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ARTICLE

Association of indices of liver and adipocyte insulin resistance with 19 confirmed susceptibility loci for type 2 diabetes in 6,733 non-diabetic Finnish men

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Abstract
Aims/hypothesis Of the confirmed type 2 diabetes susceptibility loci only a few are known to affect insulin sensitivity. We examined the association of indices of hepatic and adipocyte insulin resistance (IR) with 19 confirmed type 2 diabetes risk loci in a large population-based study.
Methods Non-diabetic participants (n=8,460, age 57.3± 7.0 years, BMI 26.8±3.8 kg/m^2; mean ± SD) from a population-based cohort underwent an OGTT. Of them, 6,733 non-diabetic men were genotyped for single nucleotide polymorphisms (SNPs) in or near *PPARG2* (also known as *PPARG*), *KCNJ11*, *TCF7L2*, *SLC30A8*, *HHEX*, *CDKN2B*, *IGF2BP2*, *CDKAL1*, *HNF1B*, *WFS1*, *JAZF1, CDC123*, *TSPAN8*, *THADA*, *ADAMTS9*, *NOTCH2*, *KCNQ1*, *MTNR1B* and SNP rs7480010. We investigated hepatic IR with a new index of liver IR. The adipocyte IR index was defined as a product of fasting NEFA and plasma insulin levels.
Results Type 2 diabetes risk SNPs in or near *KCNJ11* and *HHEX* were significantly (p<0.0013), and those in or near *CDKN2B*, *NOTCH2* and *MTNR1B* were nominally (p<0.05), associated with decreased liver IR index. The Pro12 allele of *PPARG2* was significantly associated with a high adipocyte IR index and nominally associated with high liver IR.
Conclusions/interpretation The Pro12 allele of *PPARG2* seems to impair insulin's antilipolytic effect, leading to high NEFA release in the fasting state and IR. In addition, the type 2 diabetes risk alleles of *KCNJ11* and *HHEX*, which are known to impair insulin secretion, were associated with increased hepatic insulin sensitivity.

Keywords Adipocyte insulin resistance · Genetics · Liver insulin resistance · Non-esterified fatty acids · Type 2 diabetes

Abbreviations

2hPG	2 h Plasma glucose
ALT	Alanine aminotransferase
FPG	Fasting plasma glucose
HNF1α	Hepatocyte nuclear factor 1α
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
METSIM	Metabolic Syndrome in Men study
PPAR	Peroxisome proliferator-activated receptor
RISC	Relationship between Insulin Sensitivity and Cardiovascular Disease study
SNP	Single nucleotide polymorphism

Introduction

Recent genome-wide association studies have identified several genetic variants that enhance the risk of type 2

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diabetes. The majority of them were found to affect beta cell function and cause impaired insulin secretion. Only a few single nucleotide polymorphisms (SNPs) in type 2 diabetes risk genes have been related to 'common' insulin resistance (IR), examples being the Pro12 allele of *PPARG2* [1] and SNPs of *FTO* [2]. More recently, large-scale meta-analyses of genome-wide association studies have identified three new SNPs associated with IR [3, 4].

The low number of genes contributing to IR, when compared with genes affecting insulin secretion, might reflect a lower heritability of IR than of insulin secretion, different study designs, confounding effects of acquired IR and low physical activity, or poor surrogate markers of IR applied in previous studies [5].

Insulin resistance in the liver results in reduced glycogen synthesis and failure to suppress gluconeogenesis and glycogenolysis, leading to unsuppressed hepatic glucose production and hence also to hyperglycaemia [6, 7]. Impaired suppression of hepatic glucose production has been found not only in overt type 2 diabetes [8], but also in prediabetic states [9, 10]. Adipocyte metabolism is also known to be altered in type 2 diabetes [11]. Insulin resistance in fat cells leads to impairment of insulin's anti-lipolytic effect, contributing to increased flux of NEFA into the circulation, which in turn stimulates gluconeogenesis, decreases glucose uptake, induces hepatic and muscle IR, and impairs insulin secretion [12].

Since IR occurs in different tissues, some of the known type 2 diabetes risk variants may have tissue-specific effects on IR. We therefore investigated, in a large population-based sample of Finnish men, the possibility that 19 confirmed type 2 diabetes susceptibility loci regulate tissue-specific insulin sensitivity. In large-scale genetic studies it is not possible to apply the gold-standard methods, such as clamp techniques, to assess insulin sensitivity in different tissues [13]. Therefore, we estimated hepatic IR with a new liver IR index based on glucose and insulin levels during an OGTT, and on clinical and laboratory measurements [14]. We estimated adipocyte IR with an index published previously [15].

