The *TCF7L2* rs7903146 polymorphism is associated with diabetes and obesity in an elderly cohort from Brazil

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Background. Type 2 diabetes mellitus (T2DM) and obesity are complex diseases considered pandemics in the 21st century. The T allele rs7903146 in the *TCF7L2* gene is worldwide recognized as a strong GWAS signal associated with T2DM. However, the association between C allele and obesity is still poorly explored and needs to be replicated in other populations. Thus, the main objectives of this study were to evaluate the TCF7L2 rs7903146 association with T2DM according to BMI status and to verify if this variant is related to obesity and BMI variation in a cohort of elderly Brazilians.

Methods. A total of 1,023 participants from an elderly census-based cohort called SABE (Saúde, Bem Estar e Envelhecimento - Health, Well-Being and Aging) were stratified by BMI status and type 2 diabetes presence. The *TCF7L2* genotypes were filtered from the Online Archive of Brazilian Mutations (ABraOM - Online Archive of Brazilian Mutations) database: a web-based public database with sequencing data of samples of the SABE's participants. Logistic regression models and interaction analysis were performed. The BMI variation (ΔBMI) was calculated from anthropometric data collected in up to two time-points with a ten-year assessment interval.

Results. The association between rs7903146 T allele and T2DM was inversely proportional to the BMI status, with an increased risk in the normal-weight group (OR 3.36; 95% CI 1.46-7.74; P=0.004). We confirmed the T allele association with risk for T2DM after adjusting for possible confounders factors (OR 2.35; 95% CI 1.28 – 4.32; P=0.006). Interaction analysis showed that the increased risk for T2DM conferred by T allele is modified by BMI ($P_{interaction}$=0.008), age ($P_{interaction}$=0.005) and gender ($P_{interaction}$=0.026). A T allele protective effect against obesity was observed (OR 0.71; 95% CI 0.54-0.94; P=0.016). The C allele increased obesity risk (OR 1.40; 95% CI 1.06-1.84; P=0.017) and the CC genotype showed a borderline association with abdominal obesity risk (OR 1.28; 95% CI 1.06-1.67; P=0.045). The CC genotype increased the obesity risk after adjusting for possible confounders factors (OR 1.41; 95% CI 1.06 – 1.86; P=0.017). An increase of the TT genotype in the second tertile of ΔBMI values was observed in participants without type 2 diabetes (OR 5.13; 95% CI 1.40-18.93; P=0.009) in the recessive genetic model. **Conclusion.** We confirmed that the rs7903146 is both associated with T2DM and obesity. The *TCF7L2* rs7903146 T allele increased T2DM risk in the normal-weight group and interacts with sex age BMI, while the C allele increased obesity risk. The TT genotype was associated with a lesser extent of BMI

variation over the ten years of the SABE study.

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Abstract

Background. Type 2 diabetes mellitus (T2DM) and obesity are complex diseases considered pandemics in the 21st century. The T allele rs7903146 in the *TCF7L2* gene is worldwide recognized as a strong GWAS signal associated with T2DM. However, the association between C allele and obesity is still poorly explored and needs to be replicated in other populations. Thus, the main objectives of this study were to evaluate the *TCF7L2* rs7903146 association with T2DM according to BMI status and to verify if this variant is related to obesity and BMI variation in a cohort of elderly Brazilians.

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Conclusion. We confirmed that the rs7903146 is both associated with T2DM and obesity. The *TCF7L2* rs7903146 T allele increased T2DM risk in the normal-weight group and interacts with sex age BMI, while the C allele increased obesity risk. The TT genotype was associated with a lesser extent of BMI variation over the ten years of the SABE study.

Introduction

Type 2 diabetes mellitus (T2DM) and obesity are pandemic diseases interconnected by insulin mechanisms and characterized by complex interactions between environmental and genetic factors (Haupt et al., 2010; Chen et al., 2018; Grant, 2019). In this context, important T2DM genes involved in insulin production, processing, trafficking and secretion can also play a significant role in obesity development (Noordam et al., 2017; Fernández-Rhodes et al., 2018). The *TCF7L2* (10q25.2), one of these genes, encodes a transcription factor member of Wnt signaling pathway that is known to act on vital functions of β cells and glucose metabolizing tissues (Cropano et al., 2017).

The rs7903146 T allele in *TCF7L2* is the stronger GWAS signal for T2DM risk in different populations across the world and is associated with mechanisms related to insulin synthesis, processing, secretion and action (Grant et al., 2006; Cauchi et al., 2008b; Bouhaha et al., 2010; Zhou et al., 2014; Corella et al., 2016; Cropano et al., 2017). The genetic susceptibility for T2DM is modulated by BMI suggesting a potential relationship between the rs7903146 variant and risk for obesity, which may be related to the *TCF7L2* expression and the Wnt pathway regulation in adipose tissue (Ross et al., 2000; Grant et al., 2006; Zhou et al., 2014; Cropano et al., 2017; Chen et al., 2018).

