

Type 2 Diabetes and KLF14 RS972283 (G/A) Polymorphism: Evidence From San Luis, Argentina

Elena Volkova¹, Natalia Petrova¹, Sofia Ivanovna², Irina Smirnova², Maria Novikova², Alina Fedorova² & Olga Dmitrieva¹

¹Laboratory of Endocrinology, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia

²Central Clinical Hospital, Moscow, Russia

Abstract

Keywords: gene, diabetes mellitus, polymorphism.

Background and objective: Type 2 Diabetes Mellitus (T2DM) is a complex disorder caused by the interaction between genetic predisposition, lifestyle and environmental risk factors. Though the association between the Kruppel Like Factor 14 gene (KLF14) polymorphism rs972283 and T2DM has been analyzed in different ethnic groups, the results have been inconsistent.

The aim of this study was to evaluate the possible association between KLF14 gene rs972283 polymorphism and Type 2 Diabetes Mellitus in a population of diabetic patients living in San Luis, Argentina.

Methods: A total of 26 unrelated patients with T2DM and 42 healthy controls were included in the study. Genomic DNA was extracted from blood and genotyped for the single nucleotide polymorphism of KLF14 rs972283 (G/A) by Tetra Primer ARMS-PCR method.

Results: The genotype distribution and the relative allelic frequencies for the KLF14 polymorphism rs972283 were not significantly different between T2DM and controls: in T2DM patients the frequencies of the GG, GA, and AA genotypes were 38.0, 46.0, and 15.0 per cent, respectively, and in controls the genotype frequencies were 26.0, 56.0, and 18.0 per cent, respectively. The association between KLF14 gene polymorphism and T2DM was studied in two models of inheritance, i.e., dominant and recessive. In these two models there were no significant associations between KLF14 rs972283 polymorphism and T2DM.

Conclusion: The present study provides statistical evidence indicating a lack of association between Krüppel-like factor 14 rs972283 polymorphism and Type 2 Diabetes Mellitus. More studies with larger sample size are needed to confirm these null associations.

Introduction

Type 2 diabetes mellitus (T2DM) formerly named non-insulin-dependent or adult-onset is chronic disease, accounting for more than 90% of all diabetes cases in the global population. Without proper monitoring, it can lead to serious complications such as cardiovascular, kidney, nervous, and eye diseases; including death. For that reason T2DM is a life-long condition that requires careful nutritional and pharmacologic managements.

The prevalence of T2DM has increased rapidly not only in affluent societies, but also in developing countries over the last 20 years [Van Dieren et al., 2010]. This rise is likely fuelled by an aging population, economic development and increasing urbanization leading to more sedentary lifestyles and greater consumption of unhealthy foods linked with obesity. [Basu Sanjay et al., 2013]. In 2015, 425 million adults globally had the condition and it is expected that prevalence will rise by almost 48% - up to 629 million cases by 2045 [International Diabetes Federation, 2015].

Metabolic syndrome is defined as a cluster of metabolic disturbances that include insulin resistance, abdominal obesity, hyperglycemia, hypertension, dyslipidemia and T2DM. This syndrome is common in poorly controlled T2DM patients and it has been associated with an almost 5-fold increase in cardiovascular disease risk [Bonora et al., 2004]. It is important note that insulin resistance and abnormal insulin secretion are the hallmark of T2DM. On

the other hand, although environmental factors, particularly caloric excess and physical inactivity, play major roles in metabolic syndrome, the traits are highly heritable [Lusis et al., 2008].

GWAS (genome-wide association studies) have identified numerous loci influencing these traits individually, but to date, no loci have been found that affect the entire spectrum of metabolic syndrome traits. The Krüppel-like factor 14 (KLF14) gene encodes for a transcription factor which acts in trans to regulate the expression of a network of genes associated with metabolic traits, (body mass index, glucose levels, high density lipoprotein levels, index of insulin sensitivity, insulin levels, low density lipoprotein levels, triglyceride levels, T2DM, waist-hip ratio) [Civelek and Lusis, 2011; de Assuncao et al., 2014].

GWAS have implicated a group of highly- correlated SNPs including rs972283 (G/A) upstream of the transcription-starting codon of the KLF14 gene [Voight et al., 2010]. The allele G of rs972283 in KLF14 may be a risk factor for metabolic disease and had a nominal association with T2DM in a global population (Ohshige et al., 2011; Small et al., 2011; Wang et al., 2014. However, the exact impact of this SNP on lipid metabolisms of T2DM patients remain unknown.

The present study was to detect the distribution of rs972283 (G/A) SNP and to evaluate the possible association between KLF14 gene rs972283 polymorphism and Type 2 Diabetes Mellitus and lipid metabolism in a population of diabetic patients living in San Luis, Argentina.