Methods

Study participants

For the study of liver and adipose tissue insulin sensitivity (Study 1), 8,460 non-diabetic participants (Table 1) from our ongoing population-based cross-sectional Metabolic Syndrome in Men (METSIM) study [16, 17] were included. Participants, aged from 45 to 70 years, were randomly selected from the population register of Kuopio town, Eastern Finland (population 95,000). Of those included, 2,951 participants had normal glucose tolerance, 4,181 isolated impaired fasting glucose (IFG), 302 isolated impaired glucose tolerance (IGT) and 1,026 IFG and IGT according to ADA criteria [18]. For the genetic association study (Study 2), the first 6,733 non-diabetic men (age 57.0±6.9 years, BMI 26.8±3.8 kg/m^2; mean ± SD) examined in the METSIM study were included. The gene expression study (Study 3) included 41 obese participants (age 44.2±8.3 years, BMI 45.5±6.1 kg/m^2, 11 men, 18 type 2 diabetic patients; mean ± SD) from an ongoing study, including participants who were undergoing bariatric surgery at the Kuopio University Hospital and who also underwent liver biopsy. All studies were approved by the ethics committee of the University of Kuopio and Kuopio University Hospital, and were carried out in accordance with the Helsinki Declaration. All study participants gave written informed consent.

Table 1 Clinical and laboratory characteristics of non-diabetic men participating in the METSIM study [16]

Variable	Mean ± SD
n	8,460
Age (years)	57.3±7.0
BMI (kg/m^2)	26.8±3.8
WHR	0.97±0.06
FPG (mmol/l)	5.7±0.5
2hPG (mmol/l)	6.1±1.7
Fasting plasma insulin (pmol/l)	49.5±34.9
Fasting serum NEFA (mmol/l)	0.37±0.14
Total triacylglycerol (mmol/l)	1.40±0.96
HDL-cholesterol (mmol/l)	1.46±0.39
ALT (U/l)	31.2±20.2

OGTT

A 2 h OGTT (75 g glucose) was performed and samples for plasma glucose, insulin and NEFA were drawn at 0, 30 and 120 min.

Laboratory measurements

Plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab System Reagents; Thermo Fischer Scientific, Vantaa, Finland); plasma insulin by immunoassay (Advia Centaur Insulin IRI, number 02230141; Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA); serum NEFA by enzymatic colorimetric method (Konelab 20XTi Clinical Chemistry Analyzer; Thermo Fischer Scientific); HDL-cholesterol by enzymatic colorimetric test (Konelab System Reagents); and alanine aminotransferase (ALT) and total triacylglycerol by standard methods. Body composition was determined by bioelectrical impedance (Bioimpedance Analyzer Model BIA101; Akern

Srl, Florence, Italy) with participants in the supine position after a 12 h fast [19].

Genotyping

Genotyping of 19 SNPs (*PPARG2* rs1801282, *KCNJ11* rs5219, *TCF7L2* rs7903146, *SLC30A8* rs13266634, *HHEX* rs1111875, rs7480010 [previously assigned to gene locus *LOC387761*], *CDKN2B* rs10811661, *IGF2BP2* rs4402960, *CDKAL1* rs7754840, *HNF1B* rs7501939, *WFS1* rs10010131, *JAZF1* rs864745, *CDC123* rs12779790, *TSPAN8* rs7961581, *THADA* rs7578597, *ADAMTS9* rs4607103, *NOTCH2* rs10923931, *KCNQ1* rs2283228) was performed with the TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA, USA) and Sequenom iPlex Gold SBE (for *MTNR1B* rs10830963; Sequenom, San Diego, CA, USA). The TaqMan genotyping call rate was 100%, with an error rate of 0% in 4.5% of DNA samples genotyped in duplicate. The Sequenom iPlex call rate for rs10830963 was 96.8%; the error rate in 4.2% of DNA samples genotyped in duplicate was 0%. All SNPs were consistent with Hardy–Weinberg equilibrium ($p>0.001$).

Gene expression data

Tissue-specific expression data were obtained from GeneSapiens [20], version IST4, which contains expression data from 15 liver and 16 adipose tissue samples from healthy human tissue, measured with Affymetrix (Santa Clara, CA, USA) gene expression microarrays. The following mean relative expression level values from GeneSapiens were used to classify expression levels: <300 for low, 300 to 700 for medium and >700 for high expression.

Liver histological analysis

Liver biopsies were performed using Tru-Cut (Radiplast, Uppsala, Sweden) during the elective gastric bypass operation. Histological assessment of liver was performed according to Brunt et al. [21]. Steatosis was graded into four categories (<5%, 5–32%, 33–66% and >66%) [21].

