Role of genetic variation in insulin-like growth factor 1 receptor on insulin resistance and arterial hypertension

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Objective **To perform a two-stage study to explore the role of gene variants in the risk of insulin resistance and arterial hypertension.**

Methods and results **The selection of variants was performed by a first stage of in-silico analysis of the original genome-wide association data sets on genes involved in metabolic syndrome components, granted by the Diabetes Genetics Initiative and the Wellcome Trust Case–Control Consortium. We started by identifying single-nucleotide polymorphisms with a cutoff for association ($P<0.05$) in both data sets after the application of a computational algorithm of gene prioritization. Among the more promising variants, six single-nucleotide polymorphisms in *IGF1R* (rs11247362, rs10902606, rs1317459, rs11854132, rs2684761, and rs2715416) were selected for further evaluation in our population. Altogether, 1094 men, aged 34.4 ± 8.6 years, were included in a population-based study. Genotypes of rs2684761 showed significant association with insulin resistance (as a discrete trait, odds ratio per G allele 1.27, 95% confidence interval 1.03–1.56, $P=0.026$; and homeostasis model assessment-insulin resistance as a continuous trait, $P=0.01$). A significant association of rs2684761 with arterial hypertension was also observed (odds ratio per G allele 1.29, 95% confidence interval 1.02–1.64, $P=0.037$) after adjusting for age and homeostasis model assessment-insulin resistance.**

Conclusion **Our study suggests for the first time a putative role of *IGF1R* variants in individual susceptibility to metabolic syndrome-related phenotypes, in particular on the risk of having insulin resistance and arterial hypertension. *J Hypertens* 28:1194–1202 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Abbreviations: DGI, Diabetes Genetics Initiative; GWA, genome-wide association; HOMA, homeostasis model assessment; IGF1R, insulin-like growth factor 1 receptor precursor; SBP, systolic blood pressure; T2D, type 2 diabetes; WTCCC, Wellcome Trust Case–Control Consortium

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Introduction

The metabolic syndrome is characterized by insulin resistance and includes a constellation of complex diseases such as type 2 diabetes (T2D), dyslipidemias, central obesity, arterial hypertension, prothrombotic and proinflammatory states, ovarian polycystosis, and fatty liver disease. The current evidence indicates that each main component of metabolic syndrome has a genetic basis, but the genetics of each of these diseases is complex by itself, and vary in spectrum from monogenic and syndromic forms, usually rare, to the most common polygenic and multifactorial forms [1].

Advances in genome analysis, including the first wave of large-scale, high-density genome-wide association (GWA) studies, have significantly contributed to the understanding of the genetic architecture of human complex diseases, including metabolic syndrome-related phenotypes. Although genome-wide platforms yielded several promising signals of association between gene variants and either human disease or related quantitative traits, some challenges still remain. For example, the selection of statistically significant associations with those single-nucleotide polymorphisms (SNPs) that show the most extreme P value (as small as 10^{-7}) followed by a robust replication that enables identification of a true-positive signal [2] is not cost-effective. Unfortunately, although the application of stringent statistical thresholds in GWA studies is needed to safely confirm association, it may dismiss many SNPs that will not be carried forward in the second stage of the analysis.

An additional disadvantage to constraining the variant selection to those with small P values for association is the potential exclusion of those SNPs that are biologically important for the pathogenesis of the disease. Hence, another approach to circumvent the drawbacks of genome-wide significance may be the strategy of gene prioritization offered by several bioinformatic tools that prioritize SNPs based on the biological plausibility of a

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gene–disease association, an approach we have used that rendered new loci associated to T2D [3].

In view of the evidence described above, we performed a two-stage study to explore the role of gene variants in metabolic syndrome-related phenotypes, in particular insulin resistance and arterial hypertension. The first stage included an in-silico exploratory assessment of the original GWA data sets on genes implicated in common human diseases, granted by the Diabetes Genetics Initiative (DGI) and the Wellcome Trust Case Control Consortium (WTCCC), and available at public websites after obtaining a prioritized gene list based on a bioinformatic tool. The second stage was a candidate-gene association study focused on specific variants of interest in a sample of Argentinean young men.