The Wnt signaling negatively regulates adipogenesis and plays important metabolic and developmental roles in adipose tissue (Chen & Wang, 2018). Although the *TCF7L2* encodes the main effector of this signaling pathway, only a few studies investigated the association between the rs7903146 and risk for obesity (Haupt et al., 2010; Al-Daghri et al., 2014; Locke et al., 2015; Abadi et al., 2017; Muller et al., 2019). These studies reported association between rs7903146 C allele and risk for obesity. In this sense, it is important to confirm this association in other populations (Grant, 2019).

We hypothesize that rs7903146 variant is associated not only with T2DM but also with obesity. We purpose to investigate if the T2DM risk conferred by rs7903146 SNP is related to BMI status and to verify differences among the rs7903146 genotypes on BMI variation over a ten-year period. Furthermore, we performed interaction analysis of this genetic variant with BMI, age and gender.

Materials & Methods

Study Cohort

The sample belongs to elderly volunteers from a health survey called SABE (Saúde, Bem Estar e Envelhecimento - Health, Well-Being and Aging) carried out in the city of São Paulo, Brazil, under coordinated by the Pan American Health Organization. It was initiated as a multicenter health survey and well-being of older people in seven urban centers in the Caribbean and Latin America (Bridgetown, Barbados; Buenos Aires, Argentina; Havana, Cuba; Mexico City, Mexico; Montevideo, Uruguay; Santiago, Chile; and São Paulo, Brazil). Thereafter the Brazilian center has followed a longitudinal approach with a re-collection every five years, under the coordination of the Public Health School at the University of São Paulo (Lebrão & Laurenti, 2005).

The Ethics in Research Committee of the School of Public Health of the University of São Paulo and the Brazilian National Committee for Ethics in Research approved the SABE study (protocol number 2015/12837/1.015.223). All participants signed consent forms following the Brazilian regulatory requirements of research with human subjects (Lebrão & Laurenti, 2005).
The SABE Study was approved by the Institutional Review Board of the University of São Paulo School of Public Health (CAAE: 47683115.4.0000.5421, Review: 3.600.782). A detailed description of the study population including demographic characteristics, clinical and anthropometric data, medical history and socioeconomic background is presented elsewhere (Lebrão & Laurenti, 2005). All subjects in the genomic dataset have agreed on participating in this study on written consent forms approved by CEP/CONEP (Brazilian local and national ethical committee boards).

Clinical and Anthropometric Characteristics

The data collection was done in participants' households by trained interviewers, using a specific standardized questionnaire (C10) proposed by the Pan American Health Organization (PAHO), translated and adapted for use in Brazil (Naslavsky et al., 2017). The T2DM was self-reported by responding to the question "has a doctor or nurse ever told you that you have diabetes or high blood sugar levels?". Blood was withdrawn and submitted to biochemical analysis and genomic analyses.

The following demographic and health variables were assessed: gender, age, fasting plasma glucose (mg/dL), glycated hemoglobin (%), total cholesterol (mg/dL), fasting triglyceride (mg / dL), LDL cholesterol (mg/dL), HDL cholesterol (mg/dL), systolic pressure (mmHg), diastolic pressure (mmHg), BMI (kg/m²), waist circumference (cm), hip circumference (cm), hip-waist ratio (cm / cm). All participants with T2DM and/or with blood glucose levels above 100 mg/dL were considered to be in a hyperglycemic state. For anthropometric evaluation, the weight was

measured using a portable scale (Seca, Germany), and the height with an anthropometer (Harpenden, England). Waist circumference was measured with an inelastic measurement tape placed on the midpoint between the lower margin of the last palpable riband and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks.

The BMI was calculated from weight and height measured at baseline by dividing body weight in kg by height in meters squared. We stratified individuals in three groups according to the BMI classification from World Health Organization (WHO): normal-weight (18.5 – 24.9 kg/m2), overweight (25.0 – 29.9 kg/m2) and obesity (≥ 30.0 kg/m2). Abdominal obesity was defined by the waist circumference > 88 cm for women and > 102 cm for men. Anthropometric data were analyzed twice over ten-years (2000 and 2010). The BMI variation (ΔBMI) of each elderly was calculated from the difference between the BMI measured in the collection years 2010 and 2000.

After the exclusion of subjects with incomplete data, this study was performed on a multiethnic population of 1,023 elderly individuals, including men and women whose anthropometric, biochemical, and genetic information were evaluated to verify association with T2DM, obesity, and BMI variation in ten years.

