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CRTC2 polymorphism as a risk factor for the incidence of metabolic syndrome in patients with solid organ transplantation

CRTC2 and Post-transplant metabolic syndrome.

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ABSTRACT

Metabolic syndrome after transplantation is a major concern following solid organ transplantation (SOT). The CREB-regulated transcription coactivator 2 (CRTC2) regulates glucose metabolism. The effect of *CRTC2* polymorphisms on new-onset diabetes after transplantation (NODAT) was investigated in a discovery sample of SOT recipients (n_1=197). Positive results were tested for replication in two samples from the Swiss Transplant Cohort Study (STCS, n_2=1294 and n_3=759). Obesity and other metabolic traits were also tested. Associations with metabolic traits in population-based samples (n_4=46'186, n_5=123'865, n_6>100,000) were finally analyzed. In the discovery sample, *CRTC2 rs8450-AA* genotype was associated with NODAT, fasting blood glucose and BMI ($p_{corrected}$<0.05). *CRTC2 rs8450-AA* genotype was associated with NODAT in the second STCS replication sample (OR=2.01, p=0.04). In the combined STCS replication samples, the effect of *rs8450-AA* genotype on NODAT was observed in patients having received SOT from a deceased donor and treated with tacrolimus (n=395, OR=2.08, p=0.02) and in non-kidney transplant recipients (OR=2.09, p=0.02). Moreover, *rs8450-AA* genotype was associated with overweight or obesity (n=1215, OR=1.56, p=0.02), new-onset hyperlipidemia (n=1007, OR=1.76, p=0.007), and lower HDL-cholesterol (n=1214, β=-0.08, p=0.001). In the population-based samples, a proxy of *rs8450G>A* was significantly associated with several metabolic abnormalities. *CRTC2 rs8450G>A* appears to play an important role in the high prevalence of metabolic traits observed in patients with SOT. A weak association with metabolic traits was also observed in the population-based samples.

Key terms: Transplantation, metabolic syndrome after transplantation, genetic polymorphisms.

Introduction

The introduction of calcineurin inhibitors (CNIs) - cyclosporine (CSA) and tacrolimus (TAC) - in solid organ transplantation (SOT) has reduced the incidence of acute rejection episodes and improved short-term graft survival[1, 2]. However, both drugs are not devoid of metabolic complications, such as glucose intolerance, hypertension, and hyperlipidemia which can be very pronounced and have a detrimental impact on patients' life quality, and increase the mortality risk due to cardiovascular events[3]. Indeed, cardiovascular disease is responsible for approximately 20-40% of non-graft-related death after the first post-transplantation year[4-6].

New onset diabetes mellitus after transplantation (NODAT) is a serious complication partly related to the use of CNIs, mainly TAC[7, 8]. It is also associated with increased cardiovascular events, infectious complications, and graft loss[9, 10].There are several risk factors which increase the risk of NODAT in transplantation such as obesity, increased age, male sex, deceased donor, hepatitis C (HCV) status, acute rejection, and African-American or Hispanic descent[9, 11]. The identification of genetic factors involved in the development of NODAT may greatly benefit from a more detailed understanding of the complex metabolic pathways involved in glucose metabolism. Genome-wide association studies (GWAS) conducted to date explain only 10% of type 2 diabetes heritability and more diabetic susceptibility genes remain to be discovered (reviewed in [12] and [13]). A recent GWAS investigating NODAT identified eight polymorphisms[14]. However, only one of these was replicated in another study[15]. Whereas GWAS have been extremely valuable, other approaches are needed to further understand the pathophysiology of NODAT.

The cAMP-regulated transcriptional coactivator 2 (CRTC2), a transcriptional coactivator that promotes the transcription of genes targeted by the cAMP response element-binding protein[16], is an interesting target protein in glucose metabolism. CRTC2 belongs to the CRTC family which comprises 2 other members, CRTC1 and CRTC3[17]. CRTC1 is mainly expressed in the central nervous system and we recently showed an association between *CRTC1* polymorphisms and obesity markers (body mass index (BMI) and fat mass) in psychiatric and population-based samples[18], CRTC2 is

highly expressed in the thymus, and present in both T and B-lymphocytes[17]. It is also expressed in the liver and it plays a direct role during the fasting state in the induction of gluconeogenic genes[16], It further enhances hepatic insulin signalling by stimulating expression of the *insulin receptor substrate 2* gene, thus triggering a feedback response that limits glucose output from the liver during fasting[19], Calcineurin, the target of both TAC and CSA, plays an important role in the activation of CRTC2[20, 21]. Overexpression of CRTC2 induced by a mutation at 2 regulatory sites rendered *CRTC2* constitutively active in an animal model and permitted CRTC2-target gene activation even when calcineurin was inhibited by CNIs[22]. The authors of this study also showed that CRTC2 is required for β-cell function and proliferation and promoting this pathway could ameliorate symptoms of NODAT[22].

CRTC2 single nucleotide polymorphisms (SNPs) were previously investigated in two Asian populations and one coding SNP (*R379C*), with a very low minor allele frequency (MAF), was associated with type II diabetes[23] and with lung cancer[24]. So far *CRTC2* SNPs have not been investigated in other ethnic populations or with other phenotypes. In this work, we aimed to study the influence of *CRTC2* SNPs on the incidence of NODAT in a sample of Caucasian SOT recipients, and positive results were then tested for replication in the Swiss transplant cohort Study (STCS). We also aimed to extend our analysis to obesity and other variables of the metabolic syndrome (MetS) following SOT. Finally, we aimed to test if the associations with MetS components could be found in general population-based samples (>100,000 subjects).

Materials and Methods

Discovery study sample:

This study aimed initially to investigate the effect of genetic polymorphisms of drug metabolizing enzymes and/or transporters on the incidence of different post-transplant complications and on immunosuppressive doses and blood levels[25, 26]. For this study, a total of 197 patients were enrolled between 2003 and 2005 from the outpatient clinic of the Transplant Center of the University Hospital of Lausanne, Switzerland. Patients with functional graft for more than 12 months after transplantation were eligible to participate in the study. Data regarding patient's age, gender, BMI, ethnic origin, donor's age, HLA mismatch, duration of graft cold ischemia and delayed graft function were collected

retrospectively from patients' medical files. Immunosuppressive regimens, doses and blood levels were obtained retrospectively at different time-points during the first year post-transplantation. The study was approved by the ethics committee of the University of Lausanne. Patients gave their written informed consent to participate in this pharmacogenetic study. Once included, venous blood samples were collected for DNA extraction and further genotyping analysis.

