CRY1 circadian gene variant interacts with carbohydrate intake for insulin resistance in two independent populations: Mediterranean and North American

Hassan S. Dashti[1], **Caren E. Smith**[1], **Yu-Chi Lee**[1], **Laurence D. Parnell**[1], **Chao-Qiang Lai**[1], **Donna K. Arnett**[2], **José M. Ordovás**[1,3,4], and **Marta Garaulet**[5]

[1]Nutrition and Genomics Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA

[2]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA

[3]Department of Epidemiology, Centro Nacional Investigaciones Cardiovasculares (CNIC), Madrid, Spain

[4]Instituto Madrileño de Estudios Avanzados en Alimentación (IMDEA-FOOD), Madrid, Spain

[5]Department of Physiology, Faculty of Biology, University of Murcia, Murcia, Spain

Abstract

Dysregulation in the circadian system induced by variants of clock genes has been associated with type 2 diabetes. Evidence for the role of cryptochromes, core components of the system, in regulating glucose homeostasis is not supported by *CRY1* candidate gene association studies for diabetes and insulin resistance in human, suggesting possible dietary influences. The purpose of this study was to test for interactions between a *CRY1* polymorphism, rs2287161, and carbohydrate intake on insulin resistance in two independent populations: a Mediterranean (*n*=728) and an European origin North American population (*n*=820). Linear regression interaction models were performed in two populations to test for gene–diet interactions on fasting insulin and glucose and two insulin-related traits, homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI). In addition, fixed effects meta-analyses for these interactions were performed. Cohort-specific interaction analyses showed significant interactions between the *CRY1* variant and dietary carbohydrates for insulin resistance in both populations (*p*<0.05). Findings from the meta-analyses of carbohydrate–single nucleotide polymorphism interactions indicated that an increase in carbohydrate intake (% of energy intake) was associated with a significant increase in HOMA-IR (*p*=0.011), fasting insulin (*p*=0.007) and a decrease in QUICKI (*p*=0.028), only among individuals homozygous for

Correspondence: Marta Garaulet, Department of Physiology, Faculty of Biology, University of Murcia, Campus de Espinardo, s/n. 30100, Murcia, Spain. Tel: +34 868 88 39 30. Fax: +34 868 88 39 63. garaulet@um.es.

DECLARATION OF INTEREST

the minor C allele. This novel finding supports the link between the circadian system and glucose metabolism and suggests the importance this *CRY1* locus in developing personalized nutrition programs aimed at reducing insulin resistance and diabetes risk.

Keywords

Carbohydrate intake; CRY1; diabetes; gene–diet interaction; genetic epidemiology

INTRODUCTION

The circadian biological clock governs numerous aspects of human physiology including feeding behavior, sleep/wake cycle and metabolism (Ahn et al., 2010; Major et al., 2011; Mondul et al., 2011). This system is maintained endogenously *via* the co-expression of a group of genes referred to as clock genes, and is additionally synchronized by external cues including changes in light exposure, in physical activity and in dietary intake (Duguay & Cermakian, 2009; Quintero et al., 2003; Schibler et al., 2003). Recently, the role of the circadian system in contributing to insulin resistance has been explored (Shi et al., 2013). Many hormones that modulate insulin secretion, glucose homeostasis and feeding, including orexin, leptin and glucagon, are regulated cyclically by the circadian system (Turek et al., 2005). As a result of this regulation, the loss of circadian rhythmicity caused by dysregulation of the expression of these genes can have a profound impact on glucose homeostasis. Consequently, clock variants discovered through genome-wide association studies have been associated specifically with diabetes and insulin resistance (Bouatia-Naji et al., 2009; Dupuis et al., 2010).

The cryptochromes (Cry1 and Cry2) constitute the negative limb of the core clock and appear to play an important role in regulating gluconeogenesis (Hatori & Panda, 2010). In mouse studies, circadian changes in hepatic Cry1/2 expression were sufficient to modulate gluconeogenesis (Zhang et al., 2010). The knockdown of Cry1/2 elevated circulating glucose concentrations and also increased glucose production in primary hepatocytes. Because of this known influence of cryptochromes in regulating glucose homeostasis, genetic variants in *CRY1* and *CRY2* genes may be linked with risk for insulin resistance or type 2 diabetes.