Next-Generation Sequencing Data

We filtered *TCF7L2* rs7903146 genotypes from the whole-genome sequencing dataset of SABE, the second phase of genomic analyses following the dataset deposited in ABraOM - *Arquivo Brasileiro Online de Mutações* (Online Archive of Brazilian Mutations, http://abraom.ib.usp.br). Quality control of genotypes and variants is described by Naslavsky et al., (2017) and by Naslavsky et al., (2020).

Statistical Analysis

Data were expressed as percentages and means ± standard deviation (SD) for continuous variables and percentages for categorical variables. The one-sample Kolmogorov-Smirnov test was used to test the normality. Differences between groups for categorical data were tested by $\chi 2$ analysis, while for continuous data Independent Samples Mann-Whitney U test and the Kruskal-Wallis test were used. Allele frequencies were determined by gene counting and departures from Hardy-Weinberg equilibrium were verified using a $\chi 2$ test.

Allele and genotype distributions among groups were evaluated using the $\chi 2$ test or Fisher's exact test. The level of significance adopted was $P < 0.05$. Logistic regression models were developed after adjusting for age and gender and were performed to assess the independent role of *TCF7L2* genotype. Interaction analysis was performed. The rs7903146 genotypic frequencies were compared among the ΔBMI tertiles. The SPSS (version 25.0.0.0) software was used for general statistics.

Results

The main clinical features from 1,023 participants are depicted in Table 1. The median age of participants was 71.4 years old (59–99 years old), being 64.32% women. The three groups clustered by BMI contain 280 subjects with normal-weight, 424 with overweight and 319 with obesity participants (Table 1). These groups did not differ in systolic pressure, total cholesterol, LDL cholesterol but differed in gender ratio, age, waist circumference, hip circumference, hip-waist ratio, diastolic pressure, plasma glucose, glycated hemoglobin, fasting triglyceride, HDL cholesterol and number of subjects with T2DM and arterial hypertension (Table1).

The genotypic distributions for the rs7903146 were in Hardy-Weinberg equilibrium in all groups (All P>0.05). These genotypic and allelic distributions of participants with and without T2DM according to BMI status are in Supplemental Table 1 (All P>0.05). In addition, the analysis of ancestries frequency is demonstrated in Supplemental Table 2 and a significant difference in European contribution was observed among the genotypes (P=0.007) and also between Non-T2DM and T2DM groups (P=0.020)

The TT genotype was more frequent in T2DM group (*P*=0.0001). The *TCF7L2* rs7903146 T allele association with T2DM was confirmed on the recessive genetic model (OR=1.89; 95% CI: 1.21–2.95; *P*=0.004), but no significant associations were detected with other phenotypes (Supplemental Table 3). Significant association signals were detected between hyperglycemic status and the T allele on dominant genetic model (OR=1.77; 95% CI: 2.61–1.20; *P*=0.004) and also under the log-additive model (OR=1.56; 95% CI: 2.09–1.17; *P*=0.002) (Supplemental Table 4). It was observed, conversely, a C allele protective effect against T2DM under dominant (OR=0.51; 95% CI:0.32 – 0.80; *P*=0.003), additive (OR=0.50; 95% CI:0.31 – 0.81; *P*=0.004) and allelic (OR= 0.79; 95% CI: 0.64 – 0.98; *P*=0.031) genetic models (Supplemental Table 5).

The regression analysis showed a stronger risk for T2DM on TT carriers even after adjusting for all possible confounders depicted in Table 1 (Table 2). However, the interaction analysis demonstrated that BMI modifies the association between TT genotype and T2DM risk (OR= 1.02; 95% CI: 1.01 – 1.04; $P_{interaction}$=0.008). This result reinforces the logistic regression analysis stratified by BMI status which showed that the risk for T2DM conferred by T allele is stronger in the normal-weight group, with the following odds ratios: (OR=3.36; 95% CI: 1.46–7.74; *P*=0.004) on the recessive model and (OR=3.21; 95% CI: 1.31–7.87; *P*=0.011) on the additive model (Table 3). In addition, the interaction analysis of the T allele and age demonstrated that the increased T2DM risk in TT carries is maintained and the age modified this association (OR= 1.01; 95% CI: 1.00 – 1.02; $P_{interaction}$=0.005).

Association tests separated by gender showed a borderline association between TT genotype and risk for T2DM in men (OR=2.19; 95% CI: 1.05 – 4.58; *P*=0.042) and a trend for association in women (OR=1.75; 95% CI: 1.00 – 3.07; *P*=0.055). After grouping subjects from the normal-weight and overweight groups and excluding the obese group, we observed association both in men (OR= 2.64; 95% CI: 1.16 – 5.98; *P*= 0.020) and women (OR=2.14; 95% CI: 1.08 – 4.21; *P*=0.028). However, in normal-weight group, we noticed a stronger association only in men (OR=5.48; 95% CI: 1.57 – 19.10; *P*=0.008) and no association was found in women. Furthermore, this result is also reinforced by the interaction analysis of the TT genotype and gender on risk for T2DM (OR=1.87; 95% CI:1.08 – 3.25; $P_{interaction}$=0.026).