Research design and results

First stage: in-silico analysis of genome-wide association open data

The methodology for obtaining the prioritized gene list and the in-silico analysis of the GWA data sets was carried out as we previously described [3]. Briefly, from the web site http://www.broad.mit.edu/diabetes/, we downloaded the results of the GWA study in 3000 Scandinavian individuals about the genetic variants that influence the risk of T2D and related metabolic traits (1464 patients with T2D and 1467 matched controls). We also included in the in-silico analysis the results of the GWA study on seven common diseases performed by the WTCCC (1930 patients and 2936 control samples), which were downloaded for free from http://www.wtccc.org.uk/info/summary_stats.shtml. We focused our analysis on the open data about metabolic syndrome-related traits available in each data set as follows: homeostasis model assessment (HOMA) to evaluate insulin resistance index, calculated as fasting serum insulin (μU/ml) $\times$ fasting plasma glucose (mmol/l)/22.5; BMI calculated as weight/height^2 (kg/m^2); and the dichotomous hypertension trait and systolic blood pressure (SBP) data. Data on BMI and SBP in patients and controls, and HOMA in controls were only available from the DGI database. Both data sets enable the analysis of the dichotomous hypertension trait.

We incorporated a total of 386 731 and 459 653 gene variants from the original analysis of the DGI and WTCCC studies, respectively [including those SNPs that passed the quality control filters and had a study minor allele frequency (MAF) >1%] [4].

A comprehensive analysis of candidate regions was generated by the freely accessible ENDEAVOUR software [5] available at http://homes.esat.kuleuven.be/~bioiuser/endeavour/endeavour.php (K.U. Leuven Research & Development. Leuven, Belgium), as we previously described [3]. ENDEAVOUR is a software application for the computational prioritization of candidate genes underlying biological processes or diseases based on their similarity to previously known genes involved in a disease [5]. The hypothesis of prioritization by ENDEAVOUR is that candidate test genes are ranked based on their similarity with a set of known training genes. Details about the training genes were previously published [3]. Briefly, the training genes were automatically downloaded from the software from a list generated by the following key words: obesity, hypertension, type 2 diabetes, and dyslipidemias. The training set was created by choosing 70 genes involved with either metabolic pathways related with metabolic syndrome components or with data about genes that are known to be connected with associated biological processes [3].

Results of the in-silico analysis and gene prioritization

After applying the ENDEAVOUR algorithm for gene prioritization, we performed a search in the GWA open data for the prioritized genes that showed, in both data sets, a *P* value smaller than 0.05 for the test of association with metabolic syndrome-related phenotypes (we further name this *P* value as screening *P* value). This is the step in which we merged both data sets, and the screening *P* value was a condition required to be simultaneously present in both data sets to continue further with the analysis.

The results of the first 10 ranked genes from the whole-genome prioritization (23 712 genes) by the ENDEAVOUR software are shown in Table 1. In first stage, we analyzed on the GWA data sets the results regarding all the SNPs in the first 10 prioritized genes to look for association with metabolic syndrome-related phenotypes at the screening *P* value. From all the evaluated SNPs, we selected for the candidate-gene association study in our population, the variants located in the insulin-like growth factor 1 receptor (*IGF1R*) precursor, a gene that ranked fifth in the whole-genome prioritization list. *IGF1R* variants were chosen because they consistently exhibited the screening *P* value for association with several metabolic-related phenotypes in both GWA data sets. Results for the *IGF1R* variants with *P* values less than 0.05 for association with metabolic syndrome-related phenotypes either in the DGI or in the WTCCC-GWA data set are shown in Table 2. The remaining nine genes of our whole-genome prioritization list did not show SNPs with a *P* value smaller than 0.05 for the test of association with metabolic syndrome-related phenotypes.

Second stage: a candidate gene association study – subject ascertainment, physical, anthropometric, and biochemical evaluation

Healthy individuals recruited from a factory in the Buenos Aires metropolitan area who underwent annual health examination were invited to participate in this study. Altogether, 1094 men of self-reported European ancestry were included in this study. The study group

Table 1 List of the top 10 genes prioritized from the whole genome by the ENDEAVOUR identification tool

