected and followed up in two cohorts. The first cohort came from general Dutch population. In this cohort, association with T2D was investigated using 616 T2D cases and 2890 controls; association with oral glucose tolerance test data was performed in 361 normoglycemic people. Association with fat distribution was studied in the second replication cohort, consisting of 836 people from the genetically isolated population.

Results: At the initial step, we found that the common C allele of SNP rs3745012 was associated with T2D (OR=2.01, P=0.03). This SNP is located at 3’ UTR region of LPIN2 gene, which is a plausible candidate for T2D and obesity. In the cohort from the general Dutch population, we demonstrated that rs3745012 interacts with body mass index in determination of T2D: while in subjects with high BMI the common C allele is associated with T2D, the same allele exhibits neutral or protective effect in lean subjects (P=0.05 overall effect, P=0.02 interaction). Most remarkably, rs3745012 strongly affected composite insulin sensitivity index (P=0.006 overall effect, P=0.004 interaction). In the second replication cohort, we found that allele C of rs3745012 increases trunk to legs fat mass ratio (P=0.001) and may affect other fat-related measurements.

Conclusions: rs3745012 SNP of the LPIN2 gene is associated with T2D and fat distribution.
The majority of type 2 diabetes (T2D) patients are obese and obesity by itself may be a cause of insulin resistance (1;2). Not only total fat mass, but also fat distribution is an important determinant of insulin resistance (3;4). Given the same degree of obesity, insulin sensitivity varies largely depending on visceral adipose tissue content (5;6). Abdominal obesity measures and visceral fat content may be better predictors of insulin resistance when compared to body mass index (BMI) (see e.g. (7)). Also abnormally decreased adipose tissue mass, such as observed in lipodystrophy, leads to severe insulin resistance in humans (8;9) and animal models (10-13). Several known genes for T2D are also implicated in lipodystrophy. Examples include peroxisome proliferator-activated receptor gamma (PPARG) (14), which plays a critical role in the differentiation of preadipocytes to mature fat cells (15). A common 12Pro polymorphism in this gene is associated with increased risk of T2D in general population (16;17) and rare deleterious variants lead to familial partial lipodystrophy (9;18). Another example is the lamin A gene, which is implicated in rare Dunnigan-type familial partial lipodystrophy (8). This gene also plays a role in T2D and related phenotypes (19-22). The LPIN1 gene was recently shown to be associated with serum insulin levels and BMI in a Finnish population (23). This gene appeared as a possible candidate involved in human glucose metabolism because it is one of the human homologues of mouse Lpin1 gene. Fdl mice, in which Lpin1 is deleted, have diminished adipose tissue mass and multiple pathologies including insulin resistance, fatty liver and progressive neuropathy of peripheral nerves (11;13). The protein encoded by Lpin1, Lipin, is required upstream of PPARG for normal adipocyte differentiation (24).

We reported a genome-wide scan using combined association and linkage test in a sample of T2D patients derived from a recently genetically isolated population from the Southwest of the Netherlands (25). Evidence for association with T2D was found for one region at chromosome 18p11 (P=0.001 at marker D18S63). The locus at 18p was originally found to be linked to T2D in families from Finland and Southern Sweden (26) and was confirmed in a second series derived from the Southwest of the Netherlands (27). In all three studies the effect of the 18p region was strongest in the obese sub-population.

One of the human homologues of mouse Lpin1 gene, LPIN2, is located in the 18p11 region. In this paper we examine the association between T2D and related traits and LPIN2 gene in three cohort.

RESEARCH DESIGN AND METHODS

Study population
Two series of patients and controls were studied. The first was derived from a young genetically isolated population located in the Southwest of the Netherlands. The characteristics of the population regarding linkage disequilibrium (LD) and drift are described elsewhere (28-30). The original sample was formed by 79 small families (117 patients) which could be traced to a common ancestor within 13 generations. The diagnosis of T2D was based on the American Diabetes Association criteria (31). The details of the recruitment procedure and analysis are described elsewhere (25). The study protocol was approved by the medical ethics committee of the Erasmus Medical
Centre, Rotterdam, and written consent was obtained from all participants.