Research Design and Methods

2.1. Study Population

The present study was carried out in accordance with the guidelines of the Helsinki Declaration. A total of 68 volunteers (26 T2DM patients and 42 healthy controls) participated in this study. Patients were between 35 and 70 years of age. Criteria published by the Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus were used to diagnose T2DM [Seino et al., 2010]. These patients resided in San Luis, Argentina. The study protocol was approved by the Ethics Committee of the San Luis Hospital and was in accordance with the Helsinki Declaration II. Written informed consents were obtained from all the participants before data collection. Exclusion criteria included renal, hepatic or brain-vascular disorders, endocrinal disorders, women receiving estrogen therapy and chronic disorders, as well as the use of lipid-lowering drugs, which can affect glucose metabolism and/or insulin sensitivity. All participants were recruited consecutively between March 2017 and March 2018.

2.2. Anthropometric and Clinical Data

For each enrolled participant, height (H, m) and weight (W, kg) were measured. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. The body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

2.3. Definitions

The criteria for weight classification by BMI were according to the Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults [Clinical Guidelines, 1998]. Participants were classified according to the following criteria: Underweight is defined as $\text{BMI} < 18.5 \text{ kg/m}^2$, Normal as BMI between $18.5 - 24.9 \text{ kg/m}^2$, Overweight as BMI between $25.0 - 29.9 \text{ kg/m}^2$, Type I Obesity as BMI between $30.0 - 34.9 \text{ kg/m}^2$, Type II Obesity as BMI between $35.0 - 39.9 \text{ kg/m}^2$ and Type III Obesity as $\text{BMI} \geq 40.0 \text{ kg/m}^2$.

2.4. Blood Sampling

Blood samples were obtained from patients that had fasted overnight for a minimum of 12 h. Blood was collected in tubes containing 0.1% ethylene diaminetetraacetic acid. Plasma and blood cells were separated by centrifugation at $1400 \times g$ for 20 min at room temperature. Plasma and packed blood cells were aliquoted and stored at -20°C until use.

2.5. Biochemical Measurement

Fasting plasma glucose (FPG) was measured by using a glucose oxidase method with a commercial enzymatic kit (Wiener Laboratories, Rosario, Argentina). Total cholesterol (TC), triglycerides (TG) and HDL-c concentrations

were measured using commercial kits by following the manufacturer's instructions (Wiener Laboratories, Rosario, Argentina). LDL-cholesterol (LDL-c) and VLDL-cholesterol (VLDL-c) were calculated with the Friedewald formula: $LDL-c = TC - (HDL-c + TG/5)$ in mg/dL and $VLDL-c = TG/5$ in mg/dL [Friedewald et al., 1972].

2.6. Definitions

The criteria for dyslipidemia were according to the National Cholesterol Education Program [Expert Panel on Detection, 2001]. Briefly, participants were diagnosed with dyslipidemia if they had one or more of the following criteria: a plasma concentration of TC of ≥ 6.24 mmol/L (≥ 240 mg/dL), plasma concentration of TG ≥ 2.26 mmol/L (≥ 198 mg/dL); plasma concentration of HDL-c of < 1.04 mmol/L (< 40 mg/dL) for men or < 1.30 mmol/L (< 50 mg/dL) for women; and a plasma concentration of LDL-c ≥ 4.14 mmol/L (≥ 160 mg/dL).

The TG / HDL-c ratio ≥ 3 was used as an alternative marker of insulin, while the CT / HDL ratio ≥ 4.5 was used as atherosclerosis and cardiovascular risk prediction marker [Li et al., 2008; Acevedo et al., 2012; Gimeno-Oma et al., 2005]

2.7. DNA analysis

Genomic DNA was isolated from T2DM patients and healthy volunteers using conventional protocol by Qiagen kits (Qiagen, Inc., Valencia, CA). DNA concentration was measured by UV-VIS spectroscopy and diluted to a final concentration of 20 ng/ μ L.

Genotyping of the KLF14 polymorphism rs972283 (A/G) was performed by Tetra Primer ARMS-PCR method as described previously (Zabala et al., 2017). Briefly, each PCR reaction was carried out in a total volume of 20 μ L, containing 50-100 ng of template DNA, 0.04 μ M of Reverse inner primer (A allele) and both Outer primers, 0.16 μ M of Forward inner primer (G allele), 200 μ M dNTPs (Invitrogen, CA, USA), 2.5 mM MgCl₂, 1x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), and 0.7 U Taq polymerase (Productos Biológicos, Argentina). The template DNA was denatured for 3 minutes at 94°C before undergoing 30 cycles of denaturation for 1 minute at 94°C, primer annealing for 1 minute at 60°C and extension for 1 minute at 72°C, and final extension at 72°C for 10 minutes (Table 1).

