Association of rs4731702(C/T) Polymorphism of Kruppel like Factor 14 (KLF14) Gene with ketosis-Prone Diabetes (KPD) in a Cameroonian Population

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Abstract

Aims: This study was to assess the association between the rs4731702(C/T) polymorphism of the Krüppel like Factor 14 (KLF14) gene and ketosis-prone diabetes (KPD) in a Cameroonian population.

Methods: This case-control study included 34 KPD patients and 71 healthy normoglycemic controls who were all unrelated Cameroonian adults (aged ≥ 24 years). Demographic, clinical and biological data were collected to determine phenotypic traits. Biochemical analyses were performed with Chronolab kits using a spectrophotometer. KLF14 rs4731702(CT) genotypes were determined using the Polymerase Chain Reaction follow by Restriction Fragment Length Polymorphism (PCR-RFLP).

Results: From the KLF14 rs4731702(C/T) polymorphism, the T allele was associated to KPD (70.59% KPD patients vs. 30.99% healthy controls, OR=5.345 and p<0.0001) whereas the C allele was protective (29.41% KPD patients vs. 69.01% healthy controls, OR=0.187 and p<0.0001). The susceptibility to KPD was higher among participants who had the TT genotype with OR=4.756 (95%CI [1.984 – 11.40], p=0.0005).

Conclusion: This study showed, for the first time, the association between KLF14 rs4731702(C/T) gene polymorphism with KPD in a Cameroonian population.

Keywords: Ketosis-prone diabetes, polymorphism, Krüppel-Like Factor 14, sub-Saharan Africa, Cameroon.

Introduction

Diabetes, a metabolic disorder characterized by chronic hyperglycaemia resulting, mainly, from insulin resistance in peripheral tissues along with impaired insulin secretion from pancreatic β-cells, is one of the largest global health emergencies of the 21st century [1].

Traditionally, this disease has existed in two forms. The first from, Type 1 Diabetes (T1D) is associated with an autoimmune attack and destruction of pancreatic β cells, resulting in total insulin deficiency. The second one, Type 2 Diabetes (T2D) is associated with insulin resistance and features of metabolic syndrome [2].

Since the 1960s, however, ketosis-prone diabetes (KPD) has been described in many populations, especially of African origin [3-5] and classified as an idiopathic subtype of T1D by the American Diabetes Association. The onset characteristics are ketosis or ketoacidosis, insulin requirement as in type 1 diabetes, but absence of antibody mediated autoimmunity. After the initial acute insulin deficiency, 50-75% people with KPD will experience a remission, defined as the recovery of β-cell function with the possibility of withdrawing from insulin treatment while maintaining an excellent glycemic control only with a balanced diet and/or oral hypoglycemic drugs [6,7] Although KPD patients present ketosis as a common feature with T1D, they display mostly T2D features [7-9], thus making clinical classification difficult. The causes and mechanisms underlying the KPD phenotype are still unclear. Neither a metabolic [6,10] nor a metabolomic [11] approach identify the factors explaining the phenomenon of acute insulin secretion deficit with recovery of β-cell function during remission, in patients with the KPD phenotype. Genetic factors implicated in insulin secretion, β-cell differentiation, and protection against oxidative stress may contribute to the disease onset. However, none
of these factors, on their own, explain the disease [12-17]. When compared to T2D, there is no evidence of susceptibility to ketosis associated with DRB1 and DQB1 genotypes in KPD [18].

Several studies have demonstrated that metabolic diseases are complex and multifactorial because they are shaped by multiple environmental, life-style and genetic factors. All the same there is increasing evidence that genetic factors contribute to disease risk. In this regard, more than 60 polymorphisms, and increasing since the first Genome Wide Association Study (GWAS) in 2007, for increased T2D susceptibility have been identified [19]. There, however, has not been a thorough investigation in African populations [20].

Recently, a study by Civelek and Lusis showed that the KLF14 gene trans regulates a network of genes implicated in various metabolic phenotypes or disorders (in parentheses): T(1)PTM (HDLC, TG, BMI, INS, HOMA); ARSD (HDLC, TG, BMI, INS, GLU, HOMA); SLC7A10 (HDLC-C, WHR, HDLC-C, BMI); C8orf82 (T2D, BMI); APH1B (HDLC-C, INS, HOMA); PRMT2 (BMI); NINJ2 (LDLC-C); KLF13 (LDLC, WHR); GNB1 (HDLC-C, BMI, INS) and MYL5 (BMI) [21]. Thus, this gene is a target for the further investigation of the pathophysiology of these metabolic diseases.

KPD is prevalent in people of African descent [16,22], and may therefore have a genetic predisposition. There are very little data on the genetic risk of diabetes in Cameroonians [23-25].

The present investigation was aimed at studying the association of KLF14 rs4731702(C/T) gene polymorphism with KPD in a Cameroonian population.