In the present study, we used 78 patients, who were probands in our previous research, and were not or remotely (>10 meioses) related. The unrelated spouses of the T2D patients, together with the spouses of patients from other ongoing studies, were used as controls (N=101). This schema justifies the use of the sample as independent cases and controls.

There was no significant difference in sex ratio or age between patients and controls was observed (P>0.1). The BMI was significantly higher in patients (30.21) than in controls (27.53, P=0.03).

The second series was derived from the Rotterdam Study. This is a large cohort from outbred population, which is aimed to study prevalence, incidence and determinants of chronic disease in the elderly (32). The medical ethics committee of the Erasmus Medical Centre Rotterdam approved the study protocol. Baseline examination, including a detailed interview, physical examination, and blood sampling, were conducted between 1990 and 1993.

In the Rotterdam Study, 3506 people were genotyped. Diagnosis of T2D was made based on the use of anti-diabetic medication or random glucose level > 11.1 mmol/l, according to the previous work of Rietveld and colleagues (33). Of the investigated group, 616 were affected. The effect of sex was not significant. T2D people were on average older (73.46 y.o. vs. 68.09 in controls, P<0.001), had higher BMI (26.76 kg/m^2 vs. 26.14 in controls, P<0.01), and WHR (0.93 in cases and 0.90 in controls, P<0.001).

Within the Rotterdam Study, oral glucose tolerance test was performed for 361 normoglycemic people, thus making fasting glucose and insulin and postload glucose and insulin at 30 and 120 minutes available.

To study body fat distribution, we used data on 836 diabetes-free individuals from the family-based Erasmus Rucphen Family (ERF) study. The study protocol was approved by the medical ethics board of Erasmus MC Rotterdam. ERF consist of approximately 3,000 people, who are descendants of 22 couples living in the second half of the 19th century.

**Measurements**

In the Rotterdam study, at baseline examination, information concerning health status, and drug use was obtained using a computerized questionnaire. Height and weight were measured and BMI (kg/m^2) was calculated. Blood sampling and storage have been described elsewhere (34). Serum was separated by centrifugation and quickly frozen in liquid nitrogen. Baseline measurements were performed on nonfasting blood samples. Glucose levels were measured by the glucose hexokinase method in fasting serum and postload serum samples (35).

Glucose level was measured in mmol/l and insulin in mU/l. As indicator of insulin resistance we used HOMA-IR, as suggested by Matthews (36):

\[
\text{HOMA-IR} = \frac{\text{FGL} \times \text{FIL}}{22.5}
\]

As indicator of insulin sensitivity, which uses the data from the oral glucose tolerance test (OGTT), we used the index suggested by Matsuda (37):

\[
\text{ISI} = \frac{10000}{\sqrt{\text{FGL} \times \text{FIL} \times \text{MEAN_GL_IN_OGTT} \times \text{MEAN_IL_IN_OGTT}}}
\]

In the ERF study, anthropometric measurements and total-body imaging (using dual-energy X-ray absorptiometry,
DEXA) were done with participants in their underwear and without shoes. Using soft tape, with the subject in standing position, waist circumference was measured midway between the lower costal margin and the iliac crest, while hip circumference was taken at the hip’s widest diameter. Waist-to-hip ratio (WHR) was then computed. Height and weight were also measured. BMI was calculated as weight in kilograms divided by square of height in meters. All DEXA scans were performed in a calibrated ProdigyTM total body fan-beam densitometer and analysed with the enCORETM 2002 software V.6.70.021 (GE Lunar Corporation, Madison, Wisconsin). Other parameters estimated by DEXA include fat and lean mass measurements for total body, trunk and legs.

Genotyping
Genomic DNA for each individual was extracted from 10 ml of whole blood using the Puregene kit (Gentra systems, Minneapolis, MN) and protocol recommended by the manufacturer.