Table 1. PCR primers and conditions

SNP	Primer sequence (5' to 3')	Annealing Temp.	Expected products
rs972283 (G/A)	Forward outer GTCATAGGTCAAACAGCTAGATATTGGGT	60°C	Common 437 bp
	Reverse outer TCTACAGGACCAACTCAAATTATGAGGT		
	Forward inner TCATTGTATACTTGGAAAAATCCTACATG		G allele 221 bp
	Reverse inner TATGTAAAAATAAGTATGCGCCATGCCT		A allele 274 bp

The resultant DNA amplicons were separated by electrophoresis onto a 3 % agarose gel-bed containing GelRed (Biotium). The image was visualized and photographed under UV transillumination. The wild type homozygote (GG), heterozygote (GA) and mutant homozygote (AA) showed two bands (437 and 221 bp), three bands (437, 274 and 221 bp) and two bands (437 and 274 bp), respectively (Figure 1).

For quality control, 20 per cent of the samples were selected at random for repeated genotyping for cross validating initial genotypes and concordance was 100 per cent. No genotyping error was observed during cross validation

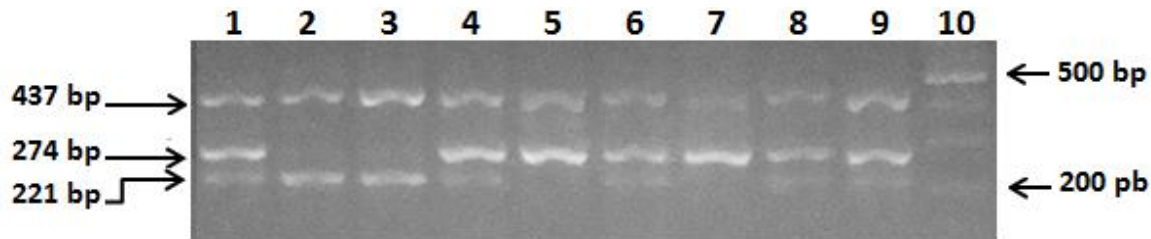


Figure 1. KLF14 genotyping by Tetra Primer ARMS-PCR.

The 437 bp band is the amplicon of the outer primers, the 274 bp band, of an outer primer and the inner primer for allele A; and the 221 bp band, of the other outer primer and the inner primer for the G allele. Lanes 1, 4, 6, 8 and 9: A/G genotype; lanes 2 and 3: G/G genotype; lanes 5 and 7: A/A genotype; lane 10: molecular weight marker (100 pb).

2.8. Statistical Analysis

The association between anthropometric, biochemical and genotypic parameters of KLF14, a statistical analysis was performed using the GraphPad Prism software (5.04). Continuous variables were analyzed through the Student's test, checking the normality of the data using the D'Agostino and Pearson test or the Kolmogorov-Smirnov test, according to the number of samples analyzed. Data are shown as mean values $\pm$ standard deviation (SD), absolute values or percentages (%). Chi-square test was used for the analysis of categorical variables. SNPSTAT software (<https://www.snpstats.net>) was used to evaluate Hardy-Weinberg equilibrium, compare allelic and genotypic frequencies, determinate the mode of inheritance and analyze haplotype variables. A $P < 0.05$ was considered statistically significant.

Results

3.1. Participant Characteristics

Table 2 shows the demographic and biochemical characteristics of both groups. 52.94% were women and 47.06% were men. T2DM patients have higher fasting plasma glucose, TG and VLDL-c concentration than controls, whereas HDL-c concentration was slightly lower in T2DM subjects compared to control. Furthermore, the TG/HDL-c ratio- an insulin resistance marker- reaches high positive values in T2DM group, in contrast with the low levels in control group.

Table 2. Clinical and biochemical characteristics in controls and T2DM patients.

	Control (n = 42)	T2DM (n = 26)	p
Sex (female/male)	22/20	14/12	0.1953
Age (years)	51.14 $\pm$ 11.24	62.69 $\pm$ 12.19	< 0.0001
Weight (kg)	75.81 $\pm$ 13.38	80.02 $\pm$ 13.75	0.2169
Height (m)	1.685 $\pm$ 0.09498	1.670 $\pm$ 0.07977	0.5069
BMI (kg/m ²)	26.69 $\pm$ 4.11	28.11 $\pm$ 3.91	0.1682
FPG (mg/dL)	88.50 $\pm$ 9.247	156.0 $\pm$ 61.50	<0.0001
TG (mg/dL)	141.5 $\pm$ 71.90	238.9 $\pm$ 117.1	< 0.0001
TC (mg/dL)	200.0 $\pm$ 47.35	190.7 $\pm$ 48.74	0.4371
HDL-c (mg/dL)	51.83 $\pm$ 10.24	48.84 $\pm$ 9.660	0.2353
LDL-c (mg/dL)	118.3 $\pm$ 38.49	94.06 $\pm$ 40.18	0.0158
VLDL-c (mg/dL)	28.31 $\pm$ 14.38	47.77 $\pm$ 23.41	< 0.0001
TG/HDL-c ratio	2.763 $\pm$ 1.343	4.960 $\pm$ 2.340	< 0.0001
TC/HDL-c ratio	3.906 $\pm$ 0.7997	3.953 $\pm$ 0.8894	0.8217
Dyslipidemia	60%	81 %	0.0688

Data are shown as mean $\pm$ standard deviation. BMI, body mass index; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; VLDL-c, very low-density lipoprotein.