For the present study, data were collected between October 2011 and April 2012 as related to the development of NODAT during the first 5 years following transplantation. Data regarding fasting blood glucose (FBG), glycated haemoglobin (HbA1c), 2hrs oral glucose tolerance test (OGTT), insulin and oral anti-diabetic treatment were collected retrospectively from the medical files at the time of transplantation, at 1, 3, 6, 9 and 12 month post-transplantation and at the yearly follow-up until 5-year post-transplantation. NODAT was diagnosed if a patient needed anti-diabetic treatment (either insulin or oral anti-diabetic agents) for at least 6 months following transplantation or had several abnormal glucose profiles during the follow-up period that fulfil the criteria given by the WHO and ADA consensuses[27], including FBG≥7.0 mmol/l (in ≥ two occasions) or 2 hours plasma glucose≥11.1 mmol/l during OGTT [27] (even if not treated with anti-diabetic drugs). Patients who had diabetes or prediabetes before transplantation or were transplanted because of diabetic nephropathy were excluded from the present study.

The Swiss Transplant Cohort Study (STCS):

The STCS is an ongoing prospective multicenter cohort project (Basel, Bern, Genève, Lausanne, St. Gallen and Zurich), aiming at a nationwide comprehensive and structured data collection in all SOT recipients. All recipients of SOTs in Switzerland are prospectively registered since May 2008. Currently more than 3500 patients are included in the STCS. No particular eligibility or exclusion criteria exist for enrolment. After transplantation, all patients are mandatorily followed in their respective transplant centers. After baseline assessment, STCS follow-up assessments take place at 6-, 12 months and yearly thereafter. Biological samples are collected in relation to the case at baseline, and at 6-, and the 12-month visits. Data regarding patient's age, gender, BMI, blood pressure and lipid profiles (total cholesterol, HDL- and LDL-cholesterol) were available for all patients at the different

time-points of the STCS follow-up. Full description of this cohort is published elsewhere[28, 29]. For the first STCS replication sample, patients transplanted from May 2008 to 8th of May 2011 with a functional graft for at least 12 months after transplantation were included in the analyses (n=1294). NODAT was diagnosed if a patient needed anti-diabetic treatments following transplantation or if such new metabolic event was reported in their case report forms. For the second STCS replication sample, patients transplanted from 9th of May 2011 to May 2013 with a functional graft for at least 12 months after transplantation were included in the analyses (n=759). For this second STCS replication sample, several random and FBG were available in the database, as well as HbA1c. Therefore, as for the discovery sample, NODAT was diagnosed if a patient needed anti-diabetic treatment (either insulin or oral anti-diabetic agents) or fulfilled the criteria given by the WHO and ADA consensuses mentioned earlier. For both STCS samples, patients who were diagnosed with diabetes or were prediabetics before transplantation or were transplanted because of diabetic nephropathy were excluded from the NODAT analyses.

For the combined STCS sample, new-onset hypertension and new-onset hyperlipidemia were diagnosed if patients needed anti-hypertensive and hypolipidemic treatment post-SOT. Patients with previous hypertension or hyperlipidemia were excluded from the new-onset hypertension and new-onset hyperlipidemia analyses, respectively. The study was approved by the ethics committee of their respective centers. Patients gave their written informed consent to participate in this pharmacogenetic study.

Recipients younger than 18 years old and recipients with multiple organ transplantation were excluded from the whole analyses. If a patient received more than one transplantation during the inclusion period, only data from the first SOT was included in the analyses.

For both the discovery and STCS samples, as abdominal obesity (waist circumference) was not available, BMI was used as a marker of obesity.

Population based samples:

We aimed to replicate results with MetS traits in several population-based samples: the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC)(n=46'186)[30], Genetic Investigation of

ANtropometric Traits (GIANT) consortium (n=123'865)[31-33], and Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides (n>100'000)[34, 35]. More details are in the Supplementary Material.

Polymorphism selection and genotyping:

Two tagging SNPs within *CRTC2* gene (*rs8450G>A* and *rs12117078G>C*) were selected using HapMap Genome Browser by limiting the search to SNPs with a MAF>5% in the Caucasian population and r^2 cutoff of 0.8. Genotyping in the discovery study sample was done using Taqman allelic discrimination assays. Genetic analyses for the STCS replication samples were done using the fluorescence-based competitive allele-specific PCR technology (KASPar)[36]. More details are in the Supplementary Material.

Statistical analysis:

Detailed statistical methods are presented in the Supplementary Material.

Discovery and STCS samples:

We assessed the association of *CRTC2* SNPs with MetS traits 1) by applying a logistic regression for binary outcomes adjusted for recipient's age at transplantation and sex. Other variables identified through the univariate analysis (P<0.10) were also added as covariates in these models, 2) by using linear mixed models for continuous variables, and 3) by fitting a Generalized Additive Mixed Model (GAMM) to include a smooth trend for the response in time (allowing multiple observations for each patient) for non-linear continuous variables (BMI and FBG).

For the discovery sample, the p-values of the models were adjusted for multiple comparisons using Bonferroni correction and the SNP that survived this correction was investigated in the STCS.

Data for both the discovery and replications samples were analyzed using Stata 12 (StataCorp, College Station TX, USA) and R version 2.13.0 software (http://www.R-project.org).

Population-based samples:

We analyzed the associations of *CRTC2* SNP with different MetS traits using multivariate linear regression with allele dosage in which potential confounding factors such as age, sex, and smoking status were added as covariates.

Results:

Discovery and STCS replication samples:

General characteristics of the discovery and STCS replication samples are presented in the Supplementary Material and Supplementary Tables 1 and 2. Supplementary Table 2 represents the global STCS sample without the exclusion of patients with previous history of diabetes or other metabolic abnormalities before transplantation.

The distribution of *CRTC2* genotypes in all studies did not deviate from the Hardy-Weinberg equilibrium (P>0.05) and the MAFs were similar to those reported in HapMap (Supplementary Table 3).