In the initial scan, all SNPs were genotyped using the Pyrosequencing technology according to the manufacturer’s instructions (Biotage, Uppsala, Sweden). For each assay, a standard PCR reaction was performed in a total volume of 10 µl using 20 ng genomic DNA, 10 pmol of each primer, one of which was biotinylated at the 5’ end, and AmpiTaq Gold DNA polymerase (Applied Biosystem, Foster City, CA). A full list of primer sequences can be found in Supplementary Table 1.

The biotinylated PCR product was bound to magnetic streptavidin-coated Dynabeads (Dynal, Oslo, Norway) by shaking at 65 °C for 15 min and then denatured in 0.5M NaOH. DNA bound to the Dynabeads was transferred between solutions using the magnetic PSQ Sample Tool (Biotage, Uppsala, Sweden). After washing to remove excess NaOH, the single-stranded template was hybridised to 15 nmol sequencing primer by incubating at 80°C for 2 min and cooling for 15 min. The pyrosequencing reaction was then carried out in a PSQ96 instrument (Biotage, Uppsala, Sweden) using the PSQ SNP Reagent Kit (Biotage, Uppsala, Sweden). Genotypes were analysed using the PSQ96 Analysis Software (Biotage, Uppsala, Sweden).

In the ERF and Rotterdam Study, genotyping was performed using Taqman allelic discrimination Assay-By-Design (Applied Biosystems, Foster City, CA) (38). Forward and reverse primer sequences and the minor groove binding probes were presented in Supplementary Table 2. We used the reverse strand design for SNP9 and SNP10. The assays utilized 5 ng of genomic DNA and 5 µl reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 minutes at 95°C preceded 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 50°C for 60 seconds. Allele-specific fluorescence was then analysed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA). Based on the analysis of 5% of blind duplicates, there was a 100% concordance in genotyping this polymorphism.

Expression analysis
To access functionality of the potential miR-371 target site at LPIN2 3’UTR we fused 780 bp of the LPIN2 3’UTR to a firefly luciferase reporter gene and
injected mRNA derived from this construct, together with a Renilla luciferase control reporter, in the zebrafish embryo. In this experiment, reporter signal is expected to decrease if miRNA-target interaction happens (39;40).

To study expression of LPIN2, whole blood samples were collected from 7 participant of the Rotterdam study. Ten milliliters of peripheral venous blood was drawn from each donor into heparin-containing Vacutainer tubes (BD Biosciences, Breda, The Netherlands). Selective lysis of red blood cells was achieved by the addition of 3 volumes of hypotonic buffer (150 mM ammonium chloride, 10 mM potassium bicarbonate, and 1 mM EDTA disodium salt) to a whole blood and incubation on ice for 20 min. Nucleated blood cells were then collected with centrifugation at 500g for 10 min at 4°C and washed twice with ice-cold phosphate-buffer saline prior to RNA extraction. Total RNA was extracted from leukocytes with RNABee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer’s protocol. RNA quality was determined with an A260/A280 ratio and capillary electrophoresis on an Agilent 2100 Bioanalyzer automated analysis system (Agilent Technologies, Palo Alto, CA). Hybridization of the RNA to Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Affymetrix Inc., Santa Clara, CA) was performed by the microarray core facility of the Erasmus MC Center for Biomics according to Affymetrix specifications. Background subtraction, probe signal summarization, and normalization were calculated according to the RMA algorithm using the R Bioconductor (41). LPIN2 gene is represented by 3 probesets on Affymetrix U133 plus2 microarray: probesets 202459_s_at and 202460_s_at target different regions of 3’ UTR; the probeset 244799_s_at targets the exon IV of LPIN2 transcript. The position of Affymetrix target sequences for LPIN2 gene on genomic alignment is shown at Supplementary Figure 1.

Statistical analysis
ANOVA and Wilcoxon tests were used for crude comparison of means between groups. For single SNPs, the crude comparison of frequencies between cases and controls was performed using the Fisher exact test. Adjusted analysis of relation between T2D status and genotypes was performed using linear regression analysis. In population-based cohorts, all analyses were performed using freely available R 1.9.0 software (http://www.r-project.org/).

