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IGF2BP1, *IGF2BP2* and *IGF2BP3* genotype, haplotype and genetic model studies in metabolic syndrome traits and diabetes

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ABSTRACT

Objective: Genetic variation at the insulin-like binding protein 2 (*IGF2BP2*) gene has been associated with type 2 diabetes (T2D) by genome-wide association studies and by replication analyses. Our aim was to explore the underlying genetic model and mechanism of action, factors accounting for non-replications of the associations, and the effect of variation from pathway-related genes *IGF2BP1* and *IGF2BP3*.
Method: We analysed here the association between T2D (and related traits) and rs4402960 and rs1470579 in *IGF2BP2*, and rs46522 and rs6949019 (marking *IGF2BP1* and *IGF2BP3* respectively) from the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study ($N \sim 2500$ aged 65–96 years). We undertook a retrospective analysis of the deviations from the multiplicative model in previous studies and the present study.
Results: We replicated an association between rs4402960 and T2D status, and reported significant associations with anthropometric traits, fasting insulin, HOMA-IR and HOMA-%B. These associations were also observed for rs1470579, but not for the SNPs marking *IGF2BP1* and *IGF2BP3*.
Conclusions: The lower fasting insulin levels and the impaired β-cell function associated with *IGF2BP2* SNPs are independent of obesity phenotypes. The action of these SNPs on T2D may result from an effect on β-cell function. This could lead to lower insulin levels, the association with anthropometric traits being secondary. We discuss possible mechanisms of action relating *IGF2BP2* with T2D traits. The occurrence of null alleles, the inclusion of T2D patients in analyses of metabolic syndrome risk traits and the genetic model, are possible factors accounting for non-replications of *IGF2BP2* associations with T2D.

1. Introduction

Type 2 diabetes (T2D) is a complex disorder with raised plasma glucose levels caused by impairment of insulin action and secretion [1]. It is substantially caused by obesity, and conditions such as hypertension and dyslipidemias associate with this disorder [2]. T2D is a major health problem, with an estimated 300 million people predicted to develop the disease by 2025 [3].

The complexity of T2D lies in the involvement of a large number of polygenes with small phenotypic effects and the combined action of environmental factors. A powerful approach for detecting candidate genes is the analysis of association involving large numbers of Single Nucleotide Polymorphisms (SNPs) densely distributed throughout the genome. In recent years, genome-wide association studies (GWAS) have identified 19 common variants associated with T2D [4]. Replication, in independent studies, of the associations found in GWAS provides further evidence of association.

One gene with a proven polygenic contribution in T2D is *IGF2BP2*. To May 2009, 31 studies testing the association between *IGF2BP2* genotype and T2D risk have been published, 19 showing significant association and 12 showing no association (reviewed in [5]). Some of the non-replication results have been linked to low powered studies [5]. Results from meta-analyses including both significant and non-significant associations provide strong evidence of an association between *IGF2BP2* and T2D risk [6,7]. A number of T2D related quantitative traits including insulin secretion, insulin sensitivity (or resistance) and β-cell function, have been studied in relation to *IGF2BP2*. Some studies have found significant associations between *IGF2BP2* and insulin secretion [8–12], insulin sensitivity (or resistance) [11,13] and β-cell function [9,10,12,14–16], although not all of these traits have shown significant association in all studies [9,13,17,18]. The most commonly studied SNPs are rs4402960 and rs1470579.

Functionally, *IGF2BP2* is a plausible candidate gene influencing T2D. It belongs to a family of proteins binding to insulin-like growth

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factor 2 (IGF-II), an important growth and insulin-signalling factor. IGF2BP1, IGF2BP2 and IGF2BP3 (also known as IMP-1, IMP-2 and IMP-3) are essential for normal growth and development. They attach to the 5′UTR from the IGF-II leader 3 mRNA and influence post-transcriptional events [5,19].

Association analyses involving *IGF2BP2* and T2D and other T2D related phenotypes are important to the determination of the role of *IGF2BP2* in this disease. In addition, the analysis of *IGF2BP2* pathway-related genes may help in unravelling the mechanism of action of *IGF2BP2*. No association attaining genome-wide significance between T2D risk or related phenotypes and either *IGF2BP1* or *IGF2BP3* has been observed in GWAS, but it has been recognised that further studies are required in order to investigate the role of both genes in relation to T2D [5].

We have studied here the association between two *IGF2BP2* SNPs previously shown to associate with T2D and both T2D status and a range of related phenotypes. We have extended these analyses to two other SNPs displaying nominal associations with T2D or coronary disease in GWAS [20], near to the related genes *IGF2BP1* and *IGF2BP3*. We have conducted the analysis using a large sample (N~2,500) of participants in the AGES-Reykjavik study.

2. Materials and methods

2.1. Study cohort

The study cohort was the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. This is a continuation of a population-based Reykjavik Study, initiated in 1967 by the Icelandic Heart Association [21]. Details on the study design and the baseline AGES-Reykjavik assessments have been previously described [22]. The study population consists of 2510 individuals, 1064 men and 1446 women, aged 65–96 years, with an overall response rate of 75% for men and 68% for women. The mean age of the AGES-Reykjavik cohort when first entering the Reykjavik Study was 50 years. AGES-Reykjavik was approved by the Icelandic National Bioethics Committee (VSN 00-063), the Icelandic Data Protection Authority, and by the Institutional Review Board of the US National Institute on Aging, National Institutes of Health. Informed consent was signed by all participants.

2.2. Phenotypes

The following anthropometric and metabolic phenotypes were analysed: Hemoglobin A1c, height, weight, body mass index (BMI, defined as weight (kg)/height squared (m^2)), waist circumference, fat-free mass (measured by bioimpedence), systolic blood pressure, diastolic blood pressure, fasting glucose, fasting insulin, cholesterol, HDL, LDL, triglycerides and coronary calcium.

Blood pressure and anthropometric data including BMI and waist circumference were collected using standardized protocols. A Xitron HYDRA ECF/ICF, Model 4200, was used to measure body composition with the bioelectrical impedance analysis (BIA) to assess the composition of the total body. From these BIA data, and additional variables such as age, gender and body weight, the fat-free mass (FFM, in kg) of the body can be estimated using prediction equations.

Fasting blood glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured on a Hitachi 912 using reagents from Roche Diagnostics and following the manufacturer's instructions. Fasting insulin was measured by an electrochemiluminescence immunoassay on a Roche Elecsys 2010 instrument, using two monoclonal antibodies and a sandwich principle. The method was standardized using the first IRP WHO Reference Standard 66/304 (NIBSC).

Two binary traits were analysed, hypertension (defined by self report, medication use or Systolic>140 mm Hg or Systolic>90 mm Hg) and T2D (defined by self report, medication use or fasting glucose ≥ 7.0 mM).