Due the very low frequencies of obesity type II and III, they were analyzed in the same category as obesity type I, referred to as obesity. The distribution of BMI, according to WHO, in control and T2DM is shown in **Figure 2**. The media BMI of overweight and obesity in control patients, was lower than in T2DM (26.30 kg/m² vs 27.49 kg/m² and 31.77 kg/m² vs 35.15 kg/m², respectively), there were no differences in the median BMI of normal category between control and T2DM group.

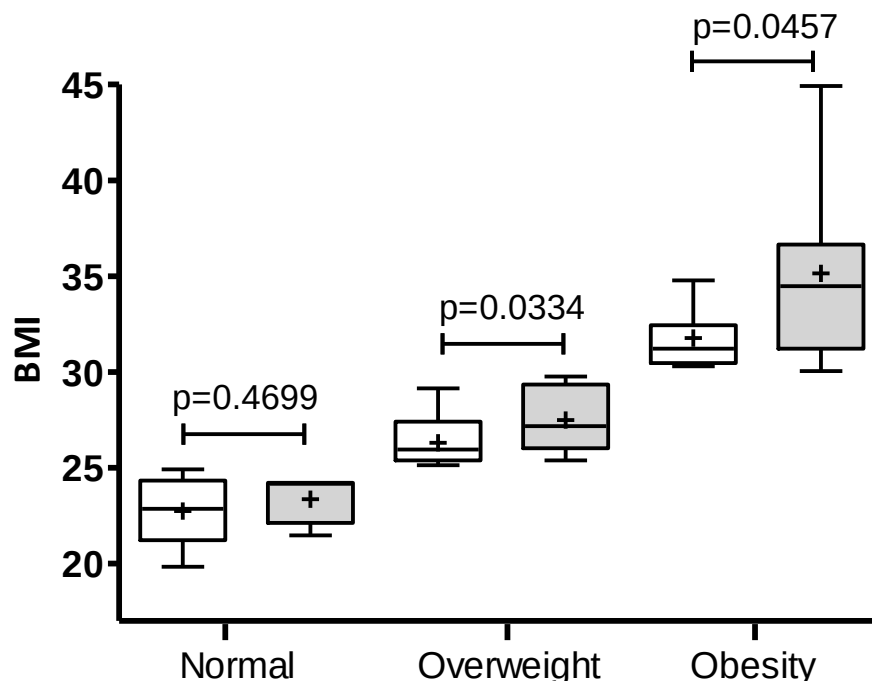


Figure 2: Vertical box plots of BMI values according the criteria for weight classification by WHO, in controls and T2DM group. For each plot, the line within the box represents the media and the cross the average. The lower and upper lines of the box represent the 25th and 75th percentiles, respectively.

The rate of overweight among diabetics was 53.84% compared to 35.71% in control, whereas the rate of normal weight among diabetics was 19.23% compared to 35.71% in control, but there was no difference in the rates of obesity between both groups (**Figure 3**).

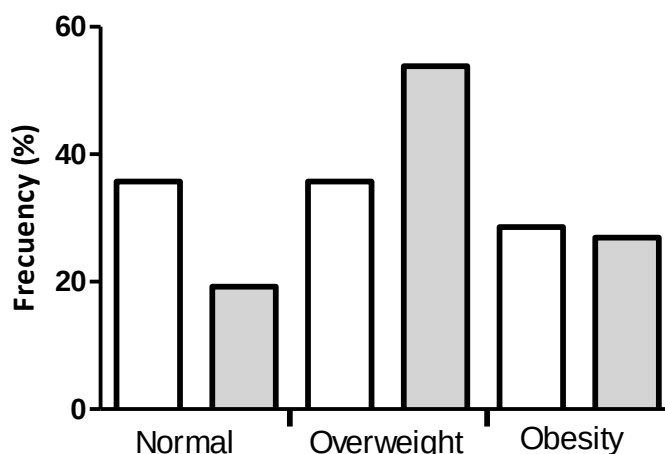


Figure 3: Percentages of classification of BMI according to the WHO in both groups.

Figure 4 shows the frequency of the four criteria for dyslipidemia, according to the National Cholesterol Education Program, in the samples of controls and diabetic subjects. High concentrations of TG are present in 80.95% of the subjects with T2DM, while 36% of control subjects have high TG levels. The second most frequent individual parameter among control and T2DM was a low concentration of HDL-c below the cutoff point, though there was no significant difference in the rates between both groups.