The association between rs7903146 variant and obesity status was analyzed and the T allele conferred protection against obesity on the dominant model (OR=0.71; 95% CI: 0.54–0.94; *P*=0.016) (Supplemental Table 6). This result leads us to verify the association of the C allele with obesity. Our analysis revealed a CC genotype association with obesity risk on the recessive model (OR 1.40; 95% CI 1.06-1.84; *P*=0.017) (Table 4). The regression analysis showed a stronger risk for obesity on CC carriers even after adjusting for all confounders possible (OR 1.41; 95% CI 1.06 – 1.86; *P*=0.017) (Table 5). Additionally, we observed a borderline association with abdominal obesity in subjects with CC genotype (OR=1.29; 95% CI: 1.28–1.67; *P*=0.045).

Analysis of BMI variation over ten years of SABE study revealed a different distribution of rs7903146 genotypes among tertiles of ΔBMI (Supplemental Table 7). We observed an increase in the TT genotype in the ΔBMI second tertile when compared to the first tertile in the total population on the recessive genetic model (OR 2.00; 95% CI 1.01 - 3.97; *P*= 0.044) and in participants without T2DM both on the additive (OR 5.13; 95% CI 1.40-18.93; *P*= 0.009) as on the recessive model (OR 5.13; 95% CI 1.43-18.37; *P*= 0.010). No significant values were found among individuals with T2DM (Supplemental Table 8).

Discussion

We evaluated the *TCF7L2* rs7903146 association with T2DM and obesity. We explored whether the strength of association with T2DM depends on BMI status (normal-weight, overweight and obesity) and also investigated differences in BMI variation over ten years on C and T allele carriers. We confirmed that the T allele risk confers risk for T2DM and it is influenced by BMI status, age and gender. The TT genotype conferred a protective effect against obesity and CC genotype was associated with risk for obesity. Moreover, the TT genotype was associated with a lower BMI variation over a ten-year period in our elderly population.

According to the Allele Frequency Aggregator (ALFA) project from National Center for Biotechnology Information (NCBI) database, the worldwide frequency for the rs7903146 T allele is around 0.29 (Phan et al., 2020). In our population, the frequency of the T allele variated from 0.27 to 0.33 among the categories, except for the diabetic elderlies with normal weight which T allele frequency was around 0.40 (Supplemental Table 1). From that, we performed regression analysis adjusted for gender and age and confirmed the rs7903146 T allele risk for T2DM in the total population (Table 3).

Other Brazilian studies also reported the rs7903146 T allele association with risk for T2DM (Barra et al., 2012; Assmann et al., 2017). However, these studies did not investigate the influence of BMI on diabetes risk or addressed this issue in an elderly population. Thus, we verified an increased risk for T2DM conferred by the rs7903146 T allele in participants with lower BMI, being stronger in the normal-weight group (OR 3.36; 95% CI 1.46-7.74; P=0.004) (Table 3). Prior studies, with populations from other countries, also reported a stronger risk for T2DM in lower BMI (Cauchi et al., 2006, 2008b; Bouhaha et al., 2010; Corella et al., 2016). Cauchi et al., (2008) and Corella et al., (2016) observed in individuals without obesity odds ratios of 1.89 (95% CI: 1.67-2.14) and 2.32 (95% CI: 1.90-2.85) respectively, while Bouhaha et al., (2010) reported odds ratio similar to our (OR 3.24; 95% CI: 1.10-9.53). Perry et al., (2012) also verified a stronger risk for T2DM in individuals of normal-weight compared to obese individuals for 29 of 36 diabetes loci.

The rs7903146 T allele may have a greater impact on individuals without obesity not through obesity-induced insulin resistance but due to pancreatic dysfunction, indicating that β-cell impairment predicts a future T2DM in subjects with lower BMI (Cauchi et al., 2008b; Bouhaha et al., 2010). In leaner subjects, the β-cell compensation is lower while in people with obesity is higher (Watanabe et al., 2007). Plasmids carrying the T allele showed stronger transcriptional activity when compared to those with the C allele and pancreatic cells of T allele carriers showed impaired proinsulin processing, resulting in a high level of pro-insulin in the plasma and an increase in the proinsulin/insulin ratio (Stolerman et al., 2009). Human islets have a higher degree of open chromatin, corroborating that the T allele leads to increased expression of

TCF7L2 and decreased insulin content and secretion (Zhou et al., 2014). Additionally, Zhou et al., (2014) demonstrated that in islets from CC genotype carriers, *TCF7L2* mRNA expression was negatively associated with the genes *ISL1*, *MAFA* and *NKX6.1* but not with *MAFA* and *NKX6.1* in CT/TT genotype carriers reinforcing the β-cell impairment in T allele risk carriers.