Calculations

The trapezoidal method was used to calculate glucose and insulin AUC (AUC 0–120 min) in an OGTT based on samples collected at 0, 30 and 120 min. The liver IR index was calculated from the formula: $-0.091 + (\log \text{insulin AUC } 0-120 \text{ min} \times 0.400) + (\log \text{fat mass } \% \times 0.346) - (\log \text{HDL-cholesterol} \times 0.408) + (\log \text{BMI} \times 0.435)$ as described in our validation study [14]. The adipocyte IR index was calculated as a product of fasting NEFA and fasting insulin ($\text{Ins}_0 \times \text{NEFA}_0$) as previously reported [15]. This marker reflecting adipocyte IR is based on a study [22] demonstrating that insulin is a strong inhibitory stimulus for lipolysis and suppresses NEFA levels. For statistical analyses of changes in liver IR and adipocyte IR indices across the fasting plasma glucose (FPG) and 2 h plasma glucose (2hPG) levels, we generated five categories of FPG using the following cut-off points (increase by 0.5 mmol/l): 5.0, 5.5, 6.0, 6.5 and 7.0 mmol/l, and seven categories of 2hPG (increase by 1.0 mmol/l): 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.1 mmol/l. Categories with FPG <5.0 mmol/l and 2hPG <5.0 mmol/l were set as the reference categories. We calculated a combined genetic risk score for liver IR as the sum of type 2 diabetes risk alleles (hereafter 'risk alleles') at five SNPs that are significantly or nominally associated (after adjustment for age, BMI and WHR) with liver IR index (at *KCNJ11*, *HHEX*, *CDKN2B*, *NOTCH2* and *MTNR1B* loci), and were weighted for their effect sizes (Table 2). Weights of the alleles were calculated by dividing the effect size of each SNP with the sum of effect sizes per risk allele of all SNPs included in the genetic risk score, then multiplying the result by the number of risk alleles at the SNP in each individual and rounding to the nearest integer. Participants with risk alleles ≤1 or ≥7 were pooled. The HOMA-IR index [23] and early-phase insulin secretion index (insulin $\text{AUC}_{0-30\ \text{min}}$/glucose $\text{AUC}_{0-30\ \text{min}}$) [16] were calculated as described previously.

Statistical analyses

Study 1 Changes in indices of IR across the categories of glucose levels were estimated by general linear model adjusted for age, BMI and WHR. A p value of <0.05 was considered statistically significant.

Study 2 Associations between SNPs and continuous traits are presented as effect sizes (β and SE) per copy of the type 2 diabetes risk allele, estimated by linear regression adjusted primarily for age, and additionally for age, BMI and WHR using untransformed dependent variables, as previously described [16]. We calculated p values using logarithmically transformed variables in all cases except for liver IR index, which was normally distributed. The effect of genetic risk score was analysed by linear regression. A p value of <0.05 was considered nominally significant and <0.0013 statistically significant (Bonferroni correction for multiple comparisons) given 38 independent tests (19 SNPs×2 phenotypes [liver IR and adipocyte IR indices]). Hardy–Weinberg equilibrium was tested by χ^2 test. Statistical analyses were conducted with the SPSS 14 (SPSS, Chicago, IL, USA). Power calculations were performed using Bioconductor's GeneticsDesign 1.14 [24]. Depending on the minor allele frequencies (from 5.1% to 48.4%) of individual SNPs, we had ≥80% power to detect changes

Table 2 Association between 19 SNPs and liver IR index in 6,733 non-diabetic participants