HOMA-IR (a measure of insulin resistance) and HOMA-%B (a measure of β-cell function) were derived according to the formulas:

$$\mathrm{HOMA-IR} = (\mathrm{FPI} \times \mathrm{FPG})/22.5$$

$$\mathrm{HOMA-\%B} = (20 \times \mathrm{FPI})/(\mathrm{FPG} - 3.5),$$

where FPI is fasting plasma insulin concentration (mU/l) and FPG is fasting plasma glucose (mmol/l) [23].

2.3. Genotyping

Four SNPs were analysed, rs46522 (in *IGF2BP1*), rs4402960 and rs1470579 (in *IGF2BP2*) and rs6949019 (in *IGF2BP3*). Both *IGF2BP2* SNPs were selected based on their previous association with T2D in several studies ([5] for review). The *IGF2BP1* and *IGF2BP3* SNPs were selected based on SNP association signals (additive model) in Wellcome Trust Case Control Consortium (WTCCC) data [20] in or within a range of ±200 kb of the respective genes.

All four SNPs were typed by the company KBiosciences, Essex, UK, with their KASPar system. This is a competitive allele specific PCR SNP genotyping system that uses Fluoresence Resonance Energy Transfer (FRET) quencher cassette oligos [24].

2.4. Statistical analyses

Hardy–Weinberg equilibrium analyses were performed with the Hardy–Weinberg equilibrium calculator (http://www.oege.org/software/hwe-mr-calc.shtml) [25]. Linkage disequilibrium analyses were performed using the web tool CubeX [26].

Fasting plasma glucose and insulin, plasma concentrations of HDL, LDL and triglycerides, fat-free mass, coronary calcium, HOMA-IR and HOMA-%β were log-transformed before statistical analyses and then re-transformed for table presentation.

Associations between genetic variants and categorical variables were tested by logistic regression. Associations between genetic variants and continuous variables were tested by linear regression. Multiple regression analyses adjusting for covariates were performed for cases of $P<0.05$. The statistical analyses were conducted using SPSS (version 15 for Windows) and STATA/IC 10.1 for Windows.

We used the software G*Power [27] to estimate the sample size required in a regression analysis for detecting (with a power of 90%), a small effect on a phenotype. This small effect was defined as the smallest effect size measures of Cohen's conventions [27].

Given a previously reported association between *IGF2BP2* and T2D, one could expect a different association with T2D traits when T2D cases are considered or excluded. Thus, all the analyses were done excluding T2D patients, and in the whole sample including T2D patients.

2.5. Comparative analysis of genetic models from published data

We did a retrospective analysis evaluating the genetic model from published data for rs4402960 and rs1470579 in relation to T2D and T2D traits. On 2/10/2009 we searched in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using the term *IGF2BP2*. We found 47 articles and we gathered the case control counts and the mean values for T2D risk traits according to *IGF2BP2* genotype for all articles showing this information.

Conformation with the multiplicative model was defined for continuous traits as $\lambda_2/\lambda_1 = 1$, where $\lambda_2 = (c/b)/(b/a)$ and c = mean value of rare homozygote, b = mean value of heterozygote, and a = mean value of common homozygote. For the case control scenario, c = number of rare homozygote cases/number of rare homozygote

controls, b = number of heterozygote cases/number of heterozygote controls, and a = number of common homozygote cases/number of common homozygote controls.

Deviations of $\lambda_2/\lambda_1 = 1$ indicate greater relative effects of the rare homozygote or the common homozygote.

We also split the 89 values observed for continuous traits into those with a $|\lambda_2/\lambda_1 - 1| \geq 0.10$ (arbitrary value) and those with $|\lambda_2/\lambda_1 - 1| < 0.10$. We then compared the numbers of significant association with *IGF2BP2* versus the numbers observed in non-significant associations. Analysis of the resulting 2×2 table was performed by a Pearson χ^2 contingency test. A similar approach was conducted for data from case control studies.

3. Results

None of the four SNPs deviated significantly from Hardy–Weinberg equilibrium (data not shown). From the six possible pairwise combinations between the SNPs, only the linked pair (rs4402960 *vs* rs1470579) showed significant LD ($D' = 0.996$, $r^2 = 0.954$, $P < 10^{-30}$). Pairwise analysis of these two *IGF2BP2* SNPs revealed significant deviations between the observed and the expected diplotype counts in a 3×3 table. The biggest difference was for diplotypes 11–12 (in the order rs1470579 and rs4402960), with 44 observed individuals (30 expected). A detailed examination of the surrounding sequence of rs1470579 revealed the presence of a second SNP (rs9869293) located 19 base pairs apart from rs1470579, lying within the sequence of the forward primers used for the amplification of rs1470579. An analysis of the frequency of T2D patients with diplotypes 11–12 revealed a significant excess ($P = 0.037$) of diabetics compared with the remaining diplotypes pooled together [22.7% (10 type 2 diabetics vs 34 non-diabetics) compared with 14.0% (302 diabetics vs 2164 non-diabetics)].

Table 1 shows the results of association between the four SNPs and T2D status, under three different genetic models (additive, dominant and recessive). rs4402960 showed a significant association under both the additive and the dominant models, with increments of 22% and 32% in disease risk, respectively. The allele conferring increased risk was T. The associations remained significant when adjusting for the covariates sex, age and BMI (Table 1). rs1470579 did not associate significantly with T2D status. It showed slightly lower magnitude of effect and in the same direction, and a trend of association ($P = 0.079$) when adjusting for covariates under the additive model. Neither of the *IGF2BP1* and *IGF2BP3* SNPs showed association with T2D status (Table 1).

The percentage of T2D patients in our sample was 12.4% (312 diabetics and 2198 non-diabetics). Observed numbers of T2D patients and non-diabetics stratified by genotype can be seen in Table 2.

Table 1
Association between *IGF2* mRNA binding protein genes 1, 2 and 3 and type 2 diabetes. Logistic regression results for each of the four SNPs analysed (rs4402960 and rs1470579 in *IGF2BP2*, rs46522 in *IGF2BP1* and rs6949019 in *IGF2BP3*). Association tests have been done under the additive (ADD), dominant (DOM) and recessive (REC) models.

Type 2 diabetes	Odds Ratio	95% CI	P_1	P_2
rs4402960 (ADD)	1.22	1.01–1.46	0.037	0.022
rs4402960 (DOM)	1.32	1.04–1.69	0.024	0.024
rs4402960 (REC)	1.18	0.79–1.76	0.421	
rs1470579 (ADD)	1.15	0.96–1.38	0.131	0.079
rs1470579 (DOM)	1.21	0.96–1.54	0.112	0.104
rs1470579 (REC)	1.15	0.77–1.71	0.506	
rs46522 (ADD)	1.03	0.87–1.22	0.763	
rs46522 (DOM)	1.19	0.91–1.55	0.199	
rs46522 (REC)	0.86	0.63–1.18	0.344	
rs6949019 (ADD)	1.06	0.90–1.26	0.479	
rs6949019 (DOM)	1.12	0.85–1.49	0.422	
rs6949019 (REC)	1.05	0.80–1.38	0.717	

P_1 = unadjusted probability; P_2 = adjusted for sex, age and BMI.