In the family-based ERF study, we assessed association using variance-components model, as implemented in the SOLAR software (42). The null hypothesis of no association was formulated using the polygenic model with covariates. The hypothesis of association was obtained by adding SNP (coded as –1 for AA, 0 for AB and 1 for BB) as a covariate. To test significance of association, the Likelihood Ratio Test was performed: under the null, twice the difference between log-likelihood of these nesting models is distributed as chi-square with 1 degree of freedom. Total, trunk and leg lean tissue mass, lean mass index and the ratio between trunk and leg fat tissue masses were log-transformed. Total, trunk and leg fat tissue mass, fat mass index and ratio between trunk and leg fat tissue masses underwent square root transformation.
During the analysis, we considered nominal P-values ≤0.05 as significant.

RESULTS

Fine-mapping of 18p11 in a young genetically isolated population

With 26 single-nucleotide polymorphisms (SNPs), we covered the telomeric region 18p11.22-18p11.32 spanning from 0.2 to 12.3 Mb (Table 1). In total, 78 T2D patients and 101 controls were genotyped. The average heterozygosity of the SNP markers was 38% with a minimum of 6% and maximum 50%. The average coverage was 1 SNP per 465 kb, with a maximal gap of 1.3 Mb (Table 1, Supplementary Figure 2 A,B [available at http://diabetes.diabetesjournals.org]).

In our previous analysis the strongest association was found for D18S63 (position 3,428,540 in the Ensembl database) and D18S1105 (position 1,823,900), flanking the LPIN2 gene region. In the current SNP analysis, association was found for 2 SNPs, SNP9 and SNP12 (Table 1). For SNP9, after adjustment for sex and age, the common allele C was associated with Odds Ratio (OR) 2.01 (95% CI 1.09 – 3.71, P= 0.03); the frequency of C was 0.84 in cases and 0.73 in controls. For SNP12, the G allele was associated with T2D (OR= 1.66, P=0.03, after adjustment for age and sex).

The region showing association with T2D (SNP9–SNP12) contains 6 hypothetical and 7 known genes: EMLIN2, LPIN2, MYOM1, MRCL3, MRCLC2, TGIF, and DLGAP1. From these seven, LPIN2 was studied further because of the association of its’ 3’UTR SNP9 with T2D and the analogy to the mouse Lpin1, the gene involved in insulin resistance in the fjdl mouse (13; 43). According to the HapMap data, LPIN2 is included into a large block of LD; the boundaries of the block coincide well with the gene boundaries (Supplementary Figure 3). We typed 8 additional SNPs in the LPIN2 gene (Table 1, SNPs with study name starting with “LPN”; Supplementary Figure 2B). However, no strong association with additional SNPs was found.

Role of LPIN2 in the general population

We next conducted a study in a large outbred population, the Rotterdam Study. We have genotyped 3506 participants (616 with T2D) of the Rotterdam Study for SNP9 and SNP10. Both SNP9 and SNP10 were in Hardy-Weinberg equilibrium (P>0.1). Given the findings that the T2D locus at the 18p11 region interacts with BMI in other populations (25-27), we allowed for interaction in the analysis. We defined BMI groups as 5 quintiles (cut-off points at BMI 23.3, 25.0, 26.7, and 28.9 kg/m²), coded as 0, 1, 2, 3 or 4. The model consisted of this covariate, SNP9, their interaction, and additional covariates. The interaction of SNP9 with BMI was significant (P=0.02, Figure 1). Also the overall test of the effect of SNP9 and BMI was significant (P=0.05). Interestingly, while the common C allele is the risk allele at high BMI (OR = 1.38, 95% CI 0.97 – 1.94 in upper quintile; frequency of C in cases 0.79, in controls – 0.74), the situation is reverse at low BMI (Figure 1). The ORs and case/control frequencies observed in the highest BMI quintile are similar to that seen in the genetically isolated population among predominantly obese patients (mean BMI of 30.21 vs. 26.76 kg/m² in participants of the Rotterdam Study).