Gene symbol SNP	Allele	MAF% [b]	Liver IR index							Gene expression in liver
			Adjusted for age only			Adjusted for age, BMI and WHR				
			β	SE	*p* value	β	SE	%	*p* value	
PPARG2 rs1801282	C[a]/G	15.3	−0.0001	0.004	0.981	0.006	0.003	0.21	0.039	Low
KCNJ11 rs5219	G/A[a]	47.4	−0.009	0.003	0.002	−0.008	0.002	−0.27	1.8E-04	Low
TCF7L2 rs7903146	C/T[a]	17.5	−0.008	0.004	0.034	−0.004	0.003	−0.16	0.090	High
SLC30A8 rs13266634	C[a]/T	39.3	−0.003	0.003	0.359	0.0003	0.002	0.01	0.879	Low
HHEX rs1111875	C[a]/T	46.6	−0.010	0.003	3.5E-04	−0.008	0.002	−0.29	5.4E-05	High
rs7480010[c]	A/G[a]	17.4	−0.007	0.004	0.083	−0.003	0.003	−0.10	0.288	–
CDKN2B rs10811661	A[a]/G	14.8	−0.005	0.004	0.191	−0.007	0.003	−0.25	0.013	Low
IGF2BP2 rs4402960	C/A[a]	32.0	−0.002	0.003	0.529	−0.001	0.002	−0.04	0.600	Low
CDKAL1 rs7754840	G/C[a]	36.9	−0.006	0.003	0.053	−0.003	0.002	−0.12	0.125	Low
WFS1 rs10010131	G[a]/A	44.6	−0.002	0.003	0.567	−0.001	0.002	−0.05	0.465	Medium
HNF1B rs7501939	C/T[a]	27.3	0.004	0.003	0.173	−0.0003	0.002	−0.01	0.865	High
JAZF1 rs864745	A[a]/G	48.4	0.004	0.003	0.192	0.004	0.002	0.14	0.054	Low
CDC123 rs12779790	A/G[a]	21.3	−0.007	0.003	0.034	−0.003	0.002	−0.11	0.212	High
TSPAN8 rs7961581	A/G[a]	19.9	−0.001	0.004	0.864	0.003	0.003	0.10	0.282	Medium
THADA rs7578597	A[a]/G	5.1	−0.005	0.007	0.427	−0.007	0.005	−0.24	0.142	Medium
ADAMTS9 rs4607103	G[a]/A	25.8	−0.001	0.003	0.792	−0.001	0.002	−0.04	0.600	Medium
NOTCH2 rs10923931	C/A[a]	14.0	−0.009	0.004	0.023	−0.007	0.003	−0.26	0.016	Medium
KCNQ1 rs2283228	A[a]/C	6.1	−0.002	0.006	0.785	−0.002	0.004	−0.06	0.704	Low
MTNR1B rs10830963	C/G[a]	35.8	−0.010	0.003	0.001	−0.005	0.002	−0.20	0.009	Low

Effect sizes (β and SE, and % of β from mean) per type 2 diabetes risk allele and corresponding *p* values are shown

Results for the additive model are based on adjustments as indicated

Tissue-specific expression of respective genes was obtained from the GeneSapiens database [20]

[a] Type 2 diabetes risk allele

[b] Minor allele frequency

[c] rs7480010 was previously assigned to gene locus *LOC387761*

from 0.5% to 1.2% per copy of a risk allele for liver IR and from 7.8% to 17.8% for the adipocyte IR index, both at a significance level of 0.05.

Study 3 Effects of genotypes on gene expression levels in liver biopsy samples were analysed by analysis of variance or Student's *t* test. Associations between gene expression and continuous traits were analysed by Spearman's correlation.

Results

Association of liver IR and adipocyte IR indices with glucose levels

As seen in Fig. 1, the liver IR index was significantly associated with FPG (increase up to 1.4%) and 2hPG levels (increase up to 3.5%) ($p = 3.1\times10^{-12}$ and 5.4×10^{-103}, respectively). The adipocyte IR index also showed a significant association with FPG and 2hPG (increase up to 88% and 108%, $p = 2.9\times10^{-122}$ and 1.0×10^{-219}, respectively).

Association between SNPs and liver IR index

Table 2 and Fig. 2a–f show that *HHEX* rs1111875 and *MTNR1B* rs10830963 were significantly, and four other SNPs (at *KCNJ11*, *TCF7L2*, *CDC123* and *NOTCH2* loci) were nominally, associated with liver IR index in analyses adjusted for age only. When additional adjustments were done for BMI and WHR, risk alleles at five SNPs decreased liver IR either significantly (*HHEX* rs1111875 $p=5.4\times10^{-5}$, *KCNJ11* rs5219 $p=1.8\times10^{-4}$) or nominally (*CDKN2B* rs10811661 $p=0.013$, *NOTCH2* rs10923931 $p=0.016$, *MTNR1B* rs10830963 $p=0.009$). The Pro12 allele (rs1801282) of *PPARG2* was associated with higher liver IR ($p=0.039$). All effect sizes were less than 0.3% per risk allele. Additional adjustment for insulin secretion (insulin AUC_{0-30}/glucose AUC_{0-30}) abolished the associations of