Given the high prevalence of type 2 diabetes, identifying modifiable factors, specifically dietary components that could modulate the risk of the disease together with clock genetic variants, could help to identify nutrition-based and genetic interventions for individuals at risk for diabetes (Ordovas & Corella, 2004). Very few studies have focused on analyzing the potential interactions between clock variants and modifiable risk factors. Earlier studies have assessed the role of carbohydrate intake as a risk factor for type 2 diabetes (Meyer et al., 2000), and more recent gene–diet interaction studies have identified genetic variants that could modify the association between carbohydrate intake and diabetes traits (Corella et al., 2006; Smith et al., 2012; Zheng et al., 2013). These studies provide promising insights for diabetes prevention and treatment.

Since evidence for the role of *CRY1* in regulating glucose homeostasis is not supported by *CRY1* candidate gene association studies for diabetes and insulin resistance in human, the investigation of potential gene–diet interactions that could explain the lack of direct association is warranted. These interactions could result from known pathways linking dietary intake to circadian genes, most commonly investigated for rs1801260, a variant located 3′ downstream of *CLOCK* (Garaulet et al., 2009). However, of particular interest is rs2287161, a variant located 3′ downstream of *CRY1*. The association between this common variant and known circadian disruption phenotypes suggests further functionality in other circadian phenotypes (Soria et al., 2010). In addition, recent advancements in the Encyclopedia of DNA Elements (ENCODE) project indicate that rs2287161 is likely to affect the binding of several transcription factors across multiple cell types, including HepG2 and adipocytes, potentially resulting in dysregulation of the gene (Raney et al., 2011).

The purpose of this study was to test for interaction between the *CRY1* rs2287161 polymorphism and carbohydrate intake on glucose and insulin-related traits. In order to increase the confidence that the interaction identified is sound, results from a Mediterranean discovery cohort were validated in an independent European origin North American population. In addition, a meta-analysis of both cohorts provided an overall effect size and significance level for these interactions.

MATERIALS AND METHODS

Study participants and study design

A total of 1548 participants from two independent populations (Mediterranean and North American) were investigated for this study. All participants provided written informed consent, and the experimental protocol conforms to international ethical standards (Portaluppi et al., 2010).

Mediterranean population—The study sample consisted of 728 subjects (81% women) (age: mean=39.3 years, SD=12.4 years; body mass index (BMI): mean=31.2 kg/m^2, SD=5.5 kg/m^2), residents of Murcia (Spain) who voluntarily attended five nutrition clinics in southeast Spain with the objective of losing weight. The participants followed a Mediterranean diet and supervised behavioral modification. All procedures were in accordance with good clinical practice. Patient data were codified to guarantee anonymity.

North American population—The study sample consisted of 820 (50.5%) women (age: mean=48.3 years, SD=15.9 years; BMI: mean=28.5 kg/m^2, SD=5.5 kg/m^2) who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. GOLDN is part of the Program for Genetic Interactions Network and is funded by the NIH through the University of Alabama at Birmingham and in collaboration with the University of Utah, Washington University, Tufts University, University of Texas, University of Michigan, University of Minnesota and Fairview University of Minnesota Medical Center. The majority of participants were re-recruited from three-generational pedigrees from two National Heart, Lung and Blood Institute Family Heart Study field centers (Minneapolis, MN and Salt Lake City, UT) (Higgins et al., 1996). Nearly all individuals were of European

descent. The details of the study are available at https://dsgweb.wustl.edu/goldn/. The protocol was previously approved by the institutional review boards at each of the above-referenced institutions.

Laboratory measurements

In both populations, blood samples were drawn after an overnight fast. Anthropometric data, including height, weight and waist circumference, were measured by using standard techniques. BMI was calculated as weight (kg)/height (m)^2. Plasma concentrations of glucose, cholesterol, triglyceride and lipoproteins were determined (Sánchez-Moreno et al., 2011; Shen et al., 2007). Insulin was determined through a solid-phase, two-site chemiluminescent immunometric assay (IMMULITE 2000 Insulin). Homeostasis model assessment of insulin resistance (HOMA-IR; fasting glucose × fasting insulin/22.5) was used to assess insulin resistance (IR). The quantitative insulin sensitivity (IS) check index (QUICKI; 1/(log (fasting insulin μU/mL) + log (fasting glucose mg/dL))) was used to assess IS (Katz et al., 2000).