We studied the association of SNP9 with early T2D pathology in 361 normoglycemic subjects who underwent
oral glucose tolerance test (descriptives shown in Supplementary Table 3). For fasting glucose, insulin and insulin resistance (HOMA-IR), the results, though insignificant, point in the same direction as the analysis of T2D: the common SNP9C allele is a risk allele at high BMI while in those with low BMI it appears to decrease fasting glucose, insulin and insulin resistance (Figure 2).

We found a significant effect of SNP9 on glucose (P=0.04) and insulin (P=0.02), when studying the area under the curve, and on the insulin sensitivity index (ISI) (P=0.006) (Figure 2). For all three measures, interaction with BMI was significant. Consistent with our previous findings on T2D, the common SNP9C allele was associated with increased risk of early pathology in participants with high BMI, while the effect was reversed in those with low BMI (Figure 2).

**SNP9 and body composition**

In the outbred population drawn randomly from the Rotterdam Study, we found that SNP9 genotype was associated with BMI, when adjusting for sex and age. The carriers of the common C allele, which was associated with T2D in the genetic isolate, had a significantly increased BMI when compared to TT homozygotes (difference of 0.54 kg/m², P=0.03). At the same time, the carriers of C had lower waist to hip ratio (WHR) when compared to TT homozygotes (P=0.02), suggesting an atypical fat distribution in TT genotype as BMI and WHR are usually positively correlated.

We further studied association between LPIN2 and body composition in 836 diabetes-free participants of the family-based cohort derived from the EFR study (Table 2). The family structure was adjusted for within variance components framework. Similar to the findings in the Rotterdam Study, when adjusting for age and sex, the SNP9C was associated with an increased BMI, albeit only marginally significantly (effect of 0.51 kg/m², P=0.08). In ERF, SNP9C increased both waist (by 1.67 cm, P=0.03) and hip circumference (by 1.03 cm, P=0.06).

In the analysis of DEXA data we found that SNP9C increased total fat mass by 1.1 kg (P=0.05). This happened almost exclusively through increase in trunk fat mass (by 0.9 kg, P=0.01). A strong positive effect of SNP9C was observed on various adiposity indexes: fat mass index (P=0.02), ratio of total fat to total tissue (P=0.01), ratio of trunk fat to total tissue (P=0.001) and ratio of trunk to legs fat mass (P=0.001) (Table 2).

After adjustment for trunk to legs fat mass ratio, the association of SNP9C with waist circumstance, fat mass, trunk fat mass, fat mass index, ratio of total fat to total tissue and the ratio of trunk fat to total tissue became insignificant, suggesting that association to these variables can be explained by the trunk to legs fat ratio. The same effect was observed when adjusting for the trunk fat to total tissue ratio. These analyses indicate that trunk to leg fat (or trunk fat to total tissue) mass ratio may be the most important endophenotype associated with SNP9 and T2D.

**Potential functionality of SNP9**

The region containing SNP9 is a potential target site for microRNA miR-371 (Supplementary Figure 4). Perfect matching of the region to the first 8 bases of the miRNA – “seed” sequence – is a strong predictor for regulation of LPIN2 by miR-371 (44). Although the target site is not conserved beyond primates, the nature of the SNP suggests conservation
at the RNA level: change of the common variant (C) sequence to the rare variant T only partially affects potential miRNA:mRNA interaction due to possible G:U pairing in the RNA duplex. Whether G:U pairing is tolerated in the seed sequence is not clear and may vary between miRNAs (39;45;46). However, the reporter signal did not decrease after co-injection of a synthetic miR-371 duplex as would be expected for a true miRNA-target interaction (39;40).

We studied expression of LPIN2 in seven healthy individuals from Rotterdam study. Four of these were homozygous for the SNP9C, and three were C/T heterozygotes. Using exact Wilcoxon’s non-parametric test, we showed that the probesets targeting 3’UTR did not differ in expression between T carriers and non-carriers (P>0.2). However, the probeset 244799_s_at, located in exon IV, did show marginally significant (P=0.06) down-regulation in the carriers of the T allele.

**DISCUSSION**

In our previous study in a young genetically isolated population, we found evidence for a type 2 diabetes locus at chromosome 18p11. This region was also previously reported by Parker et al (26) in a Scandinavian study and van Tilburg and colleagues (27) in the general Dutch population. Both studies suggested that the effect of the locus is modified by body mass index.