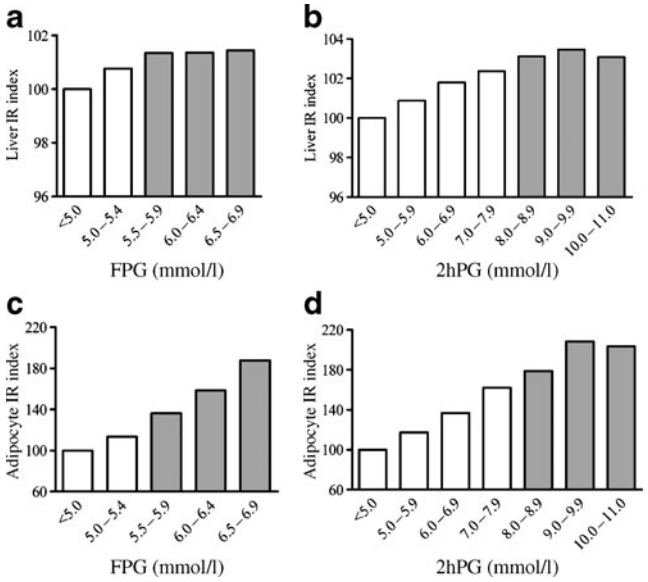

Fig. 1 Liver IR (**a**, **b**) and adipocyte IR (**c, d**) indices across the categories of FPG and 2hPG within the non-diabetic range. Bars display values relative to the reference category (FPG <5.0 mmol/l, 2hPG <5.0 mmol/l). White bars indicate normal glucose, i.e. normal fasting glucose and normal glucose tolerance. Grey bars indicate impaired glucose levels (IFG and IGT). Calculations were based on geometric means, adjusted for age, BMI and WHR with the general linear model, with *p* values obtained as follows: **a** 3.1×10^{-12}, **b** 5.4×10^{-103}, **c** 2.9×10^{-122} and **d** 1.0×10^{-219}

PPARG, *KCNJ11* and *NOTCH2* with liver IR, and weakened the association of *HHEX* with liver IR (p=0.003) (Electronic supplementary material [ESM] Table 1).

Additional analyses of the SNPs with the HOMA-IR index, an alternative measure of hepatic IR (ESM Table 2), showed effects in a similar direction to that for liver IR for *PPARG2* rs1801282 (p=0.009), *KCNJ11* rs5219 (p=0.018), *NOTCH2* rs10923931 (p=0.014) and *HHEX* rs1111875 (p= NS), and in the opposite direction for *CDKN2B* rs10811661 and *MTNR1B* rs10830963 (p=NS).

Combined effect of risk alleles on liver IR index

The risk alleles of five SNPs that significantly or nominally reduced liver IR (*KCNJ11* rs5219, *HHEX* rs1111875, *CDKN2B* rs10811661, *NOTCH2* rs10923931 and *MTNR1B* rs10830963) in adjusted analyses were combined to evaluate their joint effects. There was a small but consistent decrease (Fig. 2h) of up to 2% ($p = 6.9\times10^{-9}$) in liver IR with the increasing number of risk alleles.

Gene expression and liver steatosis

Spearman correlation analysis of liver gene expression of selected genes and hepatic steatosis in 41 morbidly obese participants (Study 3) showed a significant negative correlation between *HHEX* gene expression and liver steatosis (rho=−0.394, p=0.028). Similarly, a trend towards negative correlation was observed for *KCNJ11* (rho=−0.322, p=NS). A trend towards positive correlation was observed between *PPARG2* gene expression and hepatic steatosis (rho=0.181, p=NS). Moreover, in Study 2, the *Pro* allele of *PPARG2* was nominally associated with high ALT levels as a marker of liver steatosis (p=0.022) (ESM Table 3). Risk alleles at *KCNJ11* and *NOTCH2* were nominally associated with higher HDL-cholesterol (p=0.011 and 0.027), as was the risk allele at *NOTCH2* with lower triacylglycerol (p=0.003) (ESM Table 3).