Carbohydrate intake

Mediterranean population—To evaluate habitual dietary intake before the treatment, intake was determined by a 24-hour dietary recall, including 24-hour recalls of food intake from all days of the week in the total population. Total energy intake and macronutrient composition were analyzed with the nutritional evaluation software program, Grunumur (Murcia, Spain) (Pérez-Llamas et al., 2004), on the basis of Spanish food composition tables. Total carbohydrate included both complex carbohydrate and simple sugars and was analyzed as percentages of total energy consumption from total carbohydrate. To construct the categorical variables, carbohydrate intake (% of energy) was classified into two groups according to the median carbohydrate intake of this population.

North American population—Dietary intake was estimated using the Diet History Questionnaire (DHQ), a food-frequency questionnaire developed by the National Cancer Institute. It consists of 124 food items and includes both portion size and dietary supplement questions (Subar et al., 2001). The food list and nutrient database used with the DHQ are based on national dietary data [US Department of Agriculture (USDA) 1994–1996 Continuing Survey of Food Intakes by Individuals, available from the USDA Food Surveys Research Group]. Intake of total carbohydrate included both complex carbohydrate and simple sugars and was expressed percentages of total energy consumption from total carbohydrate. To construct the categorical variables, carbohydrate intake (% of energy) was also classified into two groups according to the median carbohydrate intake of this population.

DNA isolation and genotype analysis

DNA was isolated from blood (Qiagen, Hilden, Germany). For the Mediterranean population, we performed the genotyping for *CRY1* rs2287161 using a TaqMan assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). For the North American population, genome-wide genotyping was conducted by using Affymetrix Genome-Wide Human Single Nucleotide

Polymorphism (SNP) Array 6.0 and MACH (Markov Chain-based haplotyper V1.0.16, Ann Arbor, MI; http://www.sph.umich.edu/csg/abecasis/MaCH/, MACH 1.0, Nov.18, 2012) was used to impute the rs2287161 autosomal SNP using the phased haplotypes for CEU from HapMap (release 22, build 36) as a reference panel (high imputation quality: R^2=0.7) (Li et al., 2010). The imputed SNP genotype was determined by categorizing the SNP dosage into three groups using 0.66 and 1.33 for cutoff values. Genotype frequencies were consistent with Hardy–Weinberg equilibrium in both populations.

SNPs in the *CRY1* locus were examined *in silico* for a potential function in relevant tissues through modulation of transcription factor binding, expression quantitative trait loci (eQTL) or mRNA splicing. The rs2287161 locus was selected for this analysis because of its allele-specific disruption of transcription factor binding, as predicted by the tool RegulomeDB (Stanford, CA) (Boyle et al., 2012).

Statistical analyses

We applied analysis of variance and Student's *t* test to compare crude means. Normality of continuous variables was examined. Natural logarithmic transformations were applied to HOMA-IR, insulin and glucose values. We tested different genetic inheritance models, and a recessive model was applied in the final analyses for *CRY1* rs2287161; therefore, major G allele carriers (CG + GG) were compared against minor C allele homozygotes (CC).

First, we assessed for associations between *CRY1* rs2287161 and our outcomes of interest using multivariate linear regression models including main effects term. To study gene–diet interactions, we further included interaction terms in our models. Carbohydrate intake was considered dichotomously in the interaction models. We fitted separate models for each population including the same variables for the interaction terms and for the multivariate adjustments. In the Mediterranean population, the analyses were adjusted for sex, age, BMI, study number and clinic location. In the North American population, self-reported diabetic (*n*=60) and non-white (*n*=6) individuals were excluded from the analyses to achieve homogeneity between the two populations, and analyses were adjusted for sex, age, BMI, center location and family structure. All statistical analyses were performed using R statistical software (v. 3.0.0, Vienna, Austria). A two-tailed *p* value of <0.05 was considered statistically significant.