In the fine-typing of the 18p11 region with 34 SNPs, the association was found to be confined to the LPIN2 gene. In a small series of cases and controls, nominally significant association was found between T2D and SNP9 (rs3745012), located in 3’UTR region of the LPIN2 gene. We extended the analysis of association between LPIN2 and T2D in an independent series of patients from a large population-based outbred cohort, the Rotterdam Study. In this outbred population, we observed interaction between SNP9 and BMI. Consistent with our findings in the genetically isolated population, in which patients had a high BMI, SNP9C allele was associated with T2D in obese participants. In the Rotterdam Study SNP9 was also associated with BMI and early T2D pathology measured using OGTT. Lastly, in a family-based study conducted in the genetically isolated population we demonstrated that SNP9C is associated with increased adiposity indexes, most notably trunk fat. Together, these studies provide evidence that the SNP9C allele may be a thrifty allele (47;48), i.e., it is common and increases obesity and risk of T2D in obese subjects.

The issue of multiple testing is worth consideration when judging the results of our study. For example, in the first stage we tested 34 SNPs and found two to be associated to T2D with nominal P<0.05. When corrected for multiple testing, these results would have become insignificant. However, in our study we did not rely on experiment-wise significance achieved at any stage. As suggested by others (49), we attempted to replicate our finding: in stage one, we selected SNP9, and followed it up in two other cohorts, achieving positive results. Such replication procedure strongly reduces the chance of a false-positive finding (49).

LPIN2 gene is one of the three human homologues of the mouse Lpin1 gene, which is responsible for the lipodystrophy in fld line (13), which, among other traits, is characterized by severe insulin resistance. The product of Lpin1 is required upstream of PPARG for normal adipocyte differentiation (24).
The effects of increased Lpin1 expression on insulin resistance are opposite, depending on the tissue. Whereas Lpin1 overexpression in muscle produces obesity-associated insulin resistance, enhanced Lpin1 expression in mature adipocytes improves insulin sensitivity (43). A recent study of human homologue of mouse Lpin1 gene, LPIN1, has shown its potential involvement in glucose metabolism (23).

LPIN2, being a member of the same lipin family as the LPIN1 and bearing notable sequence similarity to both mouse Lpin1 and human LPIN1, is therefore a plausible candidate gene for T2D and body composition. Human LPIN2 contains 20 exons and spans for approximately 115kb. The deduced LPIN2 cDNA contains 6245 nucleotides. A nuclear localization signal is located close to N-terminus and is highly conserved among Lipin proteins. LPIN2 is ubiquitously expressed in different tissues including skeletal muscle (50).

The most important question is how SNP9 may be related to the risk of T2D. SNP9 is a non-coding polymorphism located in the 3’UTR of the gene at the third nucleotide downstream of the stop codon (Supplementary Figure 4). Strong computational evidences suggested that the region containing SNP9 is a potential target site for microRNA miR-371. However, our experiments on co-expression of luciferase reporter and miRNA do not reveal detectable interaction between miR-371 and LPIN2 3’ UTR. We therefore have to conclude that, in line with recent observations (46), perfect seed matching is not sufficient to confer regulation in case of miR-371. Alternatively, our heterologous expression system may be not sensitive enough to detect possible subtle effects of miR-371 on expression of LPIN2.

In our limited expression experiments, we observed increased levels of LPIN2 expression in people homozygous for the “risk” C alleles. Although these results reach only marginal significance (P=0.06), we may hypothesize that SNP9 polymorphism (or other LPIN2 polymorphism in LD with SNP9) leads to altered expression of the LPIN2 gene. This, by analogy with the mouse model, may lead to (possibly obesity-induced) T2D. Further investigation, including larger expression, and protein, studies are required to establish the link between LPIN2, obesity and type 2 diabetes.

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44. Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120:15-20, 2005
Table 1. Association between SNP located in chromosome 18p11 region and type 2 diabetes in the genetically isolated population.