Ranking order	(HGNC symbol)	Gene function	Genomic location/mapping in chromosome	Tissue distribution
1	*HNF4A*	Regulates expression of genes required for glucose transport and metabolism	42.463.324–42.493.444. 20q12–q13.1	Digestive, reproductive, respiratory, and urinary
2	*CYP3A43*	Major drug-metabolizing subfamily	99.263.572–99.302.109. 7q21.1	Liver, gastrointestinal tract, and kidney
3	*IRS4*	Mediates the biological response to insulin stimulation by binding and activating various enzymes or adaptor molecules	107.862.368–107.866.295. Xq22.3	Muscular
4	*GYS2*	Rate-limiting enzyme of the insulin-induced glycogenesis	21.580.392–21.648.821. 12p12.2–p11.2	Liver
5	*IGF1R*	Insulin-like growth factor 1 receptor with high affinity	97.010.302–97.319.034. 15q26.3	Ubiquitous
6	*PPARA*	Transcriptional activator activity	44.925.163–45.018.317. 22q13.31	Ubiquitous
7	*INSRR*	Mediates the action of a ligand that is identical with or very similar to insulin	155.076.479–155.095.434. 1q21–q23	Kidney
8	*AKT3*	Key regulator for cell growth, cell survival, and metabolic insulin action	241.718.158–242.080.053. 1q43–44	Ubiquitous
9	*ADIPOQ*	Regulates energy homeostasis and glucose and lipid metabolism	188.043.157–188.058.944. 3q27.	Adipose tissue
10	*NOS1*	Nitric oxide biosynthesis, electron transport, synaptic transmission, muscle contraction, and cell–cell signaling	116.135.362–116.283.965. 12q24.2–q24.31	Ubiquitous

HGNC, HUGO Gene Nomenclature Committee

was composed of individuals who were randomly enrolled and were willing to participate in a study of the genetic susceptibility of metabolic syndrome disease components. None of them were enrolled on the basis of phenotype.

Medical history was investigated using a self-administered questionnaire. In addition, the responses were confirmed by individual interviews conducted by occupational physicians. Health examinations included anthropometric measurements, a questionnaire on health-related behaviors, and biochemical determinations.

After a 30-min rest in a quiet room, the SBP and diastolic blood pressure (DBP) was measured on the right arm with a standard mercury sphygmomanometer while the patient was in sitting position. The blood pressure (BP) values were the means of three different measurements. BP in the fasting state was taken by the same trained person in the morning, using a mercury sphygmomanometer and an appropriately sized cuff according to standard procedures.

BMI was calculated as weight/height^2 (kg/m^2) and was used as the index for relative weight. In addition, trained staff assessed waist circumference in the standing position, midway between the highest point of the iliac crest and the lowest point of the costal margin in the midaxillary line. Hip circumference was measured at the level of the femoral greater trochanter by the same observer. Those with a BMI of 30 or more were classified as obese.

All participants were asked to fast for at least 8 h, and blood was drawn from participants who had lain in supine resting position for at least 30 min. Serum insulin, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)-cholesterol, triglycerides,

Table 2 Results for *IGF1R* variants with *P* values less than 0.05 for association with metabolic syndrome-related phenotypes either in the Diabetes Genetics Initiative or Wellcome Trust Case–Control Consortium Genome-wide association data set

NCBI SNP reference	Type	*P* value by genomic control DGI	*P* value for the additive genetic model WTCCC	*P* value for the general genetic model WTCCC	Physical position (according to NCBI build 35)	Minor allele	Major allele
IGF1R-SNPs with *P* values less than 0.05 for association with BMI in patients in the DGI-GWA data set							
rs10902606	Intronic	0.0009	NA	NA	97034517	G	C
rs4284619	Intronic	0.050	NA	NA	97040205	G	A
rs11854132	Intronic	0.02	NA	NA	97046853	A	G
rs11247362	Intronic	0.01	NA	NA	97028354	C	T
IGF1R-SNPs with *P* values less than 0.05 for association with the dichotomous hypertension trait in the DGI and WTCCC-GWA[a] data sets							
rs1317459	Intronic	0.01593	0.00235	0.00235	97046102	G	C
IGF1R-SNPs with *P* values less than 0.05 for association with HOMA in patients in the DGI-GWA data set							
rs2684761	Intronic	0.04	NA	NA	97181893	A	G
rs1317459	Intronic	0.04	NA	NA	97046102	G	C
rs2715416	Intronic	0.04	NA	NA	97271554	C	G

Contig annotation of all the SNPs in the NCBI reference assembly: *IGF1R*. DGI, Diabetes Genetics Initiative; GWA, Genome-wide association; HOMA, homeostatic model assessment; IGF1R, insulin-like growth factor 1 receptor; NA, not available; NCBI, National Center for Biotechnology Information; SNP, single-nucleotide polymorphism; WTCCC, Wellcome Trust Case–Control Consortium. [a]The WTCCC study included 2936 controls and 1930 hypertensive patients. The DGI study did not disclose the number of hypertensive patients included in the analysis.

and plasma glucose were measured by standard clinical laboratory techniques. HOMA was used to evaluate the insulin resistance index and was calculated as fasting serum insulin (μU/ml) $\times$ fasting plasma glucose (mmol/l)/22.5.