Meta-analyses

We conducted inverse variance-weighted, fixed effects meta-analyses meta-analysis for the interaction between the *CRY1* rs2287161 variant and carbohydrate intake on fasting insulin and glucose, and two insulin-related traits, HOMA-IR and QUICKI, using METAL (University of Michigan, Center for Statistical Genetics, Ann Arbor, MI; www.sph.umich.edu/csg/abecasis/metal/) (Willer et al., 2010). Cohort-specific interaction analyses included in the meta-analysis were adjusted for age, sex, BMI and other cohort-specific adjustments. Carbohydrate intake was expressed categorically. The sample size for the interaction analysis on HOMA-IR was 1482. Heterogeneity across studies was tested using Cochran's Q statistic and quantified by using the I^2 statistic. Statistical significance

was defined at a level of 0.0125, based on Bonferroni correction for four total interaction tests.

RESULTS

General characteristics of participants in both the Mediterranean (*n*=728) and North American (*n*=820) populations are listed in Table 1. Briefly, the Mediterranean population was generally younger and had a higher prevalence of obesity than the North American population. Differences between these populations were also found in the dietary carbohydrate intake, with a higher total carbohydrate intake (g/day) and percentage of carbohydrate intake found in the North American population. Significant differences were also evident in fasting insulin and fasting glucose measures, with a healthier pattern evident in the Mediterranean population, although tobacco and alcohol use was much higher than in the North American population. The frequency of rs2287161 SNP was similar in both populations.

Cohort-specific interaction analyses: Mediterranean population

Consistent with previous findings, no significant associations were evident between the *CRY1* locus and fasting insulin, glucose and two insulin-related traits (*p*>0.05). We identified a significant differential effect across the genotypes at the *CRY1* locus on HOMA-IR (*p*=0.004) when individuals were dichotomized into high and low carbohydrate intake groups based on the median carbohydrate intake (>41.65% vs <41.65%) (Figure 1). Among individuals homozygous for the minor C allele (CC), high carbohydrate intake was associated with a greater HOMA-IR as compared with low carbohydrate intake (*p*=0.002). Further adjustment for BMI did not modify the statistical significance of this interaction (*p*=0.010) (Table 2). However, regardless of carbohydrate intake, no significant differences in HOMA-IR were evident among carriers of the major G allele (GG + CG) (*p*=0.980). This *CRY1* variant also showed significant interaction with carbohydrate intake for fasting insulin (*p*=0.007) and IS (QUICKI) (*p*=0.013), while no significant interaction was found between *CRY1* rs2287161 and carbohydrate intake for fasting glucose (*p*=0.637).

Cohort-specific interaction analyses: North American population

To evaluate the plausibility of our findings, we investigated these interactions in a North American population. When dichotomized into high and low carbohydrate intake groups based on the median carbohydrate intake (>49.14% vs <49.14%), the identified differential effect across the genotypes at the *CRY1* locus on HOMA-IR was marginally significant (*p*=0.074) (Table 2). Marginal significance was also evident on fasting insulin (*p*=0.056) and IS (QUICKI) (*p*=0.12), while no significance was evident for fasting glucose (*p*=0.86) (Table 2). However, when considered continuously, we identified a significant interaction between the *CRY1* locus and carbohydrate intake on predicted HOMA-IR, consistent with that of the Mediterranean population (*p*=0.035) (Figure 2). Specifically, a greater carbohydrate intake was associated with greater HOMA-IR only among minor C homozygotes (CC) (*p*=0.009). These differences did not reach statistical significance among major G allele carriers (GG + CG) (*p*=0.360) (Figure 2). Moreover, we further identified

significant interactions on fasting insulin (p=0.024) and IS (QUICKI) (p=0.024) but not on fasting glucose (p=0.762).