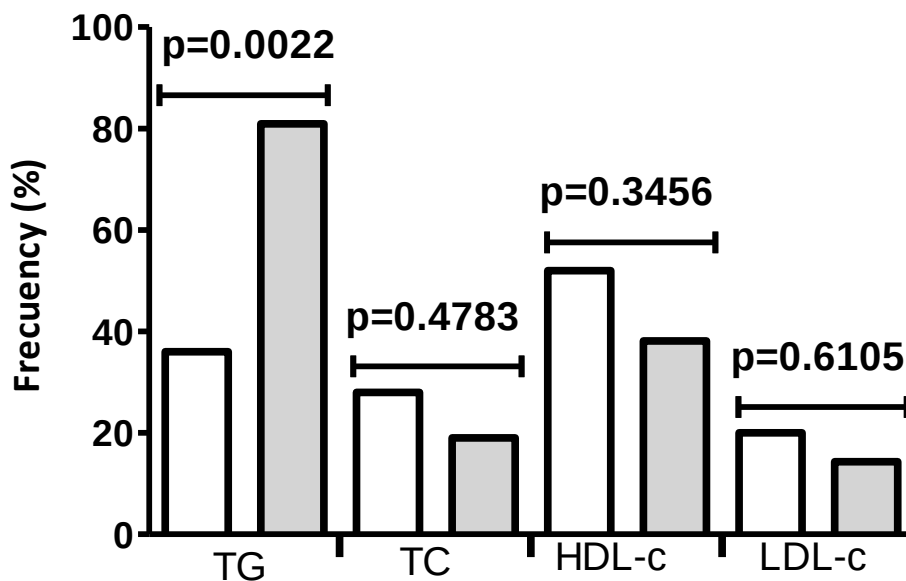


Figure 4: Percentages of the four criteria for dyslipidemia according to the National Cholesterol Education Program. TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein.

3.2. Genotype Frequencies

Table 3 shows the frequency (%) and absolute number of patients having each genotype in all patients, and in controls and T2DM patients by separate. KLF14 genotype distribution was in the Hardy-Weinberg equilibrium and comparable in both groups ($p > 0.05$). We found no-gender differences in either control or T2DM patients having the different genotypes. The frequency of the A allele of the KLF14 rs972283 polymorphism in control was not different from that in T2DM ($p = 0.189$).

Table 3. Distribution of KLF14 genotypes and allelic frequencies in controls and T2DM patients

Genotype	Total n(%)	Control n(%)	T2DM n(%)	OR(95% CI) ^a	p
GG	18 (26%)	8 (19%)	10 (38%)	1	
AG	38 (56%)	26 (62%)	12 (46%)	0.36 (0.12-1.17)	0.086
AA	12 (18%)	8 (19%)	4 (15%)	0.40 (0.08-1.826)	0.232
G	74 (54%)	42 (50%)	32 (62%)	1	
A	62 (46%)	42 (50%)	20 (38%)	0.62 (0.31- 1.26)	0.189

^aOR = odds ratio, 95% CI = 95% confidence interval.

3.3. Inheritance Model

When genotypes of KLF14 were associated with T2DM according to dominant and recessive genetic models, the polymorphism rs972283 (A/G) showed slightly association with an increased risk of type 2 diabetes, especially in a dominant model (OR 2.66 [95% CI 0.88–8.01], p=0.081) (Table 4).

Table 4. Risk analysis of the variant rs972283 KLF14 according to the model of inheritance.

Model ^a	Genotype ^b	Control n (%)	T2DM n (%)	p	OR(95% CI) ^c
Do	GG	8 (19.1%)	10 (38.5%)	0.081	1.00
	AG-AA	34 (81%)	16 (61.5%)		0.38 (0.12-1.13)
Re	GG-AG	34 (81%)	22 (84.6%)	0.700	1.00
	AA	8 (19.1%)	4 (15.4%)		0.77 (0.21-2.88)

^aInheritance models: dominant (Do), recessive (Re). ^bGenotypes and their groupings for the variant rs972283 KLF14 (G/A). ^cOR = odds ratio, 95% CI = 95% confidence interval.

3.4. Genotype Associations with Lipid Traits

The results were analyzed by comparing two subgroups of patients: a) homozygotic G/G and b) A allele carriers (G/A and A/A). This method of analysis is justified by a low number of homozygotic A/A cases and the slightly association with an increased risk of type 2 diabetes in a dominant model of inheritance (OR 2.66 [95% CI 0.88–8.01], p=0.081). No differences were identified in the anthropometric and biochemical characteristics between both groups studied (Table 5).