***CRTC2 SNPs* and NODAT, FBG, and BMI in the discovery study sample:**

Carriers of the *CRTC2 rs8450-AA* genotype showed increased risk of NODAT as compared to wild-type *GG* genotype (odd ratio (OR)=6.91, 95%CI:1.52-31.36, $p_{corrected}$=0.02). An increased risk was also observed by analyzing the *rs8450* SNP in a recessive model (Table 1). Neither cumulative prednisone dosages nor calcineurin inhibitors' doses, trough levels or concentration/dose ratios tested at 2 time periods (at 1 month or at 12 months post-SOT) were associated with NODAT in the univariate analysis or when used in the multivariate model including *CRTC2* SNPs and the other covariables (data not shown).

FBG were also available at several time-points after SOT. By applying the GAMM model, increased FBG levels were found in patients with the *CRTC2 rs8450-GA and AA* genotypes (0.24 mmol/L, $p_{corrected}$=0.06 and 0.47 mmol/L, $p_{corrected}$=0.02, respectively) as compared to wild-type *GG* genotype. We observed an equal increased of FBG levels by analyzing the *rs8450* SNP in dominant model (Table 1).

Patients with the *CRTC2 rs8450-GA* genotype showed a 1.36 kg/m2 increased BMI compared to the wild-type genotype ($p_{corrected}$=0.02). By analyzing the SNP in a dominant model, we observed a significant association between *CRTC2 rs8450* and BMI (β=1.17, 95%CI:0.17-2.35, $p_{corrected}$=0.04). We observed no association between *CRTC2 rs12117078G>C* SNP and NODAT, FBG or BMI (Table 1).

***CRTC2 SNP* in the STCS replication samples:**

The *CRTC2 rs8450G>A* SNP that survived corrections for multiple testing in the discovery study sample was investigated in the STCS. Recessive models for this SNP are presented in the text and in Tables 2 and 3 while additive and dominant models regarding NODAT are presented in Supplementary Table 4.

***CRTC2 SNP* and NODAT:**

In the univariate analyses, increased recipient's age (p=0.0003), male sex (p=0.03), positive HCV status (p=0.003), deceased donor (p=0.04), treatment with TAC (p=0.001) and baseline BMI (p=0.0002) were among the non-genetic factors associated with an increased risk of NODAT and were used as covariates in the genetic models. In the first STCS replication samples, we observed a non-significant association between *rs8450G>A* and NODAT (Table 2). However, by analyzing the SNP in the subgroup of patients having received SOT from a deceased donor and treated with TAC, a significant effect of the SNP was observed (n=281, OR=2.36, 95%CI:1.16-4.78, p=0.01)(Table 2). In the second replication sample (n=438), in which the diagnostic criteria for NODAT was the same as for the discovery sample, recipients carrying the *rs8450-AA* genotype showed a significant increased risk of NODAT (n=438, OR=2.01, 95%CI:1.03-3.91, p=0.04). In the combined STCS sample, the effect of the *CRTC2* genotype was only observed in the subgroup of patients having received SOT from a deceased donor and treated with TAC (n=395, OR=2.08, 95%CI:1.15-3.74, p=0.02) (Table 2).

***CRTC2 SNP* and BMI, new-onset hypertension/ hyperlipidemia:**

We investigated the influence of *CRTC2 rs8450G>A* on BMI and on the incidence of new-onset hypertension/hyperlipidemia in the combined STCS sample.

Increased recipient's age ($p<0.0001$), male sex ($p<0.0001$) and type of transplanted organ ($p<0.0001$) were among the non-genetic factors associated with increased BMI in the univariate analyses and were used as covariates in the genetic models. BMI values were also dichotomized into normal versus overweight or obese (BMI≥25 kg/m^2). At 12-months post-SOT, overweight or obese patients were more prevalent in the *CRTC2 rs8450-AA* genotype compared to the other genotypes (n=1215, OR=1.56, 95%CI:1.08-2.25, p=0.02). We observed the same association but with lower significance for the combined STCS sample at 6-months post-SOT (n=1389, OR=1.41, 95%CI:1.01-1.97, p=0.04), and a non-significant association with baseline BMI (n=1515, OR=1.20, 95%CI:0.87-1.65, p=0.27), suggesting that time post-SOT modulates the effect of the *CRTC2* genotypes.

By applying the GAMM model to test the association between BMI over time and *CRTC2 rs8450G>A* SNP (n=1625), patients with the *CRTC2 rs8450-AA* genotype showed a 0.47 kg/m^2 (95%CI:0.11-1.02, p=0.01) increase in BMI compared to the other genotypes.

We found no association between the *CRTC2 rs8450G>A* and the incidence of new-onset hypertension (Supplementary Material). On the other hand, carriers of the *CRTC2 rs8450-AA* genotype showed increased risk of new-onset hyperlipidemia as compared to the other genotypes (n=1007, OR=1.76, 95%CI:1.16-2.66, p=0.007). Additionally, patients with the *CRTC2 rs8450-AA* genotype also showed a significant decrease of HDL-Cholesterol levels in the combined STCS sample (n=1214, β= -0.08 mmol/L, p=0.001). *CRTC2 rs8540G>A* SNP was not associated with total cholesterol or with LDL-cholesterol blood levels in the combined STCS sample (data not shown). More details can be found in the Supplementary Material.

***CRTC2 SNP* and MetS traits in kidney and non-kidney transplant recipients:**

Patients were dichotomized into kidney versus non-kidney transplant recipients (Table 3). The *CRTC2 rs8450-AA* genotype was associated with significantly higher OR for NODAT in non-kidney

transplant recipients (n=409, OR=2.09, 95%CI:1.13-3.86, p=0.02), while the *rs8450 AA*-genotype was only associated with higher risk of new-onset hyperlipidemia in kidney transplant recipients (n=573, OR=1.95, 95%CI:1.14-3.15, p=0.01).

Population-based samples:

Several glycemic traits were available in the MAGIC study (Supplementary Table 5); each *rs1572788 C-allele* (in complete linkage disequilibrium (LD) with *rs8450 A-allele*) increased FBG levels by 0.01 mmol/l (n=46'186, p=0.004). Additionally, each *rs1572788 C-allele* decreased HOMA-B by 0.008% (n=46'186, p=0.03). No association was observed between *rs1572788T>C* and HOMA-IR or the 2 hour OGTT.

Decreased HDL-cholesterol and increased triglycerides were observed for each *rs1572788 C-allele* in the Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides (n=96'908, β=-0.008, p=0.013 and n=93'562, β=0.009, p=0.004, respectively). We observed non-significant associations for *rs1572788T>C* with total and LDL-cholesterol, as well as with obesity traits studied in the GIANT study, however, the direction of the effect is consistent with increased blood lipids and obesity with each *rs1572788 C-allele* (Supplementary Table 5).