No significant association was found between hypertension status and any of the four SNPs analysed under any genetic model (supplementary Table 1).

Linear regression results involving traits relevant to T2D are shown for each SNP in Tables 3–6, for individuals without T2D. Significant associations were found for the anthropometric traits weight, BMI and waist circumference, and for the traits fasting insulin, HOMA-IR and HOMA-%B for both rs4402960 and rs1470579. The associations were found only for the recessive genetic model with TT (rs4402960) and CC (rs1470579) rare homozygotes showing the lowest values for these traits compared with the other genotypes. None of the remaining traits associated significantly with rs4402960 or rs1470579. Likewise, neither rs46522 in *IGF2BP1* (Table 5) nor rs6949019 in *IGF2BP3* (Table 6) showed significant associations. Our power analysis for the *F*-test in linear regression analyses showed that the minimum sample size required for a power of 90% is $N = 1054$, a smaller sample size than the one used in the present work.

Fig. 1 shows the means and standard errors of the phenotypes associated with rs4402960 and rs1470579 observed for each genotype group in non-diabetic patients. Rare homozygotes for both SNPs showed lower mean values than both common homozygotes and heterozygotes. The observed reductions ranged between 13.9% and 19.7%. The maximum reduction observed applied to the comparison between rare homozygotes and heterozygotes in relation to insulin for rs1470579 (Table 4 and Fig. 1).

Fig. 2 illustrates the variation in plasma insulin, HOMA-IR and HOMA-%B observed for each of the three genotype groups for rs46522 and rs6949019 in non-diabetic patients. The absence of significant associations for these SNPs (Tables 5 and 6) can be seen graphically in Fig. 2, although rare homozygotes for rs6949019 showed the lowest values for plasma insulin, HOMA-IR and HOMA-%B.

We repeated the association analyses with quantitative traits by including the T2D patients (supplementary Tables 1–4, and supplementary Figs. 1 and 2). The results observed were essentially the same, with two exceptions. Firstly, the associations between the traits fasting insulin and HOMA-IR were weaker, with $0.011 < P < 0.031$ (rs4402960), and $0.004 < P < 0.011$ (rs1470579), both under the recessive model. And secondly, the associations with weight, BMI and waist circumference became non-significant, $0.051 < P < 0.091$ for both SNPs under the recessive model.

Table 7 summarises the strength of the associations found between each of rs4402960 and rs1470579 and three traits, when T2D patients are either included or removed from the analyses. Removing T2D patients increased the significance of the associations. Also shown in Table 7 is the *P* value observed in multiple regression analyses adjusting for sex, age and BMI. The associations with HOMA-%B remained significant in all cases. The same applied to fasting insulin, with the exception of rs4402960 when diabetic patients were included. However, the associations with HOMA-IR were ablated when adjusting for sex, age and BMI in all cases with the exception of

Table 2
Numbers of T2D patients and non-diabetic patients observed for each genotype in all four SNPs analysed in this work.

SNP	Genotype	T2D patients	Non-diabetics
4402960	GG	126 (10.83%)	1037
	GT	146 (13.79%)	913
	TT	31 (14.15%)	188
rs1470579	AA	139 (11.46%)	1074
	AC	139(13.50%)	891
	CC	31 (13.96%)	191
rs46522	TT	84 (11.08%)	674
	TC	167 (13.63%)	1058
	CC	55 (11.11%)	440
rs6940019	CC	72 (11.46%)	556
	CT	150 (12.63%)	1038
	TT	80 (12.78%)	546

Table 3
Associations observed by linear regression between rs4402960 and type 2 diabetes-related risk traits in non-diabetic patients. P_{ADD}, P_{DOM} and P_{REC} are unadjusted probabilities under the additive, dominant and recessive models respectively. 11 represent common homozygotes; 12, heterozygotes; and 22, rare homozygotes.

	11 (GG)		12 (GT)		22 (TT)		Total				
	N	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	P_{ADD}	P_{DOM}	P_{REC}
Age (y)	1037	76.67 ± 0.18	913	76.60 ± 0.20	188	76.15 ± 0.44	2138	76.60 ± 0.13	0.358	0.564	0.284
Hb A1c (mM)	876	0.47 ± 0.00	755	0.48 ± 0.00	156	0.47 ± 0.00	1787	0.47 ± 0.00	0.755	0.976	0.510
Total cholesterol (mmol/l)	1037	5.83 ± 0.04	913	5.76 ± 0.04	188	5.84 ± 0.08	2138	5.80 ± 0.02	0.503	0.263	0.651
Height (cm)	1036	166.11 ± 0.29	911	166.60 ± 0.31	188	166.11 ± 0.69	2135	166.32 ± 0.20	0.534	0.324	0.747
Weight (kg)	1037	73.90 ± 0.44	910	74.31 ± 0.46	188	71.81 ± 0.99	2135	73.89 ± 0.31	0.343	0.984	0.034
BMI (kg/m^2)	1036	26.72 ± 0.14	910	26.70 ± 0.14	188	26.00 ± 0.33	2134	26.65 ± 0.09	0.134	0.472	0.032
WC (cm)	1037	100.12 ± 0.38	912	100.40 ± 0.38	188	98.31 ± 0.84	2137	100.08 ± 0.26	0.285	0.872	0.032
SBP (mm Hg)	1036	140.91 ± 0.65	913	142.07 ± 0.72	188	141.29 ± 1.46	2137	141.44 ± 0.46	0.409	0.263	0.923
DBP (mm Hg)	1036	73.60 ± 0.28	913	73.99 ± 0.32	188	73.35 ± 0.66	2137	73.75 ± 0.20	0.788	0.488	0.542
Glucose (mmol/l)	1037	5.47 1.00	913	5.53 1.00	188	5.53 1.01	2138	5.53 1.00	0.355	0.177	0.781
Insulin (mmol/l)	1037	7.85 1.02	912	8.00 1.02	188	6.82 1.05	2137	7.85 1.01	0.145	0.850	0.003
HDL cholesterol (mmol/l)	1037	1.55 1.01	913	1.51 1.01	188	1.58 1.02	2138	1.54 1.01	0.384	0.043	0.113
LDL cholesterol (mmol/l)	1037	3.56 1.01	911	3.49 1.01	188	3.53 1.02	2136	3.53 1.01	0.440	0.318	0.996
Triglycerides (mmol/l)	1037	1.06 1.01	913	1.09 1.01	188	1.02 1.03	2138	1.07 1.01	0.980	0.381	0.108
Fat free mass (kg)	821	44.70 1.01	707	45.15 1.01	145	43.82 1.02	1673	44.70 1.01	0.738	0.333	0.339
Coronary calcium	977	137.00 1.08	864	134.29 1.08	178	154.47 1.20	2019	137.00 1.05	0.777	0.991	0.506
HOMA IR	1037	1.92 1.02	912	1.97 1.02	188	1.68 1.05	2137	1.92 1.01	0.221	0.989	0.005
HOMA %B	1035	79.84 1.02	911	80.64 1.02	188	70.11 1.04	2134	79.04 1.01	0.042	0.416	0.001

[a] Means and SE for glucose, insulin, hdl, ldl, plasma triglycerides, fat free mass, coronary calcium, HOMA IR and HOMA %B are geometric; the geometric SE are multiples of the relevant means.

rs1470579 for non diabetic patients. Adjustment just for BMI, had qualitatively the same effect as adjustment which also included sex and age as covariates (data not shown).