Table 5. Anthropometric and biochemical characteristics by the KLF14 polymorphism in controls and T2DM patients

	Control			DMT2		
	Genotypes			Genotypes		
	AA+ AG	GG	P	AA+ AG	GG	p
BMI (kg/m ²)	26.57 ± 4.22	22.20 ± 3.84	0.7009	27.84 ± 4.13	28.60 ± 3.69	0.6506
FPG (mg/dL)	88.55 ± 7.88	91.80 ± 9.53	0.3228	150.70 ± 55.51	163.90 ± 71.96	0.6107
TG (mg/dL)	144.30 ± 74.92	130.10 ± 60.77	0.6232	221.00 ± 104.70	267.50 ± 135.40	0.3340
TC (mg/dL)	192.70 ± 37.01	214.40 ± 64.45	0.2104	191.00 ± 50.70	190.10 ± 48.12	0.9622
HDL-c (mg/dL)	51.71 ± 10.22	52.38 ± 10.98	0.8703	47.14 ± 9.60	51.56 ± 9.61	0.2644
LDL-c (mg/dL)	114.10 ± 34.78	136.00 ± 50.27	0.1488	99.72 ± 36.83	85.22 ± 45.55	0.3751
VLDL-c (mg/dL)	28.86 ± 14.98	26.03 ± 12.15	0.6232	44.19 ± 20.93	53.50 ± 27.07	0.3340
TG/HDL-c ratio	2.83 ± 1.40	2.11 ± 0.53	0.1908	4.78 ± 3.32	5.26 ± 2.47	0.6206
TC/HDL-c ratio	3.86 ± 0.74	4.11 ± 1.05	0.4227	4.11 ± 1.01	3.70 ± 0.61	0.2540
Dyslipidemia	58.82%	62.50%	0.8488	81.25%	80.00%	0.9373

Data are shown as mean ± standard deviation. BMI: body mass index. FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; VLDL-c, very low-density lipoprotein.

Discussion

The current study we performed an association analysis between the variant rs972283 KLF14 (G/A) and lipid pattern in T2DM patients residing in San Luis, Argentina. The distribution of KLF14 genotypes was similar between T2DM and controls patients, which do not suggest the occurrence of any linkage disequilibrium between the KLF14 gene and those that might determine T2DM. In our investigation, no association was observed between KLF14 rs972283 (G/A) variant and type 2 diabetes, by either dominant or recessive model of inheritance. To our knowledge, the present study is the first to evaluate the KLF14 rs972283 (G/A) polymorphism in relation to type 2 diabetes in an Argentinean population.

Previous European genome-wide association study showed that rs972283 in KLF14 is linked to T2DM [Voight et al., 2010]. After the initial find, six small studies examined the *KLF14* rs972283 (G/A) polymorphism in relation to type 2 diabetes with inconsistent results (Long et al. 2012; Shi et al., 2011; Gao et al., 2016; Ohshige et al., 2011; Voight et al., 2010; Rees et al., 2011). Three studies found no statistically significant associations: Long et al. [2012] in African Americans, Shi et al. [2011] in Ningxia, China and Gao et al. (2016) in Henan Province, China. On the other hand, statistically significant associations were found in others studies as shown by Ohshige et al. [2011] in Japan, Voight et al. [2010] in European descent and Rees et al. [2011] in two Punjabi populations, Pakistan. Nevertheless, in the Japanese population studied by Ohshige et al. (2011), the association of risk allele G of KLF14 rs972283 and T2DM vanished after being adjusted for sex, age and log-transformed BMI, and there seemed to be no significant association between KLF14 rs972283 and glucose metabolism.

Wang et al. (2014) recently performed a global meta-analysis to assess the association between the KLF14 rs972283 polymorphism and type 2 diabetes susceptibility. Significant association was observed for the rs972283 (G/A) polymorphism either Asian, Afro-Americans or European participants. However, no study of South America population was included in their meta-analysis of the KLF14 rs972283 (G/A) polymorphism.

Despite strong functional evidence for the relevance of the rs972283 [Voight et al., 2010], we did not detect any association with type 2 diabetes susceptibility.

There are no previous reports about the genotypic and allelic frequencies of KLF14 SNP rs972283 in our region. According to the published data, the risk allele G frequency of this SNP in T2DM was 0.85 in African Americans [Long et al. 2012], 0.72 in Henan Province, China [Gao et al. 2016], 0.76, 0.75 and 0.73, in three studies, respectively, in Japan [Ohshige et al. 2011], 0.55 in European descent [Voight et al. 2010], 0.57 in Pakistan [Rees et al. 2011] and 0.47 in Ningxia, China [Shi et al. 2011]. In the present study, we showed that the G allele frequency of KLF14 rs972283 in T2DM group was 0.62, which was slightly higher to the data in European descent. This phenomenon may be explained by the difference in the genetic background of the study populations. It must be stated that the study group is mostly of European origin, mainly from Italy and Spain, and 2% of this population has an aboriginal ethnic ancestry suggesting that the original European allele frequency has not conserved in this area.

The fact that the polymorphism is in Hardy Weinberg equilibrium suggests that there is no significant natural selection pressure acting against individuals with the G of rs972283 variant living in San Luis, Argentina.

KLF14 has been identified as a critical regulator in lipometabolism [de Assuncao et al., 2014; Cibelek and Lusis, 2011]. KLF14 may acts in trans to regulate the expression of a network of genes associated with metabolic traits (e.g. LDL, HDL, TG and BMI) [Small et al., 2011]. In fact, because of its contribution to metabolic diseases, KLF14 has been recently referred to as a “conductor of the metabolic syndrome orchestra” [de Assuncao et al., 2014].