Metabolic syndrome was defined as the presence of three of the five main components (elevated levels of BP, serum triglycerides, waist circumference, fasting glucose level, and HDL-cholesterol) according to the National Cholesterol Education Program (NCEP) Adult Panel III guidelines [6]. Specifically, elevated BP was defined as a SBP of at least 130 mmHg and/or DBP 85 mmHg and/or receipt of antihypertensive medications. Low HDL-cholesterol was defined as less than 40 mg/dl, high serum triglycerides as at least 150 mg/dl, elevated total cholesterol as at least 200 mg/dl, and elevated plasma glucose as at least 110 mg/dl. We included a measure of insulin resistance, as it has been shown to provide incremental information regarding atherosclerotic cardiovascular disease beyond the current NCEP Adult Treatment Panel III metabolic syndrome definition [6]. Insulin resistance as a dichotomous variable was defined as a HOMA index of at least 2.4 [7]. Finally, abdominal obesity was defined as a waist circumference of at least 102 cm. We also considered the sum of the number of individual metabolic syndrome components as an additional trait.

A summary of the clinical features, anthropometric variables, and laboratory findings of the participants is shown in Table 3. In the whole population, 178 individuals were classified as hypertensive and 33 were prescribed antihypertensive medication (either beta-blockers or angiotensin I-converting enzyme inhibitors), 221 were classified as having insulin resistance, and 136 were obese, according to the aforementioned definitions.

Table 3 Clinical and biochemical characteristics of the patients in the candidate-gene association study

Variables	Mean $\pm$ SE
Age (years)	34.3 $\pm$ 0.26
BMI (kg/m^2)	26.7 $\pm$ 0.15
Waist circumference (cm)	92.7 $\pm$ 0.39
Waist–hip ratio (cm)	0.93 $\pm$ 0.002
SBP (mmHg)	121.7 $\pm$ 0.44
DBP (mmHg)	76.1 $\pm$ 0.30
Leukocyte count (cells/μl)	6862.1 $\pm$ 54.04
Erythrocyte sedimentation rate (mm/1 h)	7.2 $\pm$ 0.11
Fasting plasma glucose (mmol/l)	5.02 $\pm$ 0.02
Fasting plasma insulin (pmol/l)	59.0 $\pm$ 1.8
HOMA index	1.9 $\pm$ 0.06
Total cholesterol (mmol/l)	4.98 $\pm$ 0.03
HDL-cholesterol (mmol/l)	1.19 $\pm$ 0.01
LDL-cholesterol (mmol/l)	3.12 $\pm$ 0.03
Uric acid (mmol/l)	286 $\pm$ 2
Triglycerides (mmol/l)	1.46 $\pm$ 0.03
Cardiovascular risk[a] (%)	2.5 $\pm$ 0.12
Metabolic syndrome	1.1 $\pm$ 0.03

HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein. All measurements are in SI units. [a] Risk for developing coronary heart disease outcomes using Framingham risk scoring.

All the investigations performed in this study were conducted in accordance with the guidelines of The Declaration of Helsinki. Written consent from individuals had been obtained in accordance with the procedures approved by the Ethical Committee of our institution.

Genotype and haplotype analysis in the candidate-gene association study

The genetic analyses were done on genomic DNA extracted from white blood cells by a standard method, as previously described [8].

Genotyping was performed by a high-throughput genotyping method involving PCR amplification of genomic DNA with two-tailed allele-specific primers that introduce priming sites for universal energy-transfer-labeled primers, as previously described [9], (PreventionGenetics, Marshfield, Wisconsin, USA). To ensure genotyping quality, we included DNA samples as internal controls, hidden samples of known genotypes, and negative controls (water). No genotype with a signal below a negative control was scored. The error analysis was performed by replicating a blinded sample (always belonging to the same individual) across the templates of the project six times. Of the 248 genotypes for the 'blinded sample', we had only one nonmatched genotype (0.40% error); then the observed error rate is estimated to be less than 0.5%. Overall genotype completion rate was nearly 100%.

Haplotype frequencies, and SNP linkage disequilibrium measures and plots were performed by using the Haploview software (http://www.broad.mit.edu/mpg/haploview/) [10]. The PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) was used for assessing the association of SNPs and their haplotypes with the affection status and related quantitative traits, and for testing for Hardy–Weinberg equilibrium [11].

To explore a possible stratification in the population, we used a collection of 13 SNPs at different loci (located in chromosomes 4, 15, 17, 13, 1, and 3), then we analyzed the data with the Structure program version 2 [12] and computed the sum of χ^2 tests from each locus with a number of DF equal to the sum of the number of individual loci [13]. We found no evidence of stratification in our sample because patients and controls showed similar Q values and were assigned a similar distance to clusters by the program structure, with no further improvement in the fitting model by adding up to four clusters (the log-likelihood was maximum for $K = 1$) [14].