He, Zhong and Cui (2014) conducted an integrated approach and observed gender difference in association signals at the gene- and pathway-level. In this study, the *TCF7L2* association was found only in male and all SNPs in this gene in the female population do not show significance. Since *TCF7L2* belongs to several enriched pathways and is widely recognized as a gene conferring risk of T2DM, the authors performed the same analysis but deleting this gene in all pathways and no significant change between pathway signals with and without gene *TCF7L2* in female group, while the strong signals in male group are almost vanished after deleting the gene, suggesting potential difference in T2DM etiology in the pathway level in each gender group; they verified that the significance of the pathways in the male group is largely dominated by gene *TCF7L2* (He, Zhong & Cui, 2014). In this sense, this finding could explain the interaction between T allele and gender on diabetes risk observed in our study.

Our selected gene variant represents only a fraction of the potential variation of the studied gene and the mechanisms involving the *TCF7L2*, T2DM and obesity remain unknown and genetic studies with other variants are needed to better understand the *TCF7L2* role in these complex diseases. The rs12255372 variant in intron 4 of TFC7L2 gene showed to be in strong linkage disequilibrium (LD) with rs7903146 (Pang, Smith & Humphries, 2013). Moreover, subjects homozygous for the risk-associated showed higher gene expression in pancreatic islets and were more than twice as likely to develop T2DM as non-carriers (Lyssenko et al., 2007; Pang, Smith & Humphries, 2013).

Prior association studies reported lack of association between rs7903146 T allele and obesity status (Cauchi et al., 2008b; Stolerman et al., 2009; Bouhaha et al., 2010; Al-Safar et al., 2015). However, we verified a T allele protective effect against obesity (Supplemental Table 6) as well as the observed in more recent studies (Noordam et al., 2017; Fernández-Rhodes et al., 2018). A cross-sectional analysis conducted in middle-aged participants (mean age of 55.9 ± 6.0 years) reported a T allele association with lower BMI and mean total body fat (Noordam et al., 2017). Furthermore, Fernandez-Rhodes et al. (2018) showed an association of TT genotype with decreased waist circumference and lower mean BMI at multiple time points in the life course. This protection against obesity might be due to reduced insulin production and secretion related to the rs7903146 T allele, once insulin stimulates the increased glucose uptake in adipocytes, and plays a pro-obesogenic role both from its anabolic effect on lipid accumulation and due to compensatory eating to prevent episodes of hypoglycemia (Zhou et al., 2016).

Multiple factors are related to the changes in body composition with ageing. From the fourth decade onwards, the muscle mass decline and accounts for reduced resting metabolic rates which contribute to the gradual increase in body fat in elderlies (Gallagher et al., 1998; Sayer et al., 2008). Around 75 years old, the BMI goes through a period of apparent stability, being overestimated due to the increase in fat mass and a decrease in lean mass and bone density (Ponti et al., 2020). In this sense, is not easy to differentiate lean and obese elderlies due to sarcopenic obesity and the BMI cutoff points are still controversial for this range age. Because of this, BMI classification is a limiting factor for our cohort. However, the BMI variation is an important risk predictor for elderlies and the rs7903146 T allele protective effect against obesity deserves attention because thinness is an important risk factor for health in old age and weight loss is closely related to frailty syndrome and other health complications (Aune et al., 2016; Di Angelantonio et al., 2016; Ponti et al., 2020).

Studies have been reported the clinical implications of sarcopenic obesity in subjects with T2DM (Khadra et al.; Ghoch, Calugi & Grave, 2018; Kim & Park, 2018). A recent meta-analysis observed that the presence of sarcopenic obesity increases the T2DM risk by 38% with respect to those without sarcopenic obesity (OR = 1.38, 95%CI: 1.27-1.50) (Khadra et al., 2019). The more accepted mechanism interconnecting T2DM and sarcopenic obesity involve increase in fat mass, decrease in lean mass chronic inflammation and insulin resistance, however, it is still unclear (Srikanthan, Hevener & Karlamangla, 2010). Thereby, the interaction between T allele and age on diabetes risk observed in our elderly cohort could be related to the sarcopenic obesity in older adults and the age-related decline in resting metabolic rates.