Notably, SNPs at KLF14 genetic locus have been identified to closely associate with both lipid disorder and T2DM through GWAS large-scale association analysis [Small et al., 2011, Nair et al., 2016; Voight et al., 2010]. Among the identified loci, the most compelling signal was at rs972283, which is strongly associated with the expression of KLF14 in adipose tissue. This suggests that the variation may result in a change of KLF14 expression level which mirrors the parent-of-origin effects for T2DM susceptibility at this locus [Teslovich et al., 2010].

The study by Kong et al. revealed that the allele G of rs972283 near KLF14 was associated with a higher risk for elevated TG in T2DM patients of Chinese participants [Kong et al., 2015]. By examining expression-QTL data in

23,720 transcripts for subcutaneous adipose tissue, Voight et al. discovered that KLF14 SNP at rs972283 is strongly associated with expression of KLF14 in adipose tissue [Voight et al., 2010].

The present study showed no significant association between the rs972283 SNP and serum lipid parameters by either control and T2DM populations. Our results are in disagree with the study by Kong et al. (2015), who reported higher levels of TG in patients carriers of G allele with T2DM. The biological function and detailed role of KLF14 rs972283 SNP in lipid metabolism need to be further explored.

Conclusions

In the present study, polymorphism rs972283 of KLF14 did not have significant association with T2DM susceptibility. We were unable to reproduce previous studies which correlated KLF14 polymorphism with susceptibility to T2DM. This inconsistency could be explain by the differences in ethnicity, the sample size, environmental risk factors or other causes as yet undetermined, between the different studies. Further evaluation in this area is still needed. The present study also did not detect the association between KLF14 rs972283 lipid metabolism. The biological function and detailed role of KLF14 rs972283 SNP in lipid metabolism need to be further explored.

Therefore, the lack of association between rs972283 of KLF14 polymorphism and type 2 diabetes susceptibility an lipid metabolism in Argentinean population cannot rule out the role of the KLF14 gene in the pathophysiology of type 2 diabetes. Comprehensive assessment of variation across the KLF14 gene (gene-based association) in large samples should be investigated in the future.

References

1. Acevedo, M., Krämer, V., Tagle, R., Corbalán, R., Arnaíz, P., Berríos, X., & Navarrete, C. (2012). Relación colesterol total a HDL y colesterol no HDL: los mejores indicadores lipídicos de aumento de grosor de la íntima media carotídea. *Revista médica de Chile*, 140(8), 969-976.
2. Basu, S., Yoffe, P., Hills, N., & Lustig, R. H. (2013). The relationship of sugar to population-level diabetes prevalence: an econometric analysis of repeated cross-sectional data. *PloS one*, 8(2), e57873.
3. Bonora, E., Targher, G., Formentini, G., Calcaterra, F., Lombardi, S., Marini, et al. (2004). The Metabolic Syndrome is an independent predictor of cardiovascular disease in Type 2 diabetic subjects. Prospective data from the Verona Diabetes Complications Study. *Diabetic Medicine*, 21(1), 52-58.
4. Civelek M.,Lusis A.J. Conducting the metabolic syndrome orchestra. (2011). *Nat Genet*, 43(6): 506–508Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998) The Evidence Report. National Institutes of Health. Obesity Research, 6, 51S-209S.
5. de Assuncao T.M., Lomberg G., Cao S., Yaqoob U., Mathison A., Simonetto D.A., et al. (2014) New role for Kruppel-like factor 14 as a transcriptional activator involved in the generation of signaling lipids. *J Biol Chem* 289: 15798–15809.
6. Expert Panel on Detection (2001) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association*, 285, 2486-2497.
7. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without Use of the Preparative Ultracentrifuge. *Clinical Chemistry* , 18, 499-502.
8. Gao K., Wang J., Li L., Zhai Y., Ren Y., You H., et al. (2016). Polymorphisms in Four Genes (KCNQ1 rs151290, KLF14 rs972283, GCKR rs780094 and MTNR1B rs10830963) and Their Correlation with Type 2 Diabetes Mellitus in Han Chinese in Henan Province, China. *Int J Environ Res Public Health*, 26;13(3).
9. Gimeno-Orna, J. A., Faure-Nogueras, E., & Sancho-Serrano, M. A. (2005). Usefulness of total cholesterol/HDL-cholesterol ratio in the management of diabetic dyslipidaemia. *Diabetic medicine*, 22(1), 26-31.
10. International Diabetes Federation: IDF Diabetes Atlas (2015) (7thedn). Available at: www.diabetesatlas.org.
11. Kong, X., Zhang, X., Xing, X., Zhang, B., Hong, J., & Yang, W. (2015). The association of type 2 diabetes loci identified in genome-wide association studies with metabolic syndrome and its components in a Chinese population with type 2 diabetes. *PLoS One*, 10(11), e0143607.