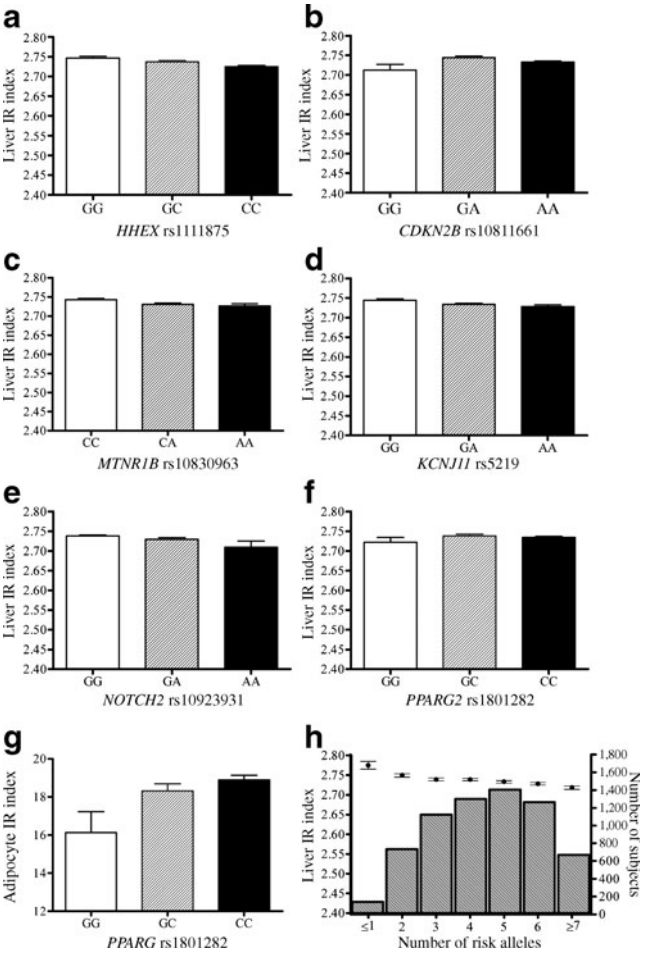

Fig. 2 Liver IR (**a–f**) and adipocyte IR (**g**) indices according to the genotypes of associated SNPs (see Tables 2 and 3), $p<0.05$. Bars show the means of liver IR and adipocyte IR indices in each genotype group. White bars, non-risk genotypes; hatched bars, carriers of one type 2 diabetes risk allele; black bars, carriers of two type 2 diabetes risk alleles. *p* values adjusted for age, BMI and WHR were obtained as follows: **a** 5.4×10^{-5}, **b** 0.013, **c** 0.009, **d** 1.8×10^{-4}, **e** 0.016, **f** 0.039, **g** 6.2×10^{-5}. **h** Liver IR index according to the number of type 2 diabetes risk alleles combined for five SNPs (*KCNJ11* rs5219, *HHEX* rs1111875, *CDKN2B* rs10811661, *NOTCH2* rs10923931 and *MTNR1B* rs10830963). Data are adjusted for age, BMI and WHR. Black circles with error bars indicate means and SE; grey bars show the number of participants in each category. The effect of the number of risk alleles on liver IR index was significant ($p = 6.9\times10^{-9}$)

Table 3 Association between 19 SNPs and adipocyte IR index in 6,733 non-diabetic participants

Gene symbol SNP	Allele	MAF% [b]	Adipocyte IR index							Gene expression in adipose tissue
			Adjusted for age only			Adjusted for age, BMI and WHR				
			β	SE	p value	β	SE	%	p value	
PPARG2 rs1801282	C[a]/G	15.3	0.824	0.402	0.026	1.226	0.349	6.58	6.2E-05	High
KCNJ11 rs5219	G/A[a]	47.4	−0.634	0.290	0.081	−0.517	0.251	−2.77	0.124	Low
TCF7L2 rs7903146	C/T[a]	17.5	−0.582	0.377	0.038	−0.344	0.327	−1.85	0.089	High
SLC30A8 rs13266634	C[a]/T	39.3	−0.498	0.295	0.080	−0.300	0.256	−1.61	0.213	Low
HHEX rs1111875	C[a]/T	46.6	−0.559	0.287	0.098	−0.426	0.249	−2.29	0.169	High
rs7480010[c]	A/G[a]	17.4	−0.896	0.383	0.071	−0.602	0.332	−3.23	0.236	–
CDKN2B rs10811661	A[a]/G	14.8	0.736	0.407	0.384	0.614	0.353	3.29	0.482	Low
IGF2BP2 rs4402960	C/A[a]	32.0	−0.462	0.307	0.310	−0.419	0.266	−2.25	0.278	Low
CDKAL1 rs7754840	G/C[a]	36.9	−0.233	0.299	0.119	−0.021	0.259	−0.11	0.361	Low
WFS1 rs10010131	G[a]/A	44.6	−0.495	0.289	0.297	−0.483	0.25	−2.59	0.224	High
HNF1B rs7501939	C/T[a]	27.3	0.241	0.333	0.510	−0.100	0.289	−0.54	0.546	Medium
JAZF1 rs864745	A[a]/G	48.4	0.247	0.286	0.126	0.233	0.248	1.25	0.076	Medium
CDC123 rs12779790	A/G[a]	21.3	−0.490	0.350	0.231	−0.194	0.304	−1.04	0.730	High
TSPAN8 rs7961581	A/G[a]	19.9	−0.319	0.361	0.767	−0.075	0.312	−0.40	0.614	Low
THADA rs7578597	A[a]/G	5.1	−0.111	0.657	0.960	−0.217	0.569	−1.16	0.907	Medium
ADAMTS9 rs4607103	G[a]/A	25.8	−0.475	0.332	0.168	−0.518	0.288	−2.78	0.075	Medium
NOTCH2 rs10923931	C/A[a]	14.0	−0.937	0.418	0.044	−0.771	0.362	−4.14	0.063	High
KCNQ1 rs2283228	A[a]/C	6.1	−0.079	0.600	0.861	−0.126	0.519	−0.68	0.762	Low
MTNR1B rs10830963	C/G[a]	35.8	−0.435	0.299	0.212	−0.162	0.259	−0.87	0.795	Low