<table>
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<th>Study name</th>
<th>dbSNP name</th>
<th>Position</th>
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<th>Controls</th>
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Significant P-values (≤0.05) and odds ratios (OR) are indicated in bold.

P-values comes from the Fisher Exact Test

Study name starting with “SNP”: SNPs typed in the first batch; starting with “LPN”: SNPs typed in the second batch

Position – in base pairs, as given in Ensembl database

OR – odds ratio from allelic 2x2 table, the allele listed first in “Change” column is used as a reference
**Table 2.** Association between SNP9C and DEXA body measurements in the genetically isolated population. Analysis is adjusted for sex, age, age$^2$ and family structure.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean (±S.D.)</th>
<th>Heritability</th>
<th>Effect of SNP9C</th>
<th>%Variance explained</th>
<th>P-value</th>
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<tr>
<td><strong>Weights, kg</strong></td>
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<td>Lean</td>
<td>46.64 (10.26)</td>
<td>0.52</td>
<td>-0.27 kg</td>
<td>0.12</td>
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<td>Fat</td>
<td>24.28 (9.26)</td>
<td>0.27</td>
<td>1.1 kg</td>
<td>0.46</td>
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<tr>
<td>Trunk Fat</td>
<td>13.98 (5.54)</td>
<td>0.21</td>
<td>0.92 kg</td>
<td>0.78</td>
<td><strong>0.012</strong></td>
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<td>Trunk Lean</td>
<td>23.30 (4.80)</td>
<td>0.42</td>
<td>-0.09 kg</td>
<td>0.05</td>
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<td>Leg Fat</td>
<td>7.53 (3.46)</td>
<td>0.45</td>
<td>0.13 kg</td>
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<td>Leg Lean</td>
<td>14.80 (3.66)</td>
<td>0.57</td>
<td>-0.12 kg</td>
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<td>9.01 (3.59)</td>
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<td>0.05 kg/m$^2$</td>
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<td>Fat/Tissue</td>
<td>0.34 (0.09)</td>
<td>0.30</td>
<td>1.22 %</td>
<td>0.80</td>
<td><strong>0.013</strong></td>
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<tr>
<td>Trunk Fat/Tissue</td>
<td>0.19 (0.05)</td>
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<td>1.03 %</td>
<td>1.35</td>
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<td>Trunk Fat/Leg Fat</td>
<td>2.02 (0.75)</td>
<td>0.54</td>
<td>0.14</td>
<td>1.35</td>
<td><strong>0.001</strong></td>
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Heritability – as reported by SOLAR

P-values ≤0.05 are indicated in bold

LMI – lean mass index, kg/m$^2$

FMI – fat mass index, kg/m$^2$
Legends to Figures

Figure 1. Effect of SNP9C allele (adjusted for age, age^2, sex and waist-to-hip ratio) on the risk of type 2 diabetes by BMI quintiles. Odds ratio and 95% confidence interval are shown by BMI quintile. Line indicates the odds ratios expected from the model of additive effect of SNP9C in linear interaction with BMI quintile. P corresponds to the P-value of the test of significance of SNP9 effect and its interaction with BMI (2 degrees of freedom). P_{int} corresponds to the P-value of the test of interaction (1 degree of freedom).

Figure 2. Effect of SNP9C allele (adjusted for age, age^2, sex and waist-to-hip ratio) on glucose and insulin measurements by BMI quintiles. Mean effect and 95% confidence interval are shown by BMI quintile. Line shows the mean expected from the model.
Figure 1

\[ P = 0.05 \]
\[ P_{\text{int}} = 0.02 \]
Figure 2

- **Fasting Glucose Level**: 
  - P = 0.4
  - P_{int} = 0.2

- **Fasting Insulin Level**: 
  - P = 0.16
  - P_{int} = 0.07

- **HOMAIR**: 
  - P = 0.15
  - P_{int} = 0.66

- **Glucose AUC**: 
  - P = 0.04
  - P_{int} = 0.61

- **Insulin AUC**: 
  - P = 0.02
  - P_{int} = 0.03

- **Composite ISI**: 
  - P = 0.004
  - P_{int} = 0.006