12. Li, C., Ford, E. S., Meng, Y. X., Mokdad, A. H., & Reaven, G. M. (2008). Does the association of the triglyceride to high-density lipoprotein cholesterol ratio with fasting serum insulin differ by race/ethnicity?. *Cardiovascular diabetology*, 7(1), 4.
13. Long, J., Edwards, T., Signorello, L. B., Cai, Q., Zheng, W., Shu, X. O., & Blot, W. J. (2012). Evaluation of genome-wide association study-identified type 2 diabetes loci in African Americans. *American journal of epidemiology*, 176(11), 995-1001..
14. Lusis, A.J., Attie, A.D. and Reue, K. (2008) Metabolic Syndrome: From Epidemiology to Systems Biology. *Nature Reviews Genetics* , 9, 819-830.
15. Nair, A. K., Piaggi, P., McLean, N. A., Kaur, M., Kobes, S., Knowler, et al. (2016). Assessment of established HDL-C loci for association with HDL-C levels and type 2 diabetes in Pima Indians. *Diabetologia*, 59(3), 481-491.
16. Ohshige T., Iwata M., Omori S., Tanaka Y., Hirose H., Kaku K., et al. (2011). Association of new loci identified in European genome-wide association studies with susceptibility to type 2 diabetes in the Japanese. *PLoS One*, 6(10):e26911
17. Rees, S. D., Hydrie, M. Z. I., Shera, A. S., Kumar, S., O'Hare, J. P., Barnett, et al.(2011). Replication of 13 genome-wide association (GWA)-validated risk variants for type 2 diabetes in Pakistani populations. *Diabetologia*, 54(6), 1368-1374.
18. Seino, Y., Nanjo, K., Tajima, N., Kadowaki, T., Kashiwagi, A., Araki, E., et al . (2010) Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Journal of Diabetes Investigation*, 1, 212-228.
19. Shi Jing, Qiang Dan, Xie Xiaomin. (2011) Relationship between polymorphism of KLF14rs972283 and type 2 diabetes in Ningxia. *Ningxia Medical Journal* 33,1151
20. Small, K.S., Hedman, A.K., Grundberg, E., Nica, A.C., Thorleifsson, G., Kong, A., et al. (2011) Identification of an Imprinted Master Trans Regulator at the KLF14 Locus Related to Multiple Metabolic Phenotypes. *Nature Genetics*, 43, 561-564.
21. Teslovich, T. M., Musunuru, K., Smith, A. V., Edmondson, A. C., Stylianou, I. M., Koseki, et al.(2010). Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*, 466(7307), 707.
22. Van Dieren S., Beulens J.W., van der Schouw Y.T., Grobbee D.E., Neal B. (2010). The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil* , 17: 3-8.
23. Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., et al. (2010) Twelve Type 2 Diabetes Susceptibility Loci Identified through Large-Scale Association Analysis. *Nature Genetics*, 42, 579-589.
24. Wang J., Zhang J., Shen J., Hu D., Yan G., Liu X., et al. (2014). Association of KCNQ1 and KLF14 polymorphisms and risk of type 2 diabetes mellitus: A global meta-analysis. *Hum Immunol*, 75(4):342-347.
25. Zabala AS, Vasquez Gomez ME, Álvarez MF, Siewert S. Tetra Primer ARMS PCR Optimization to Detect Single Nucleotide Polymorphism of the KLF14 Gene. *Open Access Library Journal* 2017, Volume 4, e4145 ISSN Online: 2333-9721.

Universities and knowledge sharing: Evaluating progress to openness at the institutional level

Dr. Lucia M. Cortes,

School of Information Studies, University of Queensland,
Brisbane, Australia

Dr. Connor J. Mason,

Centre for Open Science, University of Edinburgh,
Edinburgh, Scotland

Prof. Richard T. Holmes,

Department of Digital Humanities, University of Sydney,
Sydney, Australia

Dr. Kai-Ho Chen,

Institute of Data Science, National Taiwan University,
Taipei, Taiwan

Dr. Alara E. Demir,

School of Library and Information Science, University of
Istanbul, Istanbul, Turkey

Universities are key sites of knowledge creation. Governments and research funders are increasingly interested in ensuring that their investments in the production of new knowledge deliver a quantifiable return on investment, including in the form of ‘impact’. Ensuring that research outputs are not locked behind paywalls, and that research data can be interrogated and built upon are increasingly central to efforts to improve the effectiveness of global research landscapes. We argue that mandating and promoting open access (OA) for published research outputs, as well as the sharing of research data are important elements of building a vibrant open knowledge system, but they are not enough. Supporting diversity within knowledge-making institutions; enabling collaboration across boundaries between universities and wider communities; and addressing inequalities in access to knowledge resources and in opportunities to contribute to knowledge making processes are also important. New tools are needed to help universities, funders, and communities to understand the extent to which a university is operating as an effective open knowledge institution; as well as the steps