Statistical analysis in the candidate-gene association study

Quantitative data were expressed as mean $\pm$ SE. For univariate analysis, differences between groups were assessed by analysis of variance on log-transformed

variables when the variable variance was homogenous as assessed by Levene's test.

For testing the association between genotypes and the number of metabolic syndrome components, we used analysis of covariance (ANCOVA) for an ordinal multinomial distribution (Probit Link function) for the number of metabolic syndrome components as the dependent (response) variable coding controls to fully metabolic syndrome patients as 0, 1, 2, 3, 4, and 5, respectively, with age as a continuous predictor variable and genotype as a categorical factor variable.

Logistic regression was used to test the multivariate association among insulin resistance (as a dichotomous trait as defined above), obesity or arterial hypertension as dichotomous variables, and genotypes and haplotypes, adjusting for covariates as indicated. We used the CSS/Statistica program package StatSoft V 6.0 (Tulsa, Oklahoma, USA) to perform these analyses.

We used ANCOVA for a normal distribution of log-transformed quantitative variables, such as HOMA-insulin resistance (HOMA-IR), and genotype as a categorical factor variable, adjusting for covariates as indicated.

Results of genotyping of the *IGF1R* variants in the candidate-gene association study

No marker showed a departure from Hardy–Weinberg equilibrium ($P > 0.1$), indicating robust genotyping performance. The genotyping success rate was 98% for rs11247362, 97% for rs10902606, 97% for rs1317459, 96% for rs11854132, 96% for rs2684761, and 97% for rs2715416. Figure 1 shows a basic linkage disequilibrium plot between the studied SNPs across the *IGF1R* gene in our population.

The minor allele frequencies of the evaluated SNPs in our population were as follows: rs11247362, 13.3%; rs10902606, 6.6%; rs1317459, 8.0%; rs11854132, 27.6%; rs2684761, 47.8%; and rs2715416, 42.7%.

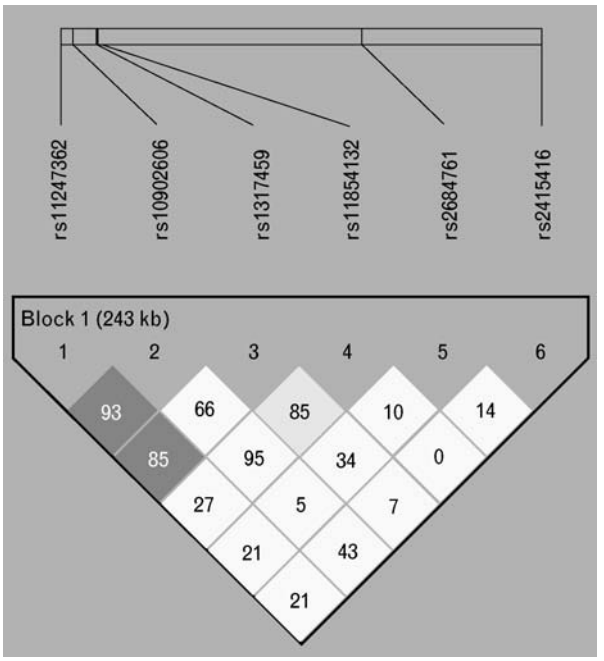
We evaluated the putative association of metabolic syndrome components with these *IGF1R* variants. We observed that rs2684761 was associated with the cumulative number of metabolic syndrome components, with G being the risk allele (Wald statistic 8.13, $P = 0.017$, adjusted for age). The mean ± SE of the number of metabolic syndrome components by genotype were AA 0.94 ± 0.08 ($n = 252$), AG 1.19 ± 0.06 ($n = 496$), and 1.19 ± 0.07 ($n = 293$). No significant association was observed with any of the other SNPs.