Our data suggest a differential effect of rs7903146 genotypes in BMI variation only in elderlies without T2DM. It was observed less variation in BMI during the ten years of SABE study which could be concluded from the increased number of TT genotype carriers on the second tertile of ΔBMI values (Supplemental Table 7). The same result is found in interventional studies that verified lower BMI variation in rs7903146 T allele carriers (Haupt et al., 2010; Kaminska et al., 2012; Roswall et al., 2014). Mattei et al., 2012 observed a greater loss of lean mass for CC carriers who consumed the low-fat diet compared with TT (Mattei et al., 2012). Similarly, less weight gain per year was observed in patients with the T allele compared to the C allele, after the adherence to the Mediterranean diet (Roswall et al., 2014). According to Fisher et al., (2012), the rs7903146 C allele arose during a transition from hunter-gatherer to agricultural practices (with reduced protein sources), carriers of the rs7906146 T allele were selectively adapted to maintain weight stability under low-protein conditions (Fisher et al., 2012).

Helgason et al. (2007) reported that the rs7903146 T allele probably was the ancestral allele, serving for a better subjacent mutation, and identified a haplotype with the C allele (HapA). This haplotype shows evidence of positive selection besides the association with BMI and altered concentrations of ghrelin and leptin, indicating that the selective advantage of HapA may have

been mediated through effects on energy metabolism (Helgason et al., 2007). Corroborating with this, the largest GWAS meta-analysis for BMI so far (~300,000 subjects), reported the C allele association with BMI (Locke et al., 2015). Although the extent of clinical variability associated with the C allele is not fully known, significant associations between the rs7903146 C allele with BMI and/or waist circumference were observed in a Saudi population (Al-Daghri et al., 2014), in European adults (Abadi et al., 2017) and American Indians (Muller et al., 2019).

The adipose tissue of CC genotype carriers expressed more transcripts containing the alternative spliced exons (13 and 13a) associated with BMI and percent body fat than T allele carriers (Kaminska et al., 2012). Further, five in seven *TCF7L2* splice forms and nine diabetes-associated genes were differentially expressed by comparing leukocyte cells of carriers of the CC and CT/TT genotypes which might reflect a significant change in gene interactions and responsible networks as glucose homeostasis, adipogenesis and other (Vaquero et al., 2012). In this sense, the *TCF7L2* alternative splicing in adipose tissue could be regulated by health, disease, weight loss and insulin resistance (Mondal et al., 2010; Kaminska et al., 2012; Vaquero et al., 2012; Zhou et al., 2014; Chen et al., 2018).

The *TCF7L2* gene plays important metabolic and developmental roles in adipose tissue, and it is largely hypothesized that the Wnt signaling is critical for obesity development (Chen & Wang, 2018; Chen et al., 2018). This gene is differentially methylated in adipose tissue, exhibiting relevant epigenetic changes to the development of both diabetes as obesity (Nilsson et al., 2014). The *TCF7L2* protein inactivation is associated with increased subcutaneous adipose tissue mass, adipocyte hypertrophy and inflammation (Chen et al., 2018). Furthermore, besides alternative splicing, other regulatory changes seem to be genotype-specific and influence the *TCF7L2* role in adipose tissue. Several protein factors, including GATA3, a transcription factor that controls the preadipocyte-to-adipocyte transition, bind only to the rs7903146 C allele but not to the T allele under calorie restriction (Cauchi et al., 2008a).

The evidence above supports the CC genotype association with the risk for obesity and abdominal obesity found in our population. Thus, we speculate that the inverse effects of rs7903146 T and C alleles on risks for diabetes and obesity observed in our study might be related to the *TCF7L2* expression and its genotype-specific effects on the WNT signaling pathway in adipose tissue and others. Despite advances in knowledge regarding the production, processing, trafficking and secretion of insulin, the mechanisms interconnecting the *TCF7L2* rs7903146 variant, T2DM and obesity are not clear and more studies are required.

The main strength of this study is that the median age of our population exceeds the-age of onset of diabetes and obesity, thus minimizing a typical bias in the selection of the control group. As far as we know, this is one of the few association studies that reported an association of rs7903146 variant with BMI variation during a decade assessment interval, and with obesity

status in an exclusively elderly population. Dietary factors play an important role in T2DM etiology and the gene-diet interaction could influence T2DM pathogenesis (Ouhaibi-Djellouli et al., 2014; Hindy et al., 2016). Therefore, the lack of assessment of dietary as well physical activity could be a limitation of our study, however, we could detect and confirm in our population the association between T2DM and rs7903146 T allele which is worldwide recognized as the stronger GWAS signal for diabetes risk (Grant, 2019). Despite our population size, we were able to reproduce significant results following more recent studies performed on larger populations (Locke et al., 2015; Abadi et al., 2017; Fernández-Rhodes et al., 2018).

Conclusions

We confirmed that the rs7903146 variant is both associated with T2DM and obesity. This observation is supported by evolutive aspects and functional studies concerning the T and C allele and it contributes to expanding the knowledge about this barely explored association. In addition, we found a TT association with a lower BMI variation in elderlies over the ten years of SABE study. These findings provide a unique contribution to association studies about this polymorphism and additional studies are needed to understand the *TCF7L2* rs7903146 association with obesity and with BMI variation in different age groups of populations across the world.