We also performed multiple regression analyses in non-diabetic patients between BMI and each of the four SNPs, by adjusting for insulin. The associations found between BMI and both rs4402960 and rs1470579 were ablated ($P>0.45$) when insulin was considered as covariable (data not shown). Indeed the percentage of the variation explained for insulin was double ($R^2=0.4\%$) compared with BMI ($R^2=0.2\%$).

Supplementary Tables 6 and 7 show λ_2/λ_1 for continuous traits and for case control counts computed from results published in the literature and from the present study. The observed values ranged from 0.55 to 1.90 (Mean ± S.E.M. = 1.00 ± 0.02, $N=89$). For case control studies, the values ranged from 0.60 to 1.29, (0.96 ± 0.05, $N=22$).

Supplementary Fig. 3 shows histograms of λ_2/λ_1 for continuous traits where a significant association has been reported ($N=25$) and traits with no significant association ($N=64$). The λ_2/λ_1 distribution for significant associations did not deviate significantly from the normal distribution (Kolmogorov–Smirnov $Z=0.959$, $P=0.304$). However, for non-significant associations, there was a significant deviation from the normal distribution (Kolmogorov–Smirnov $Z=2.421$, $P=1.63\times10^{-5}$). This deviation is not explained by the presence of two outliers in the distribution (with $\lambda_2/\lambda_1=1.81$ and 1.90), since exclusion of both samples still rendered a significant deviation from normality (Kolmogorov–Smirnov $Z=1.737$, $P=0.005$).

There was a significant difference ($P=0.023$) when comparing the number of significant and non-significant associations with values with a $|\lambda_2/\lambda_1-1|\geq0.10$ or $|\lambda_2/\lambda_1-1|<0.10$. In particular, there were less instances of non-significant $|\lambda_2/\lambda_1-1|\geq0.10$ than expected (13 vs 17) and more instances of significant $|\lambda_2/\lambda_1-1|\geq0.10$ than expected (11 vs 7).

For case control studies, the numbers were smaller ($N=22$). Although the percentage of non-significant $|\lambda_2/\lambda_1-1|\geq0.10$ was considerably smaller than the percentage of significant $|\lambda_2/\lambda_1-1|\geq0.10$ (23% vs 55%),

Table 4
Associations observed by linear regression between rs1470579 and type 2 diabetes-related risk traits in non-diabetic patients. P_{ADD}, P_{DOM} and P_{REC} are unadjusted probabilities under the additive, dominant and recessive models respectively. 11 represent common homozygotes; 12, heterozygotes; and 22, rare homozygotes.

	11 (AA)		12 (AC)		22 (CC)		Total				
	N	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	P_{ADD}	P_{DOM}	P_{REC}
Age (y)	1074	76.62 ± 0.18	891	76.57 ± 0.20	191	76.07 ± 0.44	2156	76.55 ± 0.13	0.358	0.597	0.245
Hb A1c (mM)	910	0.47 ± 0.00	727	0.48 ± 0.00	155	0.47 ± 0.01	1792	0.47 ± 0.00	0.730	0.846	0.255
Total cholesterol (mmol/l)	1074	5.82 ± 0.03	891	5.78 ± 0.04	191	5.83 ± 0.08	2156	5.80 ± 0.02	0.645	0.449	0.780
Height (cm)	1073	166.27 ± 0.28	889	166.52 ± 0.32	191	166.06 ± 0.68	2153	166.36 ± 0.20	0.889	0.661	0.651
Weight (kg)	1074	73.85 ± 0.43	888	74.34 ± 0.47	191	71.75 ± 0.98	2153	73.87 ± 0.30	0.363	0.954	0.030
BMI (kg/m^2)	1073	26.65 ± 0.13	888	26.74 ± 0.14	191	26.00 ± 0.32	2152	26.63 ± 0.09	0.263	0.801	0.035
WC (cm)	1074	100.03 ± 0.37	890	100.46 ± 0.38	191	98.39 ± 0.83	2155	100.06 ± 0.25	0.420	0.903	0.040
SBP (mm Hg)	1073	141.04 ± 0.64	891	141.64 ± 0.73	191	141.03 ± 1.45	2155	141.29 ± 0.46	0.734	0.588	0.857
DBP (mm Hg)	1073	73.63 ± 0.28	891	74.05 ± 0.32	191	73.26 ± 0.65	2155	73.77 ± 0.20	0.847	0.484	0.429
Glucose (mmol/l)	1074	5.47 1.00	891	5.53 1.00	191	5.47 1.01	2156	5.53 1.00	0.378	0.193	0.777
Insulin (mmol/l)	1074	7.77 1.02	890	8.08 1.02	191	6.75 1.04	2155	7.77 1.01	0.194	0.889	0.001
HDL cholesterol (mmol/l)	1074	1.55 1.01	891	1.51 1.01	191	1.58 1.02	2156	1.54 1.01	0.483	0.077	0.131
LDL cholesterol (mmol/l)	1074	3.56 1.01	889	3.49 1.01	191	3.53 1.02	2154	3.53 1.01	0.511	0.423	0.932
Triglycerides (mmol/l)	1074	1.06 1.01	891	1.11 1.01	191	1.02 1.03	2156	1.07 1.01	0.995	0.296	0.068
Fat free mass (kg)	853	44.70 1.01	689	45.15 1.01	146	43.82 1.02	1688	44.70 1.01	0.844	0.454	0.378
Coronary calcium	1013	135.64 1.08	842	139.77 1.08	180	152.93 1.20	2035	138.38 1.05	0.599	0.716	0.576
HOMA IR	1074	1.90 1.02	890	1.97 1.02	191	1.65 1.05	2155	1.92 1.01	0.279	0.755	0.003
HOMA %B	1072	79.84 1.02	889	80.64 1.02	191	69.41 1.04	2152	79.04 1.01	0.064	0.658	0.001

[a] Means and SE for glucose, insulin, hdl, ldl, plasma triglycerides, fat free mass, coronary calcium, HOMA IR and HOMA %B are geometric; the geometric SE are multiples of the relevant means.