Meta-analyses of carbohydrate-SNP interactions on diabetes-related outcomes

Meta-analyzed estimates of *CRY1* interaction with carbohydrate intake evaluated dichotomously on insulin-related traits are listed in Table 2. The *CRY1* locus showed significant interactions with carbohydrate intake on insulin-related outcomes including HOMA-IR (p=0.011) (Figure 3), QUICKI (p=0.028) and fasting insulin (p=0.007). Significance of these interactions remained following Bonferroni correction for multiple comparisons (p<0.0125). However, there was no significant interaction on fasting plasma glucose concentrations (p=0.806). Tests for heterogeneity across studies did not reveal significant heterogeneity between the two populations for this outcome (p>0.05).

DISCUSSION

Our study has identified a novel interaction between the *CRY1* rs2287161 SNP, carbohydrate intake and glucose metabolism using fasting glucose and insulin and two insulin-related traits, HOMA-IR and QUICKI, as outcomes. Moreover, the initial finding in the Mediterranean population is supported by its replication in an independent population. An increase in the proportion of carbohydrate intake led to a significant increase in HOMA-IR, fasting insulin and a decrease in QUICKI exclusively among participants homozygous for the minor C allele. Therefore, our data support that genetic variation at core clock genes, specifically *CRY1*, interacts with type 2 diabetes susceptibility factors (i.e. carbohydrate intake) to modulate IR and type 2 diabetes risk.

Other core clock genes have also been implicated in glucose metabolism, possibly due to the circadian system's putative influence on IS *via* glucose and lipid metabolism (Jordan & Lamia, 2013; Prokopenko et al., 2009; Scott et al., 2008; Woon et al., 2007). This is the case of a variant near *CRY2* that was found to be associated with elevated fasting glucose in humans (Liu et al., 2011). In addition, genetic variants within *CLOCK, ARNTL1* and *MTNRB1* have been associated with IR or type 2 diabetes. The lack of a direct association between rs2287161 and these traits suggests plausible interactions with environmental factors.

Moreover, genetic factors have also been identified as potential sources of variability in IS in response to carbohydrate intake, including those in the *ADRB2* and *PLIN1* genes (Corella et al., 2006; Martínez et al., 2003; Smith et al., 2012). Therefore, while it has been previously shown that diets higher in carbohydrate are associated with elevated insulin concentrations and decreased IS, these previously identified loci, along with the *CRY1* locus, appear to modulate the contribution of dietary carbohydrate toward greater insulin resistance (Corella et al., 2006; Smith et al., 2012). The recognition of these interactions offers the potential to identify personalized modifiable lifestyle changes to reduce the risk of insulin resistance and type 2 diabetes (Ordovas & Corella, 2004).

Evidence from mouse knockdown studies supports the role of both *CRY1* and *CRY2* in regulating gluconeogenesis (Zhang et al., 2010). This regulation is possibly mediated

through Cry1/2 temporal regulation of glucagon signaling, which triggers CREB phosphorylation thereby activating it (Hatori & Panda, 2010). Transcriptionally active p-CREB then drives the transcription of several important gluconeogenic genes, including *PCK1* (encoding PEPCK) and *G6PC* (encoding G6Pase). These genes play integral roles in hepatic gluconeogenesis. Therefore, it is plausible that factors affecting the expression of *CRY1/2* could impair hepatic regulation of glucose homeostasis through regulation of these regulatory genes and thus increase risk for diabetes in humans.

The exact mechanism by which the rs2287161 may contribute to the observed interaction is unknown. This variant was previously identified to be significantly associated with other common circadian disruption phenotypes (Soria et al., 2010). In addition, evidence from CHIP-seq experiments suggests that the variant is likely to disrupt the binding of transcription factors across multiple cell types (Raney et al., 2011). In human adipocytes, this allelic disruption impedes E2F4 transcription factor from binding, preventing H3K56 acetylation and thereby affecting transcriptional regulation of nearby genes (Lo et al., 2011). Results from a genomewide profile of H3K56 acetylation indicate that the highest level of H3K56 acetylation is associated with proteins in the type 2 diabetes pathways (Lo et al., 2011). Similarly, it is predicted that this variant hinders the regulatory function of RFX5, a regulatory transcription factor that is under circadian control in HepG2 cells (Boyle et al., 2012; Hughes et al., 2009). RFX5 binding is required for the transcriptional regulation of nearby genes in different cell types (Sengupta et al., 2002; Villard et al., 2000). Consequently, rs2287161 could effectively alter the expression of *CRY1* in liver, resulting in gluconeogenesis dysregulation.