Discussions:

The transcriptional co-activator (CRTC) family is implicated with energy expenditure (CRTC1 and CRTC3)[37, 38] and glucose metabolism (CRTC2)[16] in animal models. In the present study, we aimed to study the effect of *CRTC2* SNPs on the development of diabetes in SOT recipients, a population at high risk of developing metabolic abnormalities, and in population-based samples. We also extended our analyses to investigate obesity and other metabolic traits. The *CRTC2 rs8450-AA* genotype was associated with increased risk of NODAT, increased FBG, and BMI in a sample of 156 patients with SOT. Results on NODAT were replicated only in the second STCS replication sample (n=438), while in the first and the combined STCS replication samples, the SNP only showed significant influence in the population at higher risk of developing NODAT, mainly in recipients having received a graft from a deceased donor and treated with TAC. We also observed a significant association for BMI in the

STCS replication sample; overweight and obesity were also more frequent in patients carrying the *rs8450-AA* genotype at 12 months post-SOT. *CRTC2 rs8450G>A* was also associated with increased risk of new-onset hyperlipidemia following SOT and with lower HDL-cholesterol blood levels. Furthermore, we also found the increased risk of the *C-allele* of *rs1572788* (in complete LD with *rs8450 A-allele*) on diabetic and lipidemic traits in two large population-based samples (MAGIC and GWAS for Total Cholesterol, HDL-C, LDL-C and triglycerides, respectively), and a consistent direction even though non-significant between *rs1572788T>C* and obesity traits in the GIANT study.

CRTC2 rs8450G>A was associated with NODAT in the total discovery sample and only in the second STCS replication sample, but not in the total STCS sample in which a significant association was observed only in the subgroup of patients at higher risk of developing NODAT. This could partly be explained by the difference of NODAT diagnostic criteria, which is only based on anti-diabetic treatment in the first STCS replication sample. Another explanation could be the high percentage of patients receiving an organ from a living donor in the first STCS replication sample (28%, Supplementary Table 2) compared to only 11.5% in the discovery sample (Supplementary Table 1). Deceased donor is a common risk factor of NODAT[11] and the impact of the SNP seems to be more prominent in the groups at higher risk of developing MetS traits. Additionally, when differentiating kidney and non-kidney transplant recipients (Table 3), we observed a significant association between the *CRTC2 rs8450-AA* genotype and NODAT in non-kidney transplant recipients. The percentage of non-kidney transplant recipients was higher in the second STCS replication sample compared to the first STCS sample (Supplementary Table 2) and it should be noted that kidney transplant recipients had higher percentages of living donors compared to non-kidney transplant recipients (43% vs. 2% respectively). Moreover, kidney transplant recipients usually have obesity and other metabolic problems already before transplantation compared to other solid organ recipients, which could explain the differential effect of the SNP depending on the type of the transplanted organ. Interestingly, in both the discovery and the combined STCS samples, the effect of the SNP was stronger in NODAT cases developed after the first year post-SOT (data not shown), It maybe that the effect of the *CRTC2* SNP on NODAT is more important in the long-term, but this remains to be further studied.

In the GWAS literature, *CRTC2* SNPs were not found to be associated with diabetes, obesity or hyperlipidemia. On the other hand, by testing the individual effect of *CRTC2 rs1572788T>C* SNP on diabetic and lipidemic traits in two population-based samples, a significant effect was observed, even though this effect is very weak and the variance explained by the SNP is very small (Supplementary Table 5) and could thus not be detected by GWAS. However, patients with SOT is a population at risk of developing MetS traits, because of the disease itself and/or the immunosuppressive medications, both can act as an important trigger, unmasking different genetic factors. So in this at risk population, the effect of *CRTC2* SNP was more pronounced, even in much smaller sample sizes compared to population-based samples. Additionally, in the GWAS of NODAT[14], *CRTC2* SNPs did not reach genome-wide level of significance. Moreover, most of the SNPs retained in this GWAS were not replicated in another study with NODAT[15]. In fact, both GWAS and candidate gene studies are important gene association approaches and both are subject to the same artifacts of spurious association findings. Depending on the methodology of these studies (sample size, definition of the phenotypes, MAF of the SNPs, corrections for multiple testing, etc…), discordances between both approaches could be seen. GWAS relies on indirect association to locate a disease-causing variant while candidate gene approach relies on an a priori hypothesis to identify this disease-causing variant. For all of these reasons, replication of positive finding in either approach is of utmost importance to validate the results.

The functional activity of this SNP, in the regulatory 3'UTR, is unknown and data from 1000 Genomes[39] showed that 78 SNPs in the *CRTC2* gene region are in high LD (r^2 threshold≥0.8) with *rs8450G>A*. We used the RegulomeDB database that annotates SNPs with known and predicted regulatory elements in the intergenic regions of the H. sapiens genome[40]. This database reveals known and predicted regulatory DNA elements including regions of DNAase hypersensitivity, binding sites of transcription factors, and promoter regions that have been biochemically characterized to regulation transcription. Source of these data include public datasets from GEO, the ENCODE project, and published literature. Regarding *CRTC2 rs8450G>A*, several of these proxy SNPs (e.g. rs6680140 with r^2=1) show cis-eQTL effect on the expression of *CRTC2* and fall into the autoimmune regulatory

(AIRE) motif, influence the binding of several proteins (*JUN*, *CREBBP* and *ELF1*), and has several histone marks (H3k09me3, H4k20me1, etc.). Altogether, these data suggest that the SNP might have a regulatory function.

Interestingly, among the analyzed clinical factors, treatment with corticosteroids was not associated with NODAT, neither in the discovery cohort nor in the STCS sample. However, in our samples most of the patients were treated with corticosteroids so it is difficult to draw major conclusions. On the other hand, the cumulative corticosteroids dosages and the duration of therapy rather than the simple corticosteroids administration could be the most influencing factors. In our discovery sample, the cumulative dosages of corticosteroids at the first year post-transplantation were not associated with NODAT outcome. In the literature, several studies did not find any influence of cumulative corticosteroid dosages on the appearance of NODAT[41-45]. The current lower dosages of corticosteroids used in different immunosuppressive regimens could contribute to the negative association with NODAT appearance in our study.