Table 5
Associations observed by linear regression between rs46522 and type 2 diabetes-related risk traits in non-diabetic patients. P_{ADD}, P_{DOM} and P_{REC} are unadjusted probabilities under the additive, dominant and recessive models respectively. 11 represent common homozygotes; 12, heterozygotes; and 22, rare homozygotes.

	11 (TT)		12 (TC)		22 (CC)		Total				
	N	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	P_{ADD}	P_{DOM}	P_{REC}
Age (y)	674	76.57 ± 0.23	1058	76.57 ± 0.18	440	76.65 ± 0.28	2172	76.59 ± 0.13	0.857	0.950	0.807
Hb A1c (mM)	558	0.48 ± 0.00	877	0.47 ± 0.00	370	0.47 ± 0.00	1805	0.47 ± 0.00	0.050	0.061	0.194
Total cholesterol (mmol/l)	674	5.75 ± 0.04	1058	5.86 ± 0.04	440	5.77 ± 0.05	2172	5.81 ± 0.02	0.517	0.096	0.439
Height (cm)	673	166.26 ± 0.35	1056	166.23 ± 0.29	440	166.59 ± 0.45	2169	166.31 ± 0.20	0.605	0.857	0.481
Weight (kg)	672	73.96 ± 0.54	1057	73.68 ± 0.43	440	74.15 ± 0.69	2169	73.86 ± 0.30	0.901	0.822	0.634
BMI (kg/m^2)	672	26.72 ± 0.17	1056	26.60 ± 0.13	440	26.63 ± 0.21	2168	26.64 ± 0.09	0.705	0.595	0.956
WC (cm)	673	100.06 ± 0.46	1058	100.02 ± 0.36	440	100.38 ± 0.58	2171	100.10 ± 0.25	0.700	0.909	0.584
SBP (mm Hg)	674	140.78 ± 0.80	1057	141.46 ± 0.65	440	141.72 ± 1.02	2171	141.30 ± 0.45	0.445	0.443	0.644
DBP (mm Hg)	674	73.88 ± 0.36	1057	73.73 ± 0.29	440	73.53 ± 0.43	2171	73.74 ± 0.20	0.541	0.626	0.606
Glucose (mmol/l)	674	5.53 1.00	1058	5.53 1.00	440	5.53 1.00	2172	5.53 1.00	0.475	0.427	0.730
Insulin (mmol/l)	674	7.85 1.02	1058	7.77 1.02	439	7.77 1.03	2171	7.77 1.01	0.874	0.885	0.910
HDL cholesterol (mmol/l)	674	1.54 1.01	1058	1.54 1.01	440	1.55 1.01	2172	1.54 1.01	0.524	0.838	0.375
LDL cholesterol (mmol/l)	674	3.49 1.01	1056	3.56 1.01	440	3.49 1.01	2170	3.53 1.01	0.684	0.155	0.358
Triglycerides (mmol/l)	674	1.06 1.02	1058	1.08 1.01	440	1.05 1.02	2172	1.07 1.01	0.896	0.422	0.248
Fat free mass (kg)	506	44.70 1.01	851	44.70 1.01	338	45.15 1.01	1695	44.70 1.01	0.592	0.997	0.347
Coronary calcium	643	126.47 1.11	1003	152.93 1.08	407	120.30 1.13	2053	137.00 1.05	0.973	0.278	0.229
HOMA IR	674	1.92 1.03	1058	1.90 1.02	439	1.90 1.03	2171	1.92 1.01	0.799	0.803	0.872
HOMA %B	673	79.04 1.02	1056	79.04 1.02	439	79.04 1.03	2168	79.04 1.01	0.848	0.815	0.946

[a] Means and SE for glucose, insulin, hdl, ldl, plasma triglycerides, fat free mass, coronary calcium, HOMA IR and HOMA %B are geometric; the geometric SE are multiples of the relevant means.

the 2×2 table comparing the number of significant and non-significant associations with values with a $|\lambda_2/\lambda_1 - 1| \geq 0.10$ or $|\lambda_2/\lambda_1 - 1| < 0.10$ rendered a non-significant association ($P = 0.655$).

4. Discussion

This study has focused on the gene family encoding *IGF2* mRNA binding proteins, *IGF2BP1*, *IGF2BP2* and *IGF2BP3*, in relation to metabolic syndrome traits. We have replicated previous associations found between *IGF2BP2* and T2D status. In non-diabetic patients, we have characterised associations with the related quantitative traits fasting insulin, HOMA-IR and HOMA-%B. However, we did not find any significant association between SNPs at *IGF2BP1* or *IGF2BP3* and T2D status or related traits.

Our regression analyses contain 17 traits, 4 SNPs and three genetic models, at worst demanding a Bonferroni correction of 204. Under this model, all significances would be eliminated. More realistically [28], many of the traits are interrelated, such as BMI and WC with weight, systolic with diastolic BP, insulin and glucose with HOMA-IR and HOMA-%B. The two *IGF2BP2* SNPs are in LD. Most importantly, known gene function and literature permits prior hypotheses on the effect of *IGF2BP2* SNPs in relation to T2D. Our *IGF2BP2* results are replications of the effect of SNPs known to influence T2D and, as such, they provide stronger evidence of the presence of a quantitative trait locus T2D in *IGF2BP2*. In addition, our results represent descriptive extensions of the mechanism of action of *IGF2BP2* on T2D and highlight the importance of the genetic model considered in genetic studies of *IGF2BP2* and T2D.

Our results add to the evidence supporting a direct involvement of genetic variation in *IGF2BP2* on T2D, with the T allele of rs4402960 being the risk allele. In our sample, the predisposition to T2D conferred by this allele is mainly associated with lower levels of insulin and impaired β-cell function. Previous evidence has shown that rs4402960 TT homozygotes associate with the lowest levels of

Table 6
Associations observed by linear regression between rs6949019 and type 2 diabetes-related risk traits in non-diabetic patients. P_{ADD}, P_{DOM} and P_{REC} are unadjusted probabilities under the additive, dominant and recessive models respectively. 11 represent common homozygotes; 12, heterozygotes; and 22, rare homozygotes.