Although no significant association was observed between the *IGF1R* variant genotypes and BMI, waist circumference, fasting plasma glucose, and plasma triglycerides, genotypes of rs2684761 were significantly associated with insulin resistance as a discrete trait [odds ratio (OR) per G allele 1.27, 95% confidence interval (CI) 1.03–1.56, $P = 0.026$] and HOMA-IR as a continuous trait. HOMA-IR values according to the rs2684761 genotypes were: AA 1.67 ± 0.14 ($n = 251$), AG 2.04 ± 0.10 ($n = 498$), and GG 2.00 ± 0.12 ($n = 297$), $P = 0.01$ (logarithmically transformed HOMA-IR values adjusted for age using ANCOVA). The proportion of the total variance attributed to the rs2684761 genotypes was 0.9%. Similarly, fasting insulin levels were higher ($P = 0.009$, using ANCOVA on fasting insulin values logarithmically transformed and adjusted for age) in GG (61.5 ± 3.5 pmol/l) and AG (61.1 ± 2.8 pmol/l) vs. AA genotype patients (51.5 ± 3.5 pmol/l); genotypes explained 0.5% of the total fasting insulin level variance.

From visual inspection, it is obvious that a dominance model best fitted the data (data not shown). In fact, although in the genotypic additive model, obesity was marginally associated with the rs2684761 G allele ($P = 0.076$), under the dominant model, G carriers showed a 1.7-fold higher risk of being obese than homozygous for the A allele (OR: 1.73, 95%CI: 1.04–2.88, $P = 0.035$, adjusted for age and arterial hypertension).

We further evaluated the linkage disequilibrium pattern for the previously mentioned SNPs, and among all the

Fig. 1



Basic linkage disequilibrium plot between the studied single-nucleotide polymorphisms across the *IGF1R* gene in our population. The white horizontal line depicts a 243.2-kb DNA segment of chromosome 15q26.3. The locations of the six SNPs genotyped in this study are indicated by hatch marks. A linkage disequilibrium plot is depicted in the bottom of the figure. Each diamond represents the magnitude of linkage disequilibrium for a single pair of markers, with black indicating strong linkage disequilibrium ($r^2 = 1.0$) and white indicating no linkage disequilibrium ($r^2 = 0$) as the extremes (gray tones indicate intermediate linkage disequilibrium). Numbers inside the diamonds stand for D′ ×100. SNP, single-nucleotide polymorphism.

haplotypes, the association with HOMA-IR was even stronger with the haplotype TCGGGG, formed by rs11247362, rs10902606, rs1317459, rs11854132, rs2684761, and rs2715416 in that order ($P = 0.00259$). In an attempt to dissect the association signal, we performed the analysis, removing individual markers one by one. The association was lost by removing either rs2684761 ($P = 0.062$) or rs2715416 ($P = 0.115$). Then, we observed that the *IGF1R* haplotype frequency of rs2684761-G/rs2715416-G explains much of the observed effect ($\beta = 0.10$, $P = 0.01$). Similarly, the haplotype composed of rs2684761-G/rs2715416-G was significantly associated with the HOMA index as a discrete trait ($P = 0.039$).

Finally, we studied the putative association of the *IGF1R* variants with arterial hypertension as a dichotomous trait. A significant association of rs2684761 with arterial hypertension was observed (OR per G allele 1.29, 95% CI 1.02–1.64, $P = 0.037$) after adjusting for age and HOMA-IR.

In the analysis of the linkage disequilibrium pattern among the studied SNPs, we observed that the haplotype TCGGGC, composed of rs11247362, rs10902606, rs1317459, rs11854132, rs2684761, and rs2715416 in that order, was significantly associated with hypertension ($P = 0.031$). Again, in attempt to dissect the association signal, we performed the analysis, removing individual markers one by one. The above-mentioned association was lost by removing either rs2684761 ($P = 0.198$) or rs2715416 ($P = 0.077$). Then, we observed that the *IGF1R* haplotype frequencies of rs2684761-G/rs2715416-C, or conversely, rs2684761-A/rs2715416-G, explain much of the observed effect ($P = 0.015$).

Discussion

We performed a two-stage study to identify SNPs associated with metabolic syndrome-related phenotypes, including a first stage of in-silico exploratory assessment of a prioritized gene list using the original GWA data sets on genes implicated in common human diseases and a second stage focused on specific variants of interest. *IGF1R* exhibited several promising variants associated with metabolic syndrome-related phenotypes in both GWA data sets at the initial screening *P* value. In second stage, we evaluated six SNPs in *IGF1R* in a sample of 1094 Argentinean men enrolled in a population-based setting.