This study has several limitations and strengths. Random and FBG levels were only available in the second STCS replication sample and the diagnosis of NODAT in the first STCS replication sample was only based on anti-diabetic treatments post-SOT. The genotype of the *rs8450G>A* SNP of the liver transplant donors is unknown, therefore the significant effect of the SNP on NODAT found in non-kidney transplant recipients, in which nearly half of them are liver transplant recipients, should be interpreted with caution. However, CRTC2 is expressed in different tissues and is implicated in glucose regulation through different mechanisms (gluconeogenesis, insulin signaling and beta-cell survival[16, 19, 22]) which could highlight the importance of recipient *CRTC2* genotype even in liver transplant recipients. Additionally, by excluding patients with liver transplantation from the non-kidney transplant recipients' analyses, a significant association between the *CRTC2* SNP and NODAT is still observed (data not shown). The present results do not allow the determination of whether the *rs8450G>A* SNP is the causative variant or merely a proxy of one or more yet unidentified variants. Despite the possible regulatory functions proposed by the RegulomeDB database, further studies are needed to elucidate which precise mechanisms underlie the observed association. This study included

people of Caucasian origin and results cannot be generalized to other ethnic groups. On the other hand, the fact that the results were replicated in two independent samples with SOT and in large population-based samples, the latter used as a proof of concept of the effect of the polymorphism, strengthens the validity of our data.

In conclusion, our results suggest that variations in *CRTC2* play an important role in the high prevalence of MetS complications observed in patients with SOT. Besides, by studying *CRTC2* SNPs in population-based samples, we still observed a weak yet significant association, thereby suggesting that the effect of *CRTC2* variations is more important in population at risk of developing different MetS traits than in the general population. This is the first study showing the importance of *CRTC2* variations with NODAT and other MetS traits in patients with SOT. The assessment of the major risk gene variants would allow predicting vulnerability for developing Mets phenotypes and thus adapt the immunosuppressive treatment from the beginning by genotyping the patients before transplantation. In the long-term, these patients could benefit from individualized immunosuppressive regimens adapted to their genetics and environment.

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CBE takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

Declaration of interests:

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Footnote

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Supplementary data:

Supplementary information is available at journal's website

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Table 1 Association between *CRTC2* SNPs and NODAT, blood glucose levels and BMI in patients with solid organ transplantation from the discovery study sample:

	NODAT (n=156)		**FBG (n=156)**		**BMI (n=156)**	
SNP	**OR (95% CI)**	**P corrected**	**Difference (mmol/L) compared to wild-type (ref) (95% CI)**	**P corrected**	**Difference (kg/m2) compared to wild-type (ref) (95% CI)**	**P corrected**
CRTC2 rs8450						
GG	ref		ref		ref	
GA	1.81 (0.71 - 4.61)	0.46	0.24 (-0.00 - 0.49)	0.06	1.36 (0.28- 2.42)	**0.02**
AA	6.91 (1.52 - 31.36)	**0.02**	0.47 (0.07 - 0.86)	**0.02**	0.37 (-1.38 - 2.06)	0.76
Dominant	2.32 (0.96 - 5.63)	0.12	0.28 (0.05 - 0.51)	**0.02**	1.17 (0.17 - 2.35)	**0.04**
Recessive	5.11 (1.24 - 21.03)	**0.04**	0.36 (-0.03 - 0.74)	0.06	-0.24 (-2.33 - 1.68)	0.80
CRTC2 rs12117078						
GG	ref		ref		ref	
GC & CC	0.36 (0.11 - 1.16)	0.18	-0.07 (-0.40 - 0.24)	0.70	0.24 (-1.41 - 1.62)	0.78

NODAT: New-onset diabetes after transplantation, FBG: Fasting blood glucose, OR: odd ratio, CI: confidence interval, BMI: body mass index.

Regarding NODAT and blood glucose levels analyses: P values were adjusted for age of recipient at transplantation, sex of the recipient and baseline BMI and type of calcineurin inhibitor.

Regarding BMI analyses: P values were adjusted for age of recipient at transplantation, sex of the recipient and type of calcineurin inhibitor.

Table 2: Association between *CRTC2 rs8450* SNP in a recessive model and NODAT in the first, second and combined population of STCS and in different subgroups at risk of NODAT:

CRTC2 rs8450		**GG&GA (n)**	**AA**	
	N total		**NODAT OR (95% CI)**	**P value**
First STCS replication sample[§]:				
Total	711	Ref (n=623)	1.02 (0.62 - 1.70)	0.93
Treatment with TAC & deceased donor	281	Ref (n=244)	2.36 (1.16 - 4.78)	**0.01**
Second STCS replication sample[§]:				
Total	438	Ref (n=385)	2.01 (1.03 - 3.91)	**0.04**
Combined STCS replication sample[§]:				
Total	1149	Ref (n=1008)	1.25 (0.84 - 1.87)	0.26
Treatment with TAC & deceased donor	395	Ref (n=340)	2.08 (1.15 - 3.74)	**0.02**

[§]Excluding patients with previous history of diabetes before transplantation

NODAT: New-onset diabetes after transplantation, TAC: tacrolimus, OR: odd ratio, CI: confidence interval

P values were adjusted (when appropriate) for age of recipient at transplantation, sex of the recipient, hepatitis C status, baseline BMI, type of calcineurin inhibitor and type of donor.

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Table 3: Association between CRTC2 rs8450 SNP and MetS traits in all kidney and non-kidney transplant recipients in the combined STCS sample

	Kidney transplant recipients			Non-kidney transplant recipients		
	n	**OR (95% CI) for rs8450-AA**	**p-value**	**n**	**OR (95% CI) for rs8450-AA**	**p-value**
NODAT	740	0.87 (0.50 - 1.52)	0.62	409	2.09 (1.13 - 3.86)	0.02
BMI≥25 kg/m2 at 12months post SOT	761	1.59 (0.98 - 2.58)	0.06	454	1.52 (0.84 - 2.75)	0.17
New-onset hyperlipidemia	573	1.95 (1.14 - 3.15)	0.01	434	1.73 (0.84 - 3.56)	0.14
New-onset hypertension	146	1.64 (0.43 - 6.30)	0.47	345	1.44 (0.75 - 2.78)	0.27

OR: odd ratio, CI: confidence interval

NODAT: New-onset diabetes after transplantation, BMI: body mass index

For NODAT analyses: P values were adjusted for age of recipient at transplantation, sex of the recipient, baseline BMI, type of calcineurin inhibitor and type of donor.