	11 (CC)		12 (CT)		22 (TT)		Total				
	N	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	*N*	Mean SE[a]	P_{ADD}	P_{DOM}	P_{REC}
Age (y)	556	76.67 ± 0.26	1038	76.51 ± 0.18	546	76.58 ± 0.25	2140	76.57 ± 0.13	0.795	0.641	0.967
Hb A1c (mM)	477	0.47 ± 0.00	863	0.48 ± 0.00	450	0.47 ± 0.00	1790	0.47 ± 0.00	0.857	0.805	0.583
Total cholesterol (mmol/l)	556	5.84 ± 0.05	1038	5.80 ± 0.03	546	5.75 ± 0.05	2140	5.80 ± 0.02	0.202	0.358	0.241
Height (cm)	555	165.98 ± 0.38	1037	166.65 ± 0.30	545	166.10 ± 0.39	2137	166.34 ± 0.20	0.819	0.294	0.497
Weight (kg)	556	73.34 ± 0.59	1037	74.28 ± 0.44	544	73.47 ± 0.61	2137	73.83 ± 0.30	0.870	0.344	0.494
BMI (kg/m^2)	555	26.57 ± 0.18	1037	26.68 ± 0.13	544	26.56 ± 0.19	2136	26.62 ± 0.09	0.984	0.735	0.708
WC (cm)	556	99.60 ± 0.49	1038	100.39 ± 0.36	546	99.72 ± 0.54	2140	100.01 ± 0.26	0.857	0.338	0.505
SBP (mm Hg)	556	141.62 ± 0.85	1037	141.73 ± 0.67	546	139.92 ± 0.90	2139	141.24 ± 0.46	0.183	0.620	0.090
DBP (mm Hg)	556	73.68 ± 0.40	1037	74.02 ± 0.30	546	73.31 ± 0.37	2139	73.75 ± 0.20	0.513	0.837	0.199
Glucose (mmol/l)	556	5.47 1.00	1038	5.53 1.00	546	5.53 1.00	2140	5.53 1.00	0.654	0.622	0.809
Insulin (mmol/l)	556	7.85 1.03	1038	7.85 1.02	545	7.61 1.03	2139	7.77 1.01	0.429	0.617	0.425
HDL cholesterol (mmol/l)	556	1.55 1.01	1038	1.54 1.01	546	1.54 1.01	2140	1.54 1.01	0.490	0.360	0.829
LDL cholesterol (mmol/l)	556	3.56 1.01	1037	3.53 1.01	545	3.46 1.01	2138	3.53 1.01	0.173	0.327	0.208
Triglycerides (mmol/l)	556	1.06 1.02	1038	1.08 1.01	546	1.05 1.02	2140	1.07 1.01	0.598	0.719	0.219
Fat free mass (kg)	426	44.26 1.01	817	45.15 1.01	429	44.26 1.01	1672	44.70 1.01	0.700	0.229	0.570
Coronary calcium	523	130.32 1.12	975	145.47 1.08	524	134.29 1.12	2022	138.38 1.05	0.892	0.533	0.688
HOMA IR	556	1.92 1.03	1038	1.92 1.02	545	1.88 1.03	2139	1.90 1.01	0.500	0.692	0.476
HOMA %B	556	80.64 1.02	1035	79.04 1.02	545	77.48 1.02	2136	79.04 1.01	0.221	0.293	0.339

[a] Means and SE for glucose, insulin, hdl, ldl, plasma triglycerides, fat free mass, coronary calcium, HOMA IR and HOMA %B are geometric; the geometric SE are multiples of the relevant means.

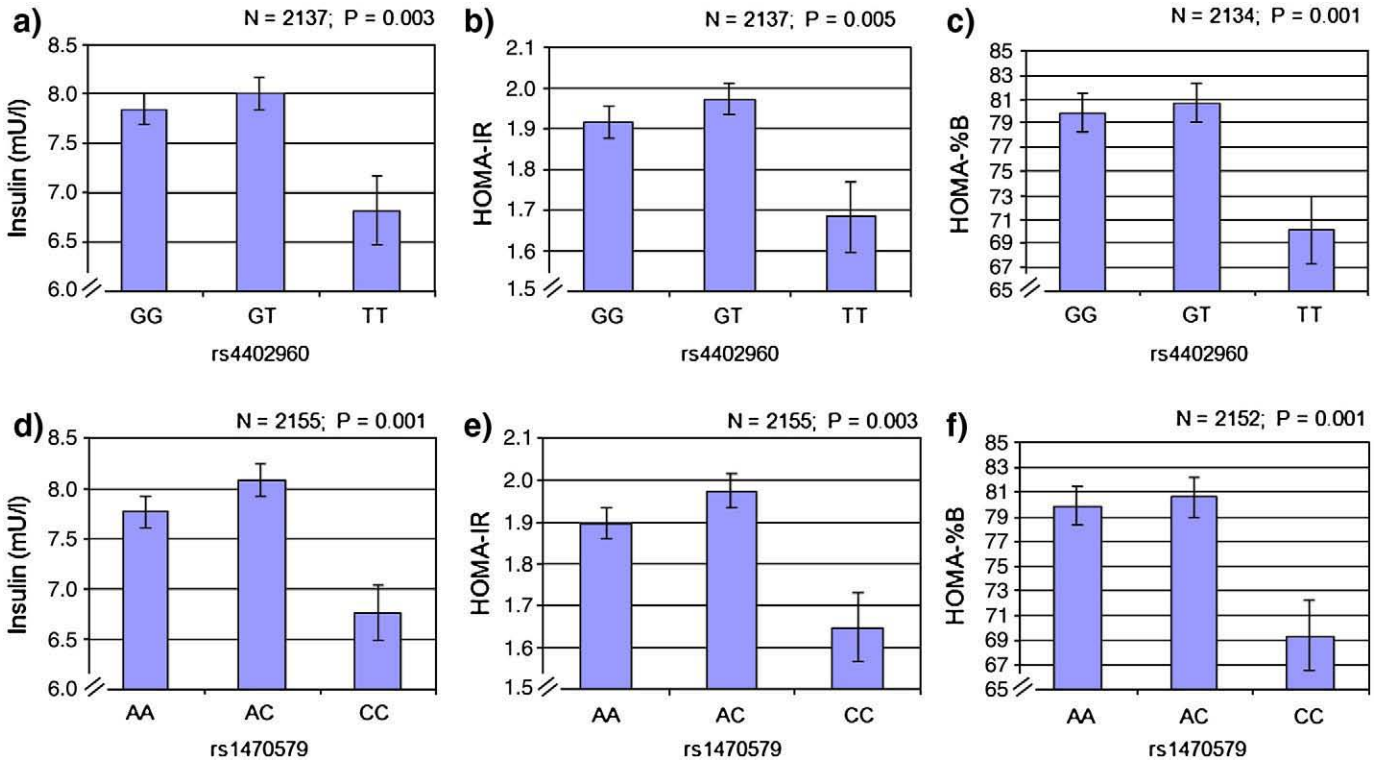

Fig. 1. Graphical summary of the significant associations found under the recessive model for quantitative traits related to T2D when T2D patients are not considered in the analysis. The bars represent the mean values observed for each genotype for rs4402960 [a), b) and c)] and for rs1470579 [d), e) and f)]. The standard error of the mean is also shown for each case.

first-phase insulin response [10,12]. The same applies to impaired β-cell function as measured by HOMA-%B or by the disposition index [9,10,12,14–16].