Accumulating evidence also suggests that core clock genes constituting the negative limb of the circadian system, such as the cryptochromes, reduce glucose output by the liver and insulin secretion by the pancreas (Bass, 2010). These findings are consistent with our results as the *CRY1* rs2287161 is predicted to alter *CRY1* regulation. Moreover, the lack of a proxy SNP for this CRY1 variant at $r^2 > 0.25$ in the CEU population (Johnson et al., 2008) implies that either the rs2287161 itself or a rare undetected variant confers these allele-specific characteristics.

Major strengths of this investigation are the consistency of the findings across two independent populations and their potential clinical significance. Generally, the replication of gene–diet interactions is uncommon due to multiple factors inherent in cohort experimental designs and biological complexity. Nevertheless, the results from the meta-analyses showed consistent interactions, with no heterogeneity, between the two studies. These interactions remained significant following Bonferroni correction for multiple testing, despite the high correlations among the outcomes of interest.

Limitations to this investigation include the use of cross-sectional data, which limit interpretation of the link between the *CRY1* variant and glucose metabolism. Longitudinal epidemiologic studies and biochemical experimentally based research are required to elucidate and strengthen the findings from this study. Despite its putative role in affecting transcription factor-binding site, as suggested by CHIP-seq experiments, there is no evidence yet from expression quantitative trait loci (cis-eQTL) analyses to suggest that the

CRY1 variant influences the rhythmicity of *CRY1* expression across relevant tissues including liver, muscle and adipose tissue. Furthermore, whether this interaction relates to changes in *CRY1* circadian rhythmicity has not been explored. In addition, the pathway linking dietary carbohydrate intake to this circadian gene is unknown. Future studies need to clarify the exact function of the *CRY1* variant in disrupting the transcription factor-binding region, and the role of carbohydrate intake, which may contribute to the development of potential future, targets for intervention.

In summary, this study revealed an interaction between a *CRY1* variant and carbohydrate intake for glucose metabolism. This novel and replicated gene–diet interaction should foster more mechanistic research to precisely identify the molecular mechanisms elucidating the observed results. Moreover, this knowledge may be applied to clinical practice and contribute to personalized therapies for the prevention and treatment of metabolic disorders.

Acknowledgments

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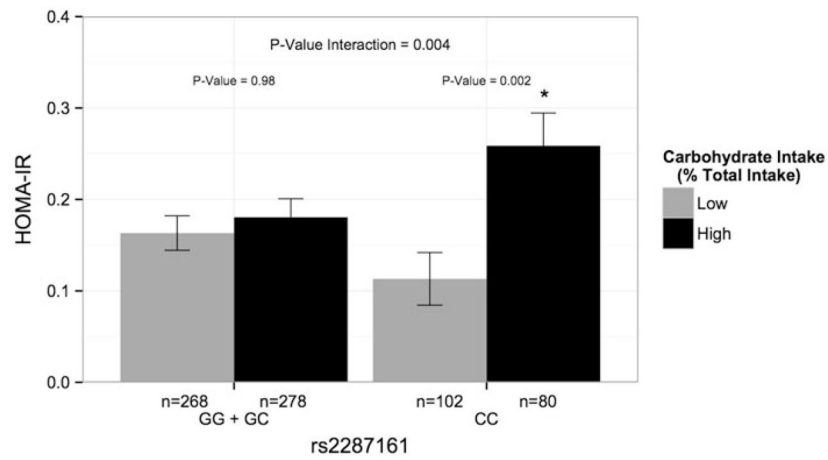
FIGURE 1.