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Table 1(on next page)

Anthropometric and biochemical characteristics according to Body Mass Index status.

Data are presented as median and range for the most variables; P-value with Kruskal-Wallis test for quantitative variables and Chi-square test for qualitative data. BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2). P, P-value; T2DM, Type 2 diabetes mellitus; M/F, Male/Female; BMI, Body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 1
Anthropometric and biochemical characteristics according to Body Mass Index status.

Variable	Unit	Normal-weight	Overweight	Obesity	P
Population size	N	280	424	319	-
Gender M/F	N/N	115/165	183/241	67/252	<0.0001
Age	years old	74.3 (65.8 - 82.6)	71.6 (64.5 - 79.2)	67.9 (64.0 - 75.2)	<0.0001
BMI	kg/m²	22.8 (21.0 - 24.0)	27.3 (26.2 - 28.4)	33.1 (31.2 - 35.9)	<0.0001
Waist circumference	cm	82.0 (77.8 - 87.0)	94.0 (89.0 - 99.0)	105.0 (100.0 - 110.0)	<0.0001
Hip circumference	cm	93.0 (90.0 - 96.0)	101.0 (98.0 - 104.0)	113.0 (108.0 - 120.0)	<0.0001
Hip-waist ratio	cm/cm	0.88 (0.83 - 0.93)	0.93 (0.88 - 0.98)	0.91 (0.87 - 0.97)	<0.0001
Systolic pressure	mmHg	134.3 (121.7 - 152.0)	138.0 (127.7 - 153.0)	138.0 (125.0 - 155.0)	0.0664
Diastolic pressure	mmHg	76.2 (68.3 - 85.8)	79.7 (72.0 - 86.3)	81.0 (74.2 - 90.0)	<0.0001
Plasma glucose	mg/dL	85.0 (78.0 - 95.0)	88.0 (81.0 - 102.3)	93.0 (84.0 - 107.0)	<0.0001
Glycated hemoglobin	%	5.7 (5.5 - 6.0)	5.8 (5.6 - 6.1)	5.9 (5.6 - 6.3)	<0.0001
Total cholesterol	mg/dL	202.5 (177.0 - 234.5)	200.0 (176.0 - 228.0)	207.0 (180.0 - 230.5)	0.3732
Fasting triglyceride	mg/dL	102.5 (75.0 - 137.3)	116.5 (89.8 - 167.3)	126.0 (94.0 - 168.5)	<0.0001
LDL cholesterol	mg/dL	126.0 (104.0 - 148.0)	124.0 (104.8 - 149.0)	130.0 (106.5 - 151.0)	0.7908
HDL cholesterol	mg/dL	52.0 (42.8 - 62.0)	45.0 (38.0 - 54.0)	47.0 (41.0 - 56.0)	<0.0001
T2DM	N (%)	51 (18)	117 (28)	92 (29)	0.0048
Hypertensive	N (%)	166 (59)	279 (66)	254 (80)	<0.0001

Data are presented as median and range for the most variables; P-value with Kruskal-Wallis test for quantitative variables and Chi-square test for qualitative data.
BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2).
P, P-value; T2DM, Type 2 diabetes mellitus; M/F, Male/Female; BMI, Body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 2(on next page)

Association between rs7903146 TT genotype and risk for Type 2 diabetes adjusted for possible confounders.

Regression logistic analysis was adopted.

1

2 **Table 2.** Association between rs7903146 TT genotype and risk for Type 2 diabetes adjusted for possible confounders.

3

Possible confounder	*P*-value	Odds Ratio	95% Confidence Interval
Age (years old)	0.005	1.89	1.21 - 2.95
Gender (N)	0.005	1.90	1.22 - 2.97
BMI (kg/m²)	0.003	1.95	1.25 - 3.06
Waist circumference (cm)	0.004	1.93	1.23 - 3.02
Diastolic pressure (mmHg)	0.006	1.88	1.20 - 2.93
Glycated hemoglobina (%)	0.004	2.34	1.31 - 4.18
Fasting triglyceride (mg/dL)	0.005	1.90	1.22 - 2.97
HDL cholesterol (mg/dL)	0.006	1.87	1.19 - 2.92
European ancestrie (%)	0.005	1.92	1.21 - 3.05
All confounders toghether	0.006	2.35	1.28 - 4.32

Regression logistic analysis was adopted.

Table 3(on next page)

Association of the rs7903146 T allele with type 2 diabetes mellitus according to Body Mass Index status. <!--[if !supportLineBreakNewLine]--> <!--[endif]-->

Volunteers without type 2 diabetes mellitus were considered as the control group. BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2). P, P-value; 95% CI, 95% confidence interval; OR, odds ratio OR adjusted for sex and age.