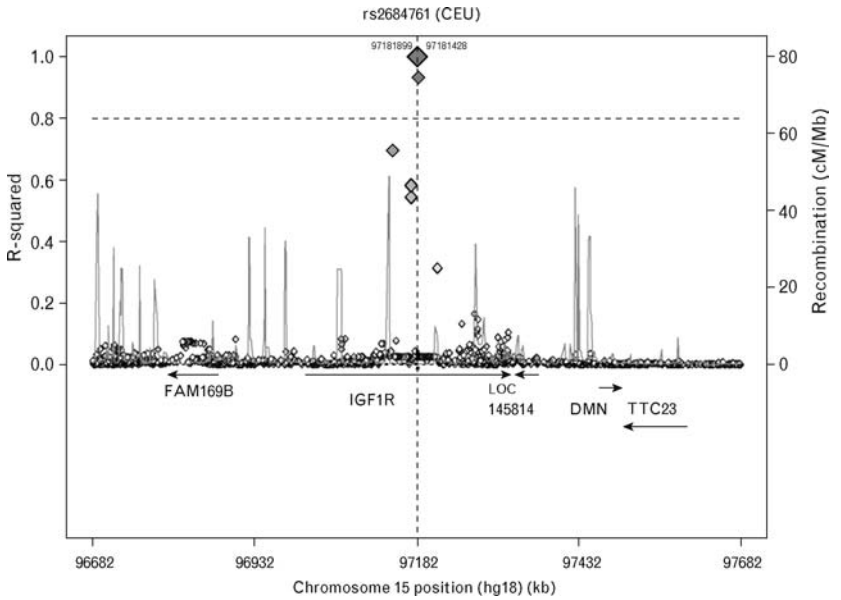
We observed that the rs2684761 G allele was significantly associated with the number of metabolic syndrome components, suggesting a putative role of *IGF1R* in individual susceptibility to these metabolic syndrome-related phenotypes. The major effects seem to be on the risk of having insulin resistance and arterial hypertension. This effect is not dependent on fasting glucose alteration but probably due to an increase in fasting insulin levels. We did not observe an association of *IGF1R* variants with SBP, DBP, or both, which may be explained by the effect of antihypertensive medication or simply a lack of power to detect a minor effect. In univariate analysis, the rs2684761-G allele was marginally associated with obesity, defined as having BMI of more than 30 kg/m^2, in the additive genotypic model, but the association was stronger and persisted after adjusting for age and arterial hypertension in the dominant model (AG + GG vs. AA).

In both insulin resistance and arterial hypertension findings, the analysis of variant haplotypes indicated a minor contribution of another variant, rs2715416, because the haplotype composed of the two variants (rs2684761 and rs2715416) was significantly associated with insulin resistance and arterial hypertension. Conversely, the removal of either SNPs from the haplotypes constructed with six variants induced loss of the positive signal. These results, along with a lack of association for the variant rs2715416 alone, probably indicate that the haplotypes composed of these two markers are associated with another functional variant(s). Because the significantly associated variants are intronic and have no obvious functional effects, further studies are necessary to identify the causal variant(s). Nevertheless, it is important to mention that intronic SNPs are not necessarily nonfunctional. They may affect gene regulation, RNA splicing, or be in linkage disequilibrium with variants that affect regulation or splicing [15]. However, annotation of nearby SNPs in linkage disequilibrium (proxies) with the rs2684761 variant based on HapMap data shows that the putatively associated variant is in fact in the *IGF1R* locus and not in a nearby locus, although a long distance effect cannot be ruled out (Fig. 2).

Moreover, it is worth mentioning that the lack of significant associations of rs10902606 and rs1317459 with phenotypes may be false-negative results due to the low MAF in our population (6.6 and 8.0%, respectively), as the analysis did not have sufficient power to detect associations according to our sample size (27 and 39%, respectively, under the multiplicative model).

The first comment about our findings is on the biological plausibility of the gene–disease association that we are reporting. The *IGF1R* is a tyrosine kinase class II receptor with potent mitogenic, antiapoptotic, and transforming activities required for oncogenic transformation. In addition, *IGF1R* has also an important role in the insulin-signaling pathway. In fact, after binding its ligand, *IGF1R* initiates metabolic cascades resulting in glucose intake, glycogen synthesis, and lipid storage. The suspected participation of *IGF1R* in the regulation of these metabolic cascades is in addition supported by previous evidence from animal models. Mutant mice lacking *IGF1R* specifically in pancreatic β cells exhibit age-dependent impairment of glucose tolerance [16]. In addition, transgenic mice with a dominant-negative

Fig. 2



Regional linkage disequilibrium plot for single-nucleotide polymorphism rs2684761 at chromosome 15. The SNP is plotted with their proxies (based on HapMap data for CEU (Utah HapMap samples)) as a function of genomic location, annotated by the recombination rate across the locus (blue-line) and nearby genes (in green). We can conclude that there are no observed variants in other loci close to *IGF1R* with any appreciable single-nucleotide polymorphism to rs2684761. The regional association plot of rs2684761 was performed by the SNAP (SNP annotation and proxy search) server available at http://www.broad.mit.edu/mpg/snap/. SNP, single-nucleotide polymorphism.