Gene–diet interaction for HOMA-IR in the Mediterranean population. Mean (± SE) HOMA-IR by CRY1 rs2287161 genotype according to total carbohydrate (% of total energy) intakes below and above the population median (41.65% of total energy). Allele was modeled recessively and estimated means were adjusted for age, sex, study number and clinic location. HOMA-IR, homeostasis model assessment-estimated insulin resistance.
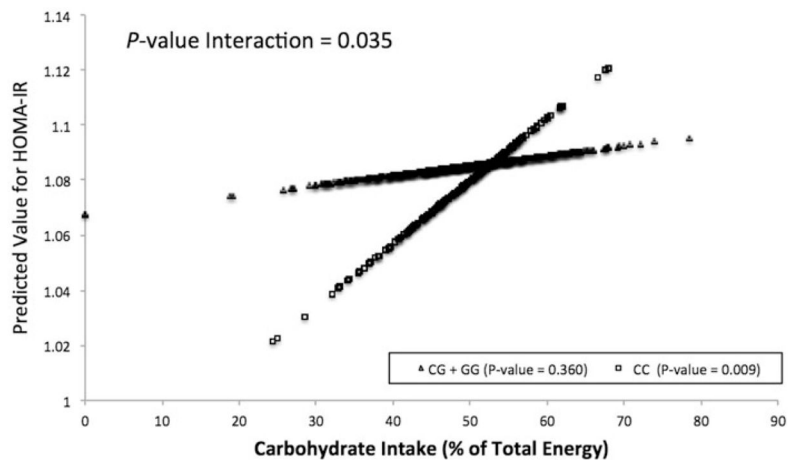
FIGURE 2.
Gene–diet interaction for HOMA-IR in the North American population. Predicted values of HOMA-IR according to CRY1 rs2287161 genotype plotted against carbohydrate intake (% of total energy) evaluated continuously. Predicted values for HOMA-IR (natural log) were calculated from the regression model after adjustment for age, sex, BMI, center location and family structure. *p* Values indicate the statistical significance of the interaction term in the adjusted regression model, the statistical significance of the adjusted regression coefficient in the regression line corresponding to CRY1 minor allele homozygotes and the statistical significance of the adjusted regression coefficient in the regression line to carriers of the CRY1 major allele. HOMA-IR, homeostasis model assessment- estimated insulin resistance.
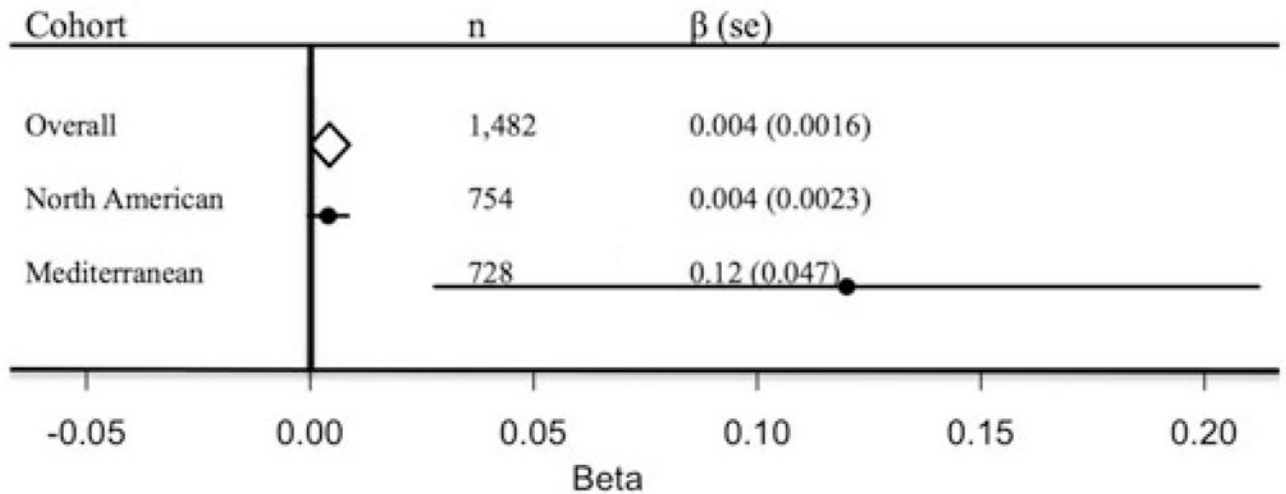
FIGURE 3.
Forest plot of the interaction between CRY1 variant (rs2287161) and carbohydrate intake (% of total energy) for HOMA-IR in two independent populations. Allele modeled recessively and carbohydrate intake (% of total energy) was evaluated dichotomously. Cohort-specific interaction analyses adjusted for age, sex, BMI and cohort-specific covariates. The estimate from each cohort study is indicated by a filled circle and the horizontal line represents the 95% CI. The overall summary estimate and its 95% CI are indicated by the open diamond.