Effect sizes (β, SE, % of β from mean) per type 2 diabetes risk allele and corresponding p values are shown

Results for the additive model are based on adjustments as indicated

p values for the adipocyte IR index were calculated using log-transformed variable due to its skewed distribution

Tissue-specific expression of respective genes was obtained from the GeneSapiens database [20]

[a] Type 2 diabetes risk allele

[b] Minor allele frequency

[c] rs7480010 was previously assigned to gene locus *LOC387761*

Association of SNPs with the adipocyte IR index

As seen in Table 3 and Fig. 2g, *PPARG2* rs1801282, *TCF7L2* rs7903146 and *NOTCH2* rs10923931 (p=0.026, 0.038 and 0.044, respectively) showed nominally significant association with the adipocyte IR index when adjusted for age only. Additional adjustment for BMI and WHR strengthened the association of the Pro12 allele of *PPARG2* with higher adipocyte IR index ($p = 6.2 \times 10^{-5}$, effect size 7% per allele) to a significant level and abolished other associations.

Discussion

This is the first population-based study aiming to evaluate the associations of liver IR and adipocyte IR indices with type 2 diabetes risk loci. As expected, both these indices were significantly associated with FPG and 2hPG levels. Of 19 type 2 diabetes risk loci tested, the risk alleles of *KCNJ11* and *HHEX* showed significant, and those of *CDKN2B*, *NOTCH2* and *MTNR1B* nominally significant, associations with lower liver IR after adjustment for age, BMI and WHR. The Pro12 allele of *PPARG2* was associated with higher liver IR and higher adipocyte IR indices.

Insulin resistance in the liver results in impaired suppression of hepatic glucose production, contributing to elevated glucose levels in fasting and postprandial states [9, 10]. Our observation of a gradual increase of the liver IR index by 1.4% to 3.5% with higher levels of FPG and 2hPG agrees with these findings. An increased adipocyte IR index indicates a loss of the antilipolytic effect of insulin. We observed an increase in the adipocyte IR index by ≥88% with increasing FPG and 2hPG levels within the non-diabetic range of glycaemia. These associations with FPG

and 2hPG levels indicate that these indices are likely to be reliable indicators of IR in the liver and adipose tissue.

We found that the Pro12 allele of *PPARG2* was significantly associated with elevated adipocyte IR index and nominally associated with increased liver IR. Peroxisome proliferator-activated receptor (PPAR)γ is a nuclear factor regulating transcriptor of various genes, particularly adipose-specific genes involved in adipocyte differentiation, and contributes to regulation of NEFA metabolism by stimulating uptake, storage and oxidation of NEFA in adipocytes [25]. The relevant SNP has been previously shown to be associated with insulin sensitivity [1, 26, 27]. Since the Pro12Ala polymorphism is present only in the PPARγ2 isoform, which is found prominently in adipose tissue [28], it is probable that this SNP exerts its effect on insulin sensitivity directly in adipose tissue. Previous studies have reported higher insulin sensitivity of lipolysis and greater suppression of NEFA levels by insulin during hyperinsulinaemic clamp in carriers of the protective *Ala* allele [29, 30]. Increased release of NEFA from insulin-resistant adipose tissue may further impair insulin sensitivity in liver [31]. This could explain the association between the Pro12 allele of *PPARG2* and higher liver IR in our study, although a direct effect on insulin action in the liver cannot be excluded. Moreover, we observed a trend towards a positive correlation between *PPARG2* expression in the liver and hepatic steatosis in 41 morbidly obese participants. The nominally significant association of the Pro12 allele of *PPARG2* with an increase in HOMA-IR, which is considered to mainly reflect hepatic IR, and elevated ALT levels provides additional support to our findings.

The type 2 diabetes risk allele in *KCNJ11* was significantly associated with a lower liver IR index, which may seem surprising. Similar results were reported in a recent study showing that homozygous carriers of the rs5219 risk allele of *KCNJ11* had increased hepatic insulin sensitivity (measured by euglycaemic–hyperinsulinaemic clamp and tracer infusion), in addition to an insulin secretion defect [32]. The risk allele of *KCNJ11* rs5219 also showed a nominal association with higher HDL-cholesterol levels in our study. In addition, a trend towards a negative correlation was found between *KCNJ11* gene expression in the liver and hepatic steatosis in 41 obese participants. Further evidence supporting our findings comes from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study, where we validated our liver IR index [14] and where hepatic IR was directly measured using tracer techniques. The standardised regression coefficients for an association of *KCNJ11* rs5219 with liver IR index (beta; adjusted for age, BMI and WHR) was −0.032 in the current study, while it was −0.035 with hepatic IR (measured as endogenous glucose production × fasting plasma insulin) in the RISC study, demonstrating similar effects towards increased hepatic insulin sensitivity (association in the RISC study was not statistically significant due to small sample size).