Table 3

Association of the rs7903146 T allele with type 2 diabetes mellitus according to Body Mass Index status.

Volunteers without type 2 diabetes mellitus were considered as the control group.

BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2).

BMI Status	N	Dominant model (CC Vs CT+TT)		Recessive Model (CC+CT Vs TT)		Additive Model (CC Vs TT)		Allelic Model (C Vs T)	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Normal-weight	280	1.23 (0.66 - 2.27)	0.512	**3.36 (1.46 - 7.74)**	**0.004**	**3.21 (1.31 - 7.87)**	**0.011**	1.52 (0.97 - 2.36)	0.066
Overweight	424	1.20 (0.78 - 1.86)	0.401	1.96 (0.99 - 3.87)	0.054	1.98 (0.96 - 4.10)	0.065	1.27 (0.92 - 1.74)	0.141
Obesity	319	1.15 (0.71 - 1.88)	0.565	1.23 (0.51 - 2.98)	0.642	1.31 (0.53 - 3.28)	0.560	1.13 (0.78 - 1.66)	0.516
Normal-weight + overweight	704	1.22 (0.86 - 1.73)	0.276	**2.31 (1.37 - 3.90)**	**0.002**	**2.34 (1.34 - 4.08)**	**0.003**	**1.34 (1.04 - 1.73)**	**0.025**
Overweight + obesity	743	1.16 (0.84 - 1.60)	0.370	1.59 (0.93 - 2.72)	0.089	1.65 (0.94 - 2.89)	0.082	1.19 (0.94 - 1.52)	0.155
Total	1023	1.16 (0.87 - 1.54)	0.305	**1.90 (1.22 - 2.97)**	**0.005**	**1.94 (1.21 - 3.10)**	**0.006**	**1.25 (1.01 - 1.54)**	**0.042**

P, P-value; 95% CI, 95% confidence interval; OR, odds ratio OR adjusted for sex and age.

Table 4(on next page)

Association of the rs7903146 C allele with the Body Mass Index Status

P, P-value; 95% CI, 95% confidence interval; OR, odds ratio OR adjusted for sex and age. BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2).

Table 4

Association of the rs7903146 C allele with the Body Mass Index Status

Control group	Case group	Dominant model (TT Vs CC+CT)		Recessive Model (TT+CT Vs CC)		Additive Model (TT Vs CC)		Allelic Model (T Vs C)	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Normal-weight	Overweight	1.24 (0.74 - 2.09)	0.410	0.92 (0.68 - 1.26)	0.611	1.15 (0.67 - 1.98)	0.615	1.00 (0.79 - 1.26)	0.984
Normal-weight	Obesity	1.60 (0.88 - 2.92)	0.122	1.41 (1.00 - 1.98)	0.052	1.73 (0.93 - 3.21)	0.081	**1.34 (1.03 - 1.75)**	**0.029**
Normal-weight	Obesity+ overweight	1.32 (0.82 - 2.13)	0.253	1.08 (0.81 - 1.43)	0.604	1.31 (0.80 - 2.15)	0.286	1.10 (0.89 - 1.36)	0.373
Normal-weight + overweight	Obesity	1.29 (0.78 - 2.11)	0.317	**1.40 (1.06 - 1.84)**	**0.017**	1.48 (0.89 - 2.47)	0.132	**1.28 (1.03 - 1.58)**	**0.024**

P, P-value; 95% CI, 95% confidence interval; OR, odds ratio OR adjusted for sex and age.

BMI classification criteria: normal-weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obesity (≥ 30.0 kg/m^2).

Table 5(on next page)

Association between rs7903146 CC genotype and risk for obesity adjusted for possible confounders.

Regression logistic analysis was adopted. *Association between rs7903146 CC genotype and obesity adjusted by fasting triglyceride: *P*=0.034; OR=1.0016; 95% Confidence Interval = 1.0001 - 1.0031.

Table 5. Association between rs7903146 CC genotype and risk for obesity adjusted for possible confounders.

Possible confounder	*P*-value	Odds Ratio	95% Confidence Interval
Age (years old)	<0.001	0.96	0.95 - 0.98
Gender (N)	<0.001	2.85	2.08 - 3.89
Diastolic pressure (mmHg)	<0.001	1.02	1.01 - 1.03
Glycated hemoglobina (%)	0.032	1.13	1.01 - 1.27
Fasting triglyceride* (mg/dL)	0.034	1.00	1.00 - 1.00
HDL cholesterol (mg/dL)	0.443	1.00	0.99 – 1.00
All confounders toghether	0.017	1.41	1.06 – 1.86

Regression logistic analysis was adopted.
*Association between rs7903146 CC genotype and obesity adjusted by fasting triglyceride: *P*=0.034; OR=1.0016; 95% Confidence Interval = 1.0001 - 1.0031.