TABLE 1

General characteristics of participants in both populations.

Characteristics/measures	Mediterranean (n = 728)		North American (n = 754)	
	M	SD	M	SD
Age (years)	39.3	12.4	48.3	15.9
Sex (% female)	81.5%		50.0%	
BMI (kg/m^2)	31.2	5.5	28.5	5.5
Waist (cm)	101.8	15.1	97.1	16.4
HOMA-IR	2.03	2.31	3.6	2.5
QUICKI	0.38	0.002	0.33	0.02
Triglycerides (mg/dL)	104.1	56.4	154.1	103.1
Total cholesterol (mg/dL)	195.4	37.8	192.6	39.8
Fasting insulin (mU/L)	8.92	8.2	14.1	8.3
Fasting glucose (mg/dL)	82.3	15.2	102.0	19.7
Dietary intake				
Total energy (kcal/day)	2077	716	2134	1261
Proteins (% total energy)	17.2	4.4	15.7	2.9
Proteins (g/day)	71.6	40.0	83.3	47.3
Carbohydrates (% total energy)	41.6	10.5	48.8	5.5
Carbohydrates (g/day)	151.5	79.0	256.8	145.7
Fiber (g/day)	18.8	11.4	19.1	10.5
Fats (% total energy)	42.4	9.5	35.4	7.0
Fats (g/day)	88.2	45.1	85.1	53.2
MUFA (% total fat)	19.3	6.4	13.3	2.9
PUFA (% total fat)	4.7	1.7	7.7	2.2
SFA (% total fat)	10.4	4.0	11.8	2.7
Other characteristics	*n*	%	*n*	%
BMI 30 kg/m^2	380	52.2	282	34.4
Drinkers	440	75.6	407	49.6
Smokers	165	22.7	67	8.2
Sedentary	332	45	288	35.1
CRY1 (rs2287161)				
CC	182	25.0	220	26.8
CG	333	45.7	391	47.7
GG	213	29.3	209	25.5

HOMA-IR, homeostasis model assessment-estimated insulin resistance; QUICKI, quantitative insulin sensitivity check index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; BMI, body mass index; M, mean; and SD, standard deviation.

TABLE 2

Interactions between *CRY1* variant (rs2287161) and carbohydrate intake (% of total energy) on glucose and insulin-related outcomes in two populations: Mediterranean and North American.

Outcome[c]	Mediterranean (n = 728)[a]			North American (n = 754)[b]			Meta-analysis (n = 1482)		
	β	SE	p Value	β	SE	p Value	β	SE	p Value
HOMA-IR[d]	0.12	0.047	0.010	0.0040	0.0023	0.074	0.0040	0.0016	0.011
QUICKI[e]	−0.19	0.007	0.013	−0.0003	0.00021	0.12	−0.0003	0.0002	0.028
Fasting insulin (mU/L)	0.12	0.04	0.007	0.0040	0.0021	0.056	0.0040	0.0015	0.007
Fasting glucose (mg/dL)	0.011	0.023	0.637	0.00019	0.0011	0.862	0.0002	0.0008	0.806

[a] Recessive model, adjusted for age, sex, BMI, study number and clinic location. Carbohydrate intake (% of total energy) dichotomized based on median intake.

[b] Recessive model, adjusted for age, sex, BMI, center location and family structure. Carbohydrate intake (% of total energy) dichotomized based on median intake.

[c] Natural logarithmic transformations were applied to HOMA-IR, fasting insulin and glucose to achieve normality.

[d] HOMA-IR=[fasting glucose (mmol/l) × fasting insulin (mU/mL)]/22.5; HOMA-IR, homeostasis model assessment-estimated insulin resistance.

[e] QUICKI=1/[log(fasting insulin (μU/mL)) + log(fasting glucose (mg/dl))]; QUICKI, quantitative insulin sensitivity check index.