The type 2 diabetes risk allele of *HHEX* also showed a significant association with lower liver IR. Results obtained from the RISC study [14] are consistent with these findings. The standardised regression coefficient (beta; adjusted for age, BMI and WHR) for an association of the risk allele of *HHEX* with hepatic insulin sensitivity measured with tracer technique was −0.022 in the RISC study (not statistically significant due to small sample size); that for an association with liver IR in the METSIM study was −0.034. Mechanisms by which *HHEX* affects liver insulin sensitivity are unknown, but hepatocyte nuclear factor 1α (HNF1α) could be involved, as *HHEX*, which is abundantly expressed in the liver, has been shown to directly activate HNF1α in mammalian hepatocytes [33]. Our study showed a significant negative correlation between *HHEX* expression in the liver and hepatic steatosis, further supporting our conclusions.

We observed a nominally significant association between the *MTNR1B* rs10830963 risk allele and a decreased liver IR index. In agreement with our result, a previous study showed an association between a different SNP and decreased HOMA-IR [34]. In contrast, a study based on the hyperinsulinaemic–euglycaemic clamp showed that the *MTNR1B* risk allele reduced suppression of hepatic glucose production, suggesting that it could be associated with hepatic IR [35]. Type 2 diabetes risk alleles of *CDKN2B* and *NOTCH2* were also nominally associated with lower liver IR index in our study. A nominally significant association was observed for *NOTCH2* rs10923931 with lower HOMA-IR, lower triacylglycerol and higher HDL-cholesterol. Both *CDKN2B* and *NOTCH2* are known to play a role in the development of the liver. However, the mechanisms by which these genes affect hepatic insulin sensitivity are unclear.

When the type 2 diabetes risk alleles of the five SNPs in *KCNJ11*, *HHEX*, *CDKN2B*, *NOTCH2* and *MTNR1B* were combined, the liver IR index significantly decreased by 2%, but for each of these SNPs alone the effect size was <0.3%. However, we noticed that the associations between risk SNPs and liver IR index either lost their statistical significance (*KCNJ11*, *CDKN2B*, *NOTCH2*) or weakened (*HHEX*) when adjusted for insulin secretion (in addition to age, BMI and WHR). In an earlier study, we reported that SNPs in or near *HHEX*, *CDKN2B*, *MTNR1B* and *KCNJ11* were associated with lower glucose-stimulated insulin release and showed comparatively larger effect sizes from 1.2% to 6.7% per risk allele [16]. Therefore, we believe that the variants of these genes primarily affect insulin secretion and that their effect on liver insulin sensitivity is of less importance and compensatory. Support for this notion is

provided by previous studies showing that *HNF1A* mutation carriers who have MODY [36] and carriers of the E1506K mutation of *ABCC8* [37] have increased insulin sensitivity, although they have a severe defect in insulin secretion. An earlier study showed that high insulin sensitivity, evaluated by the euglycaemic clamp, reflecting mostly skeletal muscle insulin sensitivity, could protect against the detrimental effect of several genes on insulin secretion [38]. We hypothesise that increased liver insulin sensitivity could be a mechanism that counterbalances impaired insulin secretion. These compensatory mechanisms might be effective in the non-diabetic range of glycaemia, but are likely to fail when frank hyperglycaemia develops.

The strengths of our study are its large sample size, the homogeneous study population and the carefully characterised phenotype. A limitation of the study is that only middle-aged Finnish men were included, so we cannot predict whether the results are valid for women, in whom adipose tissue distribution is different, or for other ethnic and racial groups. Considering the large size of our cohort, it was not feasible to use more accurate methods to evaluate liver insulin sensitivity (clamp and tracer techniques). However, our liver IR index was validated against hepatic glucose measurement using the tracer technique in a large sample of non-diabetic individuals [14]. Comparing the association of the risk alleles with liver IR index and HOMA-IR, we obtained similar results for *PPARG2*, *KCNJ11* and *NOTCH2*, which were nominally associated with HOMA-IR, and a similar trend for *HHEX*, although not statistically significant.

In conclusion, we suggest that the Pro12 risk allele of *PPARG2* reduces insulin's antilipolytic effects and leads to high release of NEFA and IR. Furthermore, type 2 diabetes risk alleles of *KCNJ11* and *HHEX*, which are known to impair insulin secretion, were associated with increased hepatic insulin sensitivity.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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