For BMI analyses: P values were adjusted for age of recipient at transplantation and sex of the recipient.

For new-onset hyperlipidemia analyses: P values were adjusted for age of recipient at transplantation, sex of the recipient, baseline BMI, the type of calcineurin inhibitor and treatment with corticosteroids.

For new-onset hypertension analyses: P values were adjusted for age of recipient at transplantation and sex of the recipient, treatment with corticosteroids, the type of calcineurin inhibitor and type of donor.

Supplementary data:

Materials and Methods

- **Population based samples:**
  - **The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC)**
  - **Genetic Investigation of ANtropometric Traits (GIANT) consortium**
  - **Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides**
- **Polymorphism selection and genotyping**
- **Statistical analysis**

Results:

- **General characteristics of the discovery sample**
- **General characteristics of the replication sample (STCS):**
- ***CRTC2* SNP and new-onset hypertension in the STCS**
- ***CRTC2* SNP and new-onset hyperlipidemia in the STCS**

Supplementary Tables 1-5

Materials and Methods

Population based samples:

The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC)

MAGIC is a large-scale meta-analyses of genome-wide data for continuous diabetes-related traits in participants without diabetes [1]. Meta-analyses of ~2.5 million directly genotyped or imputed autosomal single nucleotide polymorphisms (SNPs) were performed from genome-wide association studies (GWAS). These cohorts include up to 46'186 non-diabetic participants of European descent informative for FBG, the surrogate estimates of beta-cell function (HOMA-B) and insulin resistance (HOMA-IR) derived from fasting variables by homeostasis model assessment and up to 15'234 non-diabetic individuals informative for 2 hour oral glucose tolerance test (OGTT) [2].

Genetic Investigation of ANtropometric Traits (GIANT) consortium

The GIANT consortium performed a meta-analysis of GWAS data with a discovery set of 123'865 individuals of European ancestry from 46 studies for height [3], BMI[4] and waist-to hip ratio [5].

Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides

Data on lipid traits have been downloaded from "Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides" website [6, 7] which is a meta-analysis of 46 lipid GWASs. These studies together comprise >100,000 individuals of European descent (maximum sample size 100,184 for Total Cholesterol, 95,454 for LDL-C, 99,900 for HDL-C and 96,598 for triglycerides), ascertained in the United States, Europe or Australia.

Polymorphism selection and genotyping:

Polymorphisms within *CRTC2* were selected using HapMap Genome Browser (release 28)[8]. Two tagging SNPs were found by limiting the search to SNPs within CRTC2 gene ± 1kb with a minor allele frequency > 5% in the Caucasian population and r^2 cutoff of 0.8: *rs8450G>A* within the 3'UTR and the intronic *rs12117078G>C* SNP.

Genotyping of these 2 SNPS for the discovery study sample was done using Taqman allelic discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland) after genomic DNA extraction from whole blood. Life technologies Taqman genotyping assays C__8722376_10 and C__30997814_10 were used for *CRTC2 rs8450G>A* and *rs12117078G>C SNPs*, respectively. Genotyping were performed in the Unit of Pharmacogenetics and Clinical Psychopharmacology, in Lausanne, Switzerland.

Genetic analyses for the first STCS replication sample were performed by KBioscience Institute in United Kingdom using the novel fluorescence-based competitive allele-specific PCR technology (KASP™). Details about this technology are available at http://www.lgcgenomics.com/genotyping/kasp-genotyping-chemistry/.

Genetic analyses for the second STCS replication sample were also performed using the KASP™ technology on ABI 7500 Fast real-time thermocycler (Applied Biosystems). Genotyping were performed in the service of infectious disease in Lausanne University Hospital, Lausanne, Switzerland, according to manufacturer's protocols (LGC Genomics, UK). The KASP primers were designed by Kraken™ assay design and workflow management software and further validated by manufacturer (LGC Genomics, UK). Automated allele calling was performed using SDS software according to standard protocols (Applied Biosystems). The following primers were used for *CRTC2 rs8450G>A* genotyping in this sample: primer allele FAM: ACCCCTAGGCATCCGGAAAAG, primer allele HEX: CACCCCTAGGCATCCGGAAAAA and common primer GGGTAGAGGGGAGCCCTGGAA.

Statistical analysis:

Quantitative data are presented as median and range unless otherwise mentioned, while qualitative data are presented as frequency and percentage. For association studies, the chi-square (Chi^2) test or the Fisher exact test for binomial variables were used. Differences in allele and genotype frequencies as well as deviation from Hardy-Weinberg equilibrium were assessed using Chi^2 test.

Discovery study sample:

For NODAT analyses, logistic regression was applied adjusted for recipient's age at transplantation, sex, BMI at baseline and type of CNIs. Due to nonlinearity of our models and the absence of any linear transformation, the association between *CRTC2* SNPs with BMI and FBG levels was assessed by fitting a Generalized Additive Mixed Model (GAMM) [9, 10] to allow a smooth trend for the response in time based on multiple observations for each patient (using a thin plate regression spline basis) adjusting for recipient's age at transplantation, sex, and type of CNIs (antidiabetic treatments for FBG analyses). GAMMs were fitted using the mgcv package of R (settings were fixed at package defaults). In order to be more conservative, the uncertainty of estimated parameters were assessed by 1000 bootstraps [11] at the subject level and results were similar with those gained by 10'000 bootstraps.

Due to the small number of subjects being homozygous for the variant allele of *CRTC2 rs12117078G>C*, this SNP was analyzed in a dominant model.

Replication samples (STCS):

For the statistical analyses of NODAT, new-onset hypertension, new-onset hyperlipidemia, and overweight or obese, logistic regression models were applied adjusted for recipient's age at transplantation and sex. Other variables identified through the univariate analysis ($P<0.10$) were also added as covariates in these models. GAMM models were applied for BMI and HDL-cholesterol analyses in the STCS sample, as both models were not linear. Linear mixed models were used for systolic and diastolic blood pressure analyses and for blood total cholesterol and LDL-cholesterol analyses. In addition to recipient's age at transplantation, sex, BMI at baseline and type of CNIs, blood

pressure models and lipid models were also adjusted for antihypertensive and hypolipidemic drugs intake, respectively.