It has been suggested that *IGF2BP2* could influence on T2D indirectly. An association of *IGF2BP2* with obesity has been reported, and it has been suggested that this would influence insulin resistance,

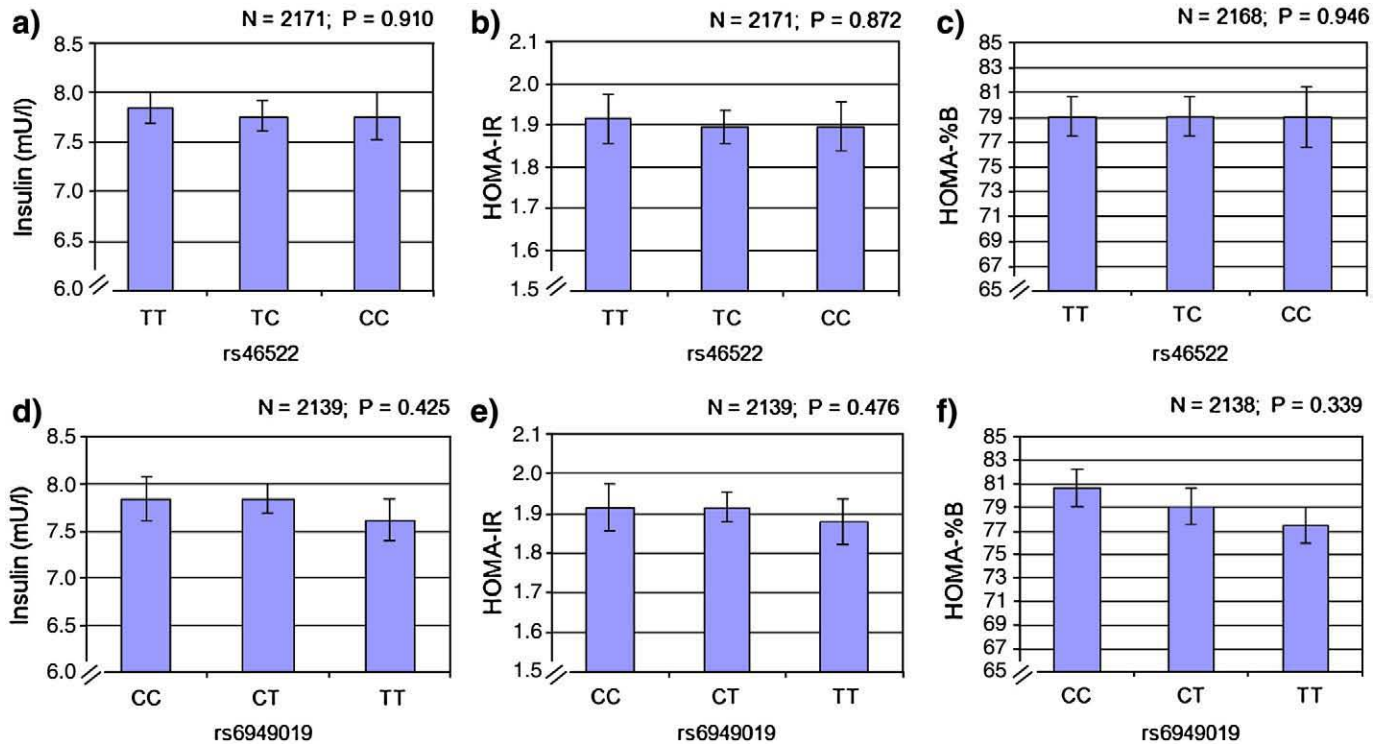

Fig. 2. Graphical summary of the significant associations found under the recessive model for quantitative traits related to T2D when T2D patients are not considered in the analysis. The bars represent the mean values observed for each genotype for rs46522 [a), b) and c)] and for rs6949019 [d), e) and f)]. The standard error of the mean is also shown for each case.

Table 7
Associations observed by linear and multiple regressions between *IGF2BP2* SNPs and type 2 diabetes-related traits in the whole sample of Icelanders and in a subset excluding T2D patients. Associations were tested under the recessive model in all cases.

	Including T2D patients				Excluding type 2 diabetics			
	rs4402960		rs1470579		rs4402960		rs1470579	
	P_1	P_2	P_1	P_2	P_1	P_2	P_1	P_2
Insulin	0.013	0.095	0.004	0.041	0.003	0.040	0.001	0.021
HOMA-IR	0.031	0.193	0.011	0.105	0.005	0.063	0.003	0.036
HOMA-%B	0.003	0.025	0.001	0.007	0.001	0.021	0.001	0.009

P_1 = unadjusted probability; P_2 = adjusted for sex, age and BMI.

ultimately leading to T2D [29]. Our results show that the significant association between insulin resistance (HOMA-IR) and *IGF2BP2* is secondary to an association of *IGF2BP2* with obesity, since adjustment for BMI ablates the association between HOMA-IR and *IGF2BP2* SNPs. These results are in agreement with previous associations between *IGF2* genetic variants and obesity indices [28,30]. We show that consideration of BMI as a covariate does not ablate the relationship between *IGF2BP2* SNPs genotypes and insulin. However, the apparent association between *IGF2BP2* SNPs genotypes and BMI is ablated when adjusting for insulin. In addition, both the lower fasting insulin levels and the impaired β-cell function are independent of obesity phenotypes. This suggests that *IGF2BP2* SNPs would have a direct effect on β-cell function, resulting in lower levels of insulin. As a consequence, the association between *IGF2BP2* SNPs and obesity indices would be secondary. This would be consistent with a scenario where lower insulin in the body would result in a reduction in the overall anabolic effects of insulin, which would lead to lower BMI due to the reduced incorporation of glucose into the tissues (Fig. 3).

In this study, we observed contrasting effects of *IGF2BP2* genotype on T2D risk compared with effect on plasma insulin levels, the former being statistically significant under a dominant model, the latter under a recessive model. Rare (TT) homozygotes for rs4402960 were distinctive in displaying a lower mean insulin level (among non-diabetic patients), the other two genotype groups being similar with each other for insulin level. By contrast, both TT and TG genotype groups showed an increased risk, of similar magnitude, for T2D, compared with common (GG) homozygotes. One possibility is that TG heterozygotes might compensate their plasma insulin level to some physiological threshold, but that ultimately this might not avert diabetic risk compared with TT homozygotes. Our analysis of the genetic model for these traits in other published studies suggests non-additive allelic effects (Supplementary Tables 6 and 7), but the data available were insufficient either to confirm or refute the generality of the observations made in our study. Factors such as age, sex, environmental differences and genetic ancestry might impact on the observed genetic model. For a more general discussion and hypothesis about setpoints and thresholds in relation to the genetic endocrinology of the metabolic syndrome, see Ref. [31].