IGFIR specifically targeted at the skeletal muscle develop insulin resistance that results in T2D [17]. Kloting *et al.* [18] demonstrated the existence of a negative IGF1R-mediated feedback mechanism of IGF-1 on its own gene expression in adipocytes, indicating an important role for adipose tissue IGF-1 signaling in the regulation of IGF-1 serum concentrations.

In addition, apart from its ubiquitous pattern of expression, *IGFIR* is expressed on vascular smooth muscle cells (VSMCs), being a critical determinant of the VSMCs growth response [19]. Hence, additional experimental evidence supports our findings about the putative influence of common variants in *IGFIR* on arterial hypertension. For example, alterations of the IGF1 system were reported in association with the pathophysiology of vascular diseases in a model of aortic coarctation hypertension in rats [20]. Indeed, Telgmann *et al.* [21] reported that the common variant $-1411C>T$ in the IGF1 promoter might play a key role in local IGF1 bioavailability, and the T allele of this molecular functional variant was associated with a decrease in DBP and SBP levels and a lower prevalence of essential hypertension.

The second comment is about the potential role of genetic variability at the human *IGF1R* in genetic susceptibility to metabolic syndrome-related phenotypes. *IGF1R* has been a candidate gene for insulin resistance and T2D in two previous studies. One report in a Finnish population evaluated common variants in several genes regulating the insulin-signaling pathway and observed that the silent polymorphism at exon *GAG1013GAA* of the *IGF1R* was associated with fasting serum insulin levels [22]. The other report is a mutational analysis of the coding regions of *IGF1R* performed on genomic DNA from probands of 82 Danish T2D families [23]. In this study (a population-based sample of 349 young healthy individuals), no significant relationships between the *GAG1013GAA* variant and the insulin sensitivity index was detected. In agreement with these observations, *IGF1R* mutations were found associated with intrauterine and postnatal growth retardation [23,24]. These data are of particular interest, as there is accumulating evidence showing that impaired intrauterine growth is an important factor that contributes to the pathogenesis of T2D [25] and other metabolic syndrome components in adult life [26].

A putative role of the *IGF1R* variants influencing arterial BP is shown in our study. Interestingly, a previous genome-wide linkage study in a large number of pedigrees in a Chinese population showed that a region on chromosome 15q was strongly associated with DBP [27]. This region (15q25.1–15q26.1) was later confirmed in a genome-wide linkage analysis of SBP in white individuals [28].

The very recently published GWA study reporting SNP hits that achieve genome-wide statistical significance in association with arterial BP revealed eight gene regions [29]. As stated by the authors, the causal gene could be any of the genes around the associated signal in

each described locus, including 15q24. In addition, the eight described loci explained a small proportion of the total variation in either SBP or DBP, which suggests that some other gene variants should contribute to genetic susceptibility to arterial hypertension. Unfortunately, this hypertension GWA data set is not freely available, so we cannot explore the possibility of an association between the *IGF1R* variants and arterial hypertension even with a less-stringent cutoff for association. In addition, sex-specific association analysis was not performed, as details about the sex of the population in the original GWA dataset are not available. This would be of interest as our study was focused only on a male population.

In conclusion, the identification of genes associated with complex diseases has enormously benefited from high-throughput functional genomic technologies, in particular GWA studies and genome-wide expression profiling evaluation. Nevertheless, the GWA strategy itself is still too expensive for routine use, and it is beyond the reach of most research groups worldwide. Moreover, large sample sizes are required. An alternative approach to circumvent these drawbacks is to provide access to full, gene-annotated GWA data sets, which could be used for further querying, analyses, or integration with other genomic information. In addition, specific biological hypotheses and candidate-gene association studies can be tested by an in-silico evaluation of the GWA data sets. This strategy was initially applied in this report, and *IGF1R* was found as a candidate gene significantly associated with two important traits related to metabolic syndrome: arterial hypertension and insulin resistance. Although *IGF1R* was not previously evaluated as a candidate gene for these clinical entities, either its role in the insulin signaling or its multiple physiologic effects on the vasculature, including proliferative, hypertrophic, and vasomotor effects, reinforce the plausible biological relevance of the association. In addition, *IGF1R* may be considered a remarkable candidate gene for further studies because of its role in the regulation of whole-body metabolism, and a potential target for therapeutic interventions.

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