Population-based samples:

The associations of *CRTC2* SNP with different MetS traits were analyzed using multivariate linear regression with allele dosage in which potential confounding factors such as age, sex and smoking status were added as covariates. FBG, OGTT, the surrogate estimates of beta-cell function (HOMA-B) and insulin resistance (HOMA-IR) were analyzed in the MAGIC study. BMI, waist circumference and waist-to hip ratio were the only adiposity traits analyzed by the GIANT Consortium. Triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were analyzed in the "Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides" study.

Results:

General characteristics of the discovery sample

Among the 197 patients included in the initial study, 17 patients were excluded because they were diagnosed with diabetes before transplantation, 11 patients had no follow-up data in their medical files, 2 patients did not sign consents for MetS-related genetic analysis, 4 patients dropped-out from the study and 7 were non-Caucasians. Finally, 156 Caucasian patients were included in the current study, 65% were kidney transplant recipients. The mean age at transplantation was 47.8 ± 11 years, 61% were male and the mean BMI pre-transplant was 24 ± 4 kg/m^2. During the five years of follow-up post-transplantation, NODAT occurred in 45 (28.9%) patients. Most NODAT cases (67%) occurred during the 1st year after transplantation. Most of the patients were treated with CSA (n=102, 65%), while 35% (n=54) were treated with TAC. As expected, NODAT was higher in the TAC-treated group compared to CSA[12] (46.3 vs. 19.6 % respectively). Full characteristics of our population and their distribution between NODAT and non-NODAT patients are presented in Supplementary Table 1. Recipient age (p=0.002), male sex (p=0.05), donor age (p=0.04), baseline BMI (p=0.0003) and the type of CNIs (p<0.001) were significantly different between patients with and without NODAT (Supplementary Table 1).

General characteristics of the replication sample (STCS):

Overall, 1294 patients from the first STCS replication sample received a SOT from May 2008 until May 2011, among them 1219 patients had both the clinical data and the genetic material available and were included in the current study. By excluding patients younger than 18 years old and patients with multiple organ transplantation, 958 patients remained and included in the analysis. The mean age at transplantation was 52.3 ± 13 years, 66% were male and pre-transplant BMI was 24.6 kg/m^2 (range:13.7-41.2). During the follow-up period post-transplantation, NODAT, new-onset HTN and new-onset hyperlipidemia occurred in 27.4%, 67.6% and 33.1% of the patients, respectively. Among the patients included in the second STCS replication sample (n=759) with both clinical and genetic data and by excluding patients younger than 18 years old and patients with multiple organ transplantation, 667 remained and were included in the analysis. Lower incidence of NODAT, new-onset HTN and new-onset hyperlipidemia were observed in this sample, which could be explained by the shorter follow-up duration compared to the first STCS replication sample (Supplementary Table 2). Full description of the first, second and combined STCS samples are presented in Supplementary Table 2. All patients from both STCS samples were of Caucasian origin.

***CRTC2 SNP* and new-onset hypertension in the STCS:**

Among the non-genetic factors, type of calcineurin inhibitor ($p<0.0001$), type of transplanted organ ($p<0.0001$), treatment with corticosteroids ($p<0.0001$) and type of donor ($p=0.02$) were associated with increased risk of new-onset hypertension after SOT in the univariate analyses and were used as covariates in the genetic models. A non-significant association was observed between *CRTC2 rs8450G>A* and new-onset hypertension, except for patients carrying the *rs8450-GA* genotype which showed a protective effect against new-onset hypertension (n=491, OR=0.62, 95%CI: 0.42 - 0.94, $p=0.02$) compared to wild type genotype.

CRTC2 rs8450G>A SNP was not associated with systolic or diastolic blood pressure levels (data not shown).

***CRTC2 SNP* and new-onset hyperlipidemia in the STCS:**

Among the non-genetic factors, increased recipient's age (p=0.003), baseline BMI (p=0.006), type of calcineurin inhibitor (p=0.005) and treatment with corticosteroids (p<0.0001) were associated with increased risk of new-onset hyperlipidemia after SOT in the univariate analyses and were used as covariates in the genetic models.

Supplementary Table 1: Clinical characteristics of the discovery study sample:

Characteristic	Total	Non-NODAT	NODAT	p-value
Recipient age at transplantation (years), median (range)	48 (22-68)	46 (22-68)	53 (28-68)	**0.002**
Recipients sex (Males) [%]	60.9	55.9	73.3	**0.05**
Living donor [%]	11.5	11.7	11.1	0.92
Donor age (years), median (range)	43.5 (10-73)	41 (10-73)	48 (13-69)	**0.04**
Donor sex (Males) [%]	56.8	63.2	54.8	0.06
Organ, n [%]				
lung	17 (10.9)	10 (9.0)	7 (15.6)	0.46
kidney	102(65.4)	75 (67.6)	27(60.0)	
liver	37(23.7)	26 (23.4)	11 (24.4)	
BMI pre-transplant (kg/m^2), median (range)	23.5 (15.8-37.3)	22.3 (15.8-36.2)	26.4 (18.8-37.3)	**0.0003**
BMI 5 year follow-up (kg/m^2), median (range)	26.0 (16.7-43.6)	25.5 (16.7-43.6)	27.1 (19.9-42.3)	0.10
Recipient CMV infection (R^+) [%]	49.3	52.5	42.2	0.25
Donor CMV infection (D^+) [%]	61.5	59.2	66.7	0.39
Recipient and Donor CMV infection (R^+/D^+) [%]	27.6	27.0	28.9	0.81
Calcineurin inhibitors, n [%]				
TAC	54 (34.6)	29 (26.1)	25 (55.6)	**<0.001**
CSA	102 (65.4)	82 (73.9)	20 (44.4)	
First transplantation [%]	80.1	77.5	86.7	0.19
Acute rejection episode during first year post-transplant [%]	47.4	47.2	47.7	0.96

NODAT: New-onset diabetes after transplantation, BMI: body mass index, CMV: cytomegalovirus, R: recipient, D: Donor, TAC: Tacrolimus, CSA: Cyclosporine.