We also report here relevant negative associations. We have not found association with hypertension or serum lipid levels, two later-stage changes observed in T2D. This is both in agreement with a previous work [32], and in accordance with a mechanism of action of IGF2BP2 influencing early-stage features of T2D. Previous evidence suggests the involvement of IGF2BP2 on first-phase insulin secretion but not on second-phase insulin secretion [10]. The absence of association between T2D (and related traits) and the two SNPs near *IGF2BP1* and *IGF2BP3* showing in GWAS nominal association with coronary artery disease and T2D respectively suggests that these signals did not represent biological effect. Our results support that this cannot be attributed to lack of power in our study. However, our observation that rare homozygotes for the SNP marking *IGF2BP3* show comparatively (although non-significant) lower values for plasma insulin, HOMA-IR and HOMA-%B than heterozygotes and common homozygotes, suggests a possible effect of *IGF2BP3* genetic variation on these traits in the same direction than that observed for *IGF2BP2* SNPs. Subject to denser scanning in large cohorts, it appears that IGF2BP2 is the only, and sufficient, contributor among the *IGF2* mRNA binding proteins to affect T2D traits.

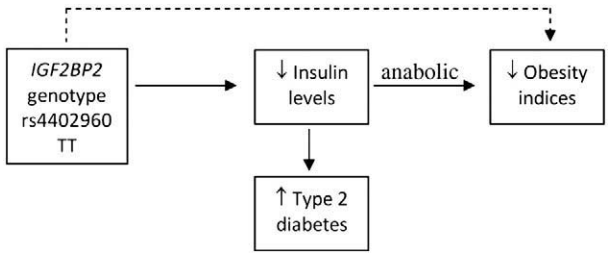

Fig. 3. Schematic representation of the relationships between *IGF2BP2* genotype, insulin levels, obesity indices and T2D.

The biological mechanism by which *IGF2BP2* would act on T2D and related traits is unknown [5]. Early-phase insulin release is triggered by Ca^{2+} and results in the release of 50–100 granules from a β-cell from a total of about 10,000 granules in the cell [33]. IGF2BP2 binds and regulates *IGF2* mRNA, and the latter has a direct effect on body mass. It is possible that IGF2BP2 would reduce the size or number of β-cells by sequestering *IGF2* mRNA in these cells, resulting in a reduced insulin secretion. In addition, the effect of early-phase insulin release on maintenance of glucose homeostasis occurs in the liver [34], and it is there where IGF-II is mainly synthesised and secreted to the circulation [35]. Another possibility is that the regulation of *IGF2* by IGF2BP2 could result in a differential expression of this growth factor in different tissues. This would alter the β-cell function ratios of other possible factors influencing T2D and enhance or reduce their effect in relation to this disease and related phenotypes.

rs4402960 is located within a long haplotype block with isofrequent alleles spanning most of intron 2 and possibly intron 1 (http://www.hapmap.org). This suggests that this is a conserved region. Actually, HMGA2, a regulator of IGF2BP2 binds a pyrimidine rich motif spanning part of intron 1 [36]. Then, alleles marked by rs4402960 could be differentially regulated by HMGA2, that could alter the function of IGF2BP2 and ultimately the expression of *IGF2*. Another possible molecular mechanism is the involvement of *INSIGF* transcripts. At least one of the hybrid transcripts combining genetic information from the neighbouring genes *INS* and *IGF2*, is expressed in the pancreas [37]. However, these isoforms do not contain *IGF2* leader 3. A hypothesis linking *INSIGF* isoforms on IGF2BP2 action requires additional evidence.

Our study shares features with previous studies that reported significant associations between *IGF2BP2* and T2D or related traits. These include large sample sizes, non-significant deviations from Hardy–Weinberg proportions and analysis of relevant phenotypes such as T2D status and β-cell function. Non-replications of *IGF2BP2* associations with T2D have been related to factors including ethnic differences [9,38] and low powered studies [5]. In addition to these possible causes, we have identified in our study other possible factors. A first factor relates to genotyping. We identified a technical null allele in rs1470579 that could originate from mispriming of the forward primers used for its amplification. Such mispriming would be produced by the presence of a second SNP in the primer region and could lead to the incorrect typing of heterozygotes as common homozygotes. Mutations in the priming region are common causes of apparent null alleles [39–41]. Our results have shown a diplotype (marked by the common homozygote genotype, AA, at rs1470579) that associated with increased T2D risk. On the contrary, common homozygotes (GG) for rs4402960, associated with lower proportion of T2D. Given that both SNPs (rs4402960 and rs1470579) are nearly in perfect LD ($r^2 = 0.954$) one would expect comparable associations

with T2D. In the absence of a possible biological basis for this observation, a possible explanation for the lack of association between T2D status and rs1470579 would be the over-representation of diabetic patients among rs1470579 common homozygotes (AA), that would be produced by a technical null allele in rs1470579.

A second factor possibly is confounding originated by including T2D cases in the study. We have shown that the exclusion of T2D patients resulted in stronger evidence of association between T2D related continuous traits and *IGF2BP2*, with up to a ten-fold difference in the magnitude of the association. Importantly, new significant associations with obesity indices (that were not detected in the whole AGES-Reykjavik sample) were found in non-diabetic patients. We have shown empirically that the consideration of mixed samples including T2D patients can alter the results found. The presence of T2D patients represents a confounding factor in the genetic analysis of T2D risk (and related phenotypes) from cohort studies.

A third factor is the genetic model considered. As we have discussed previously, we have observed associations dependent on the genetic model, with potential relevance for the interpretation of the underlying mechanism of action. Although many published studies analysing *IGF2BP2* have taken into account several genetic models, others have reported only the additive model e.g. [10–12,14,15,32,42–46]. This could lead to failure of finding and reporting significant associations. A recent review [47] has highlighted the importance of the underlying genetic model in T2D associations. The probability that the genetic inheritance model for *IGF2BP2* is additive for the association with T2D status has been calculated to be 89%, (11% for a dominant model) [47]. Our data suggests a non-multiplicative model in the *IGF2BP2* genotype association with T2D and related traits. We have also examined all other reports where genotype counts data were presented and find suggestion of deviations from the multiplicative model (Supplementary Tables 6 and 7). Such an effect could contribute to inconsistencies of reported statistical significance, but we estimate that the deviation would not be more than 20% between studies. Several reports have found association between *IGF2BP2* and T2D status under the dominant [8,48,49] or additive model [9,50,51]. These observations agree with our association results for T2D risk. Our work highlights that the underlying genetic model may differ between a binary disease status and continuous phenotypes related to the disease.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.ghir.2010.04.002.

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