Supplementary Table 2: Clinical characteristics of STCS[§]

Characteristic	First STCS replication sample (n=958)	Second STCS replication sample (n=667)	combined STCS replication sample (n=1625)
Recipient age at transplantation (years), median (range)	55 (18-79)	55 (79)	55 (18-79)
Recipients sex (Males) [%]	66.2	64.6	65.5
Period of follow-up (months), median (range)	12 (0-60)	6 (0-48)	6 (0-60)
Living donor [%]	28.7	24.0	26.7
Donor age (years)	53 (1-86)	55 (1-88)	53 (1-88)
Donor sex (Males) [%]	53.5	53.4	53.4
Organ, n [%]			
Kidney	624 (65.1)	360 (54.0)	984 (60.5)
Liver	158 (16.5)	142 (21.3)	300 (18.5)
Lung	94 (9.8)	97 (14.5)	191 (11.8)
Heart	65 (6.8)	57 (8.6)	122 (7.5)
Islets and pancreas[£]	17 (1.8)	11 (1.6)	28 (1.7)
BMI pre-transplant (kg/m^2), median (range)	24.6 (13.7-41.2)	24.9 (13.4-43.5)	24.8 (13.4-43.5)
BMI 1 year follow-up (kg/m^2), median (range)	25.3 (15.4-44.6)	24.8 (13.7-41.7)	25.2 (13.7-44.6)
Recipient CMV infection (R^+) [%]	57.5	59.5	58.2
Donor CMV infection (D^+) [%]	53.4	58.4	56.2
Recipient and Donor CMV infection (R^+/D^+) [%]	33.0	36.3	34.3
Calcineurin inhibitors, [%]			
TAC	65.5	67.1	66.3
CSA	26.7	26.1	26.4
None	7.8	6.8	7.3
Incidence of NODAT [%]	27.4	18.7	24.4
Incidence of New hypertension [%]	67.6	32.8	53.6
Incidence of New hyperlipidemia [%]	33.1	13.8	26.6

[§]patients included in this table represent the global STCS sample with the clinical data available for the analyses without the exclusion of patients with diabetes, hypertension or hyperlipidemia before transplantation.
[£]These patients had type I diabetes mellitus as an indication for transplantation and were excluded from NODAT analysis.
Abbreviations: BMI: body mass index, CMV: cytomegalovirus, R: recipient, D : Donor, TAC: Tacrolimus, CSA: Cyclosporine, NODAT: New-onset diabetes after transplantation, New hypertension: new onset hypertension after transplantation, New hyperlipidemia: new onset hyperlipidemia after transplantation.

Supplementary Table 3: Genotype frequencies in the discovery sample and in the first, the second and the combined STCS samples. Hardy-Weinberg equilibrium and a comparison between the observed minor allele frequency and HapMap minor allele frequency in the Caucasian population.

SNP	CRTC2 SNPs Frequencies n (%)			Hardy-Weinberg Equilibrium (p-value)	MAF	
					observed	HapMap
Discovery sample (n=156)						
rs8450	***GG*** 78 (50.0)	***GA*** 64 (41.0)	***AA*** 14 (9.0)	0.87	0.30	0.30
rs12117078	***GG*** 126 (80.8)	***GC*** 28 (17.9)	***CC*** 2 (1.3)	0.75	0.10	0.10
STCS						
rs8450	***GG***	***GA***	***AA***			0.30
First STCS sample (n=949)[§]	408 (43.0)	429 (44.2)	112 (11.8)	0.96	0.34	
second STCS sample (n=667)	281 (42.1)	302 (45.3)	84 (12.6)	0.84	0.35	
Combined STCS sample (n=1616)	689 (42.7)	731 (45.2)	196 (12.1)	0.92	0.35	

[§]9 missing genotypes for this sample.
MAF: minor allele frequency.

Supplementary Table 4: Association between *CRTC2 rs8450* SNP and NODAT in total population of STCS in different subgroups at risk of NODAT:

			Additive model					Dominant model	
CRTC2 rs8450		**GG**	**GA**		**AA**		**GG**	**GA&AA**	
	N		**NODAT OR (95% CI)**	**p-value**	**NODAT OR (95% CI)**	**p-value**		**NODAT OR (95% CI)**	**p-value**
First STCS replication sample:									
Total	711	ref	0.97 (0.68 - 1.38)	0.85	1.01 (0.59 - 1.73)	0.98	ref	0.97 (0.70 - 1.37)	0.88
Treatment with TAC & deceased donor	281	ref	1.08 (0.63 - 1.85)	0.79	2.45 (1.14 - 5.26)	**0.02**	ref	1.31 (0.79 - 2.17)	0.30
Second STCS replication sample:									
Total	438	Ref	0.81 (0.47 - 1.40)	0.46	1.80 (0.88 - 3.71)	0.11	ref	0.99 (0.60 - 1.62)	0.96
Combined STCS replication sample:									
Total	1149	ref	0.89 (0.66 - 1.20)	0.44	1.18 (0.76 - 1.81)	0.46	ref	0.95 (0.72 - 1.25)	0.70
Treatment with TAC & deceased donor	395	ref	0.81 (0.51 - 1.30)	0.39	1.86 (0.99 - 3.52)	0.06	ref	1.00 (0.65 - 1.54)	0.99

TAC: tacrolimus, OR: odd ratio, CI: confidence interval

P values were adjusted (when appropriate) for age of recipient at transplantation, sex of the recipient, hepatitis C status, baseline BMI, type of calcineurin inhibitor and type of donor.

Supplementary Table 5: Associations between *CRTC2 rs1572788 T>C* (in complete linkage disequilibrium (LD) with *rs8450 G>A*) and glycemic, lipidemic and obesity traits in several population-based samples:

	Effect of each *rs1572788 C-allele* (complete LD with *rs8450 A-allele*)			
phenotype	**n**	**beta**	**p-value**	**Explained variance***
Glucose[£]	46186	0.01	**0.004**	0.0002
2h glucose tolerance test[£]	15234	-0.02	0.26	
HOMA-β[£]	46186	-0.008	**0.03**	0.0001
HOMA-IR[£]	46186	0.0002	0.96	
Total cholesterol[§]	97148	0.005	0.16	
HDL-Cholesterol[§]	96908	-0.008	**0.013**	0.0001
LDL-Cholesterol[§]	92503	0.006	0.06	
Triglycerides[§]	93562	0.009	**0.004**	0.0001
BMI[$]	113955	0.004	0.37	
Waist-hip ratio[$]	55282	0.002	0.71	
Waist circumference[$]				
Male	38305	0.008	0.32	
Female	47320	0.01	0.19	

*explained variance by the polymorphism (only calculated for p<0.05).

[£] This clinical variable was analyzed in the MAGIC study.
[§] This clinical variable was analyzed in the "Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides".
[$] This clinical variable was analyzed in the GIANT study.
2h: 2 hours, HOMA: homeostatic model assessment, β: beta-cell function, IR: insulin resistance, BMI: Body mass index.

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