Methods

Study design, setting and participants

This was a case-control study involving 34 people with ketosis-prone diabetes and 71 non-diabetic volunteers, from Cameroon, aged 24 years old and above. Those with KPD were recruited at the National Obesity Center of Yaoundé Central Hospital. The condition was defined as new-onset diabetes without precipitating events, such as infection, stress or corticotherapy, with significant ketosis (urine ketones ≥ 13.7 mmol/L) requiring initial insulin treatment to achieve glucose control in the absence of cytoplasmic islet cells and glutamate decarboxylase 65 [7,9].

Non-diabetic controls were recruited from the general population and included in the study after being tested negative for diabetes. Blood samples were analyzed at the Laboratory of Molecular Medicine and Metabolism and at the Laboratory of Public Health and Research Biotechnology at the Biotechnology Center of the University of Yaoundé 1, Cameroon. Ethical clearance was granted by the National Ethics Committee of Cameroon. All the participants provided written informed consent.

Data Collection

Data were collected on sociodemographic information, medical history, clinical and biological data. Blood samples were collected for biological assessment of diabetes-associated antibodies, fasting blood glucose, lipid profile, and molecular assays. Urine samples were collected to assess urine ketones.

Anthropometric Measurements

Weight was measured to the nearest 0.1 kg using an electronic scale (CAMRY, Hong Kong, China). Height was measured to the nearest 0.1 cm using a stadiometer. Body Mass Index (BMI) was then derived as weight (kg)/height² (m²). Waist and hip circumferences were measured to the nearest 0.1 cm using measuring tapes, allowing for the waist-to-hip ratio (WHR) to be calculated. Blood pressure was measured with a validated automated blood pressure measuring device (OMRON HEM-757). Total body fat mass was estimated by bio-impedance (TANITA BC 420 MA, TANITA Corp., Tokyo, Japan).

Biochemical Measurements

All assays were performed twice using the same batch of kits in each case. Plasma glucose was measured by the hexokinase method (Roche Diagnostics, Mannheim, Germany). Serum total cholesterol, HDL cholesterol and triglycerides were measured using standard colorimetric enzymatic techniques. LDL-cholesterol was calculated using Friedwald’s formula [26].

Cytoplasmic islet cell titers were analyzed by immunofluorescence methods on group “0” donor pancreas sections by comparing consecutive dilutions of the testing serum with the Juvenile Diabetes Foundation standard curve, as described previously [7,27].

Glutamate decarboxylase 65 autoantibodies were measured by radio ligand binding immunoassay using their respective radiolabeled recombinant antigen molecules (CIS Bio International).

Molecular Genotyping

Genomic DNA was extracted from whole blood on filter paper by the Chelex method. By PCR-RFLP 105 participants were genotyped for KLF14 rs4731702(C/T). The polymorphism was genotyped using its corresponding number and size of appearing bands with the following primers: Forward 5'-AATCCAAAGGCATCTATC-3', Reverse 5'-CTTGGATTTTTGATCACG-3' (SIGMA-ALDRICH, St. Louis, Missouri, United States). Each 25 μL PCR reaction mixture consisted of 3 μL of genomic DNA, 0.25 μL of each primer (10 pmol/L) and 12.5 μL of 2 × Taq PCR Master Mix (constituents: 20 mM Tris-HCl, pH 9.3, 100 mM KCl, 3 mM MgCl₂, 0.1 U Taq polymerase/μL, 500 μM dNTP each (QIAGEN), and 9 μL of ddH₂O (DNase/RNase-free). The PCR was carried out using a BIOMETRA T3 Thermal Cycler under the following conditions: 1 cycle at 94°C for 5 minutes for an initial denaturation followed by 35 cycles of denaturation for 45 seconds at 94°C, primer annealing for 45 seconds at 53°C, primer extension for 45 seconds at 72°C and a final extension for 7 minutes at 72°C. The amplicons were analyzed by agarose gel electrophoresis on a 3% agarose gel stained with ethidium bromide, then visualized and photographed under a UV transilluminator.

Statistical Analyses

Allele and genotype frequencies in cases and controls were estimated by direct counting. Qualitative variables were analyzed by Chi square (χ²) test with Yates’ continuity correction or the Fisher’s exact test when appropriate, using Epi Info version 6 (USD, Stone Mountain, USA). Data were coded, entered and analyzed
using Statistical Package for Social Sciences (SPSS) version 20.0 for Windows (SPSS, Chicago, Illinois, USA). Continuous variables were compared using non-parametric tests (Mann Whitney) and described using median and inter quartile domain (IQR, 25th-75th percentiles), and categorical variables using their frequency and percentage. The Hardy Weinberg equilibrium was determined using the Goodness-of-Fit Chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression. A p value (two-tailed) less than 0.05 was considered statistically significant.

Results

General characteristics of the study population

In total, 105 participants were enrolled in the study (34 people with KPD and 71 normoglycemic healthy controls). The demographic, clinical and biochemical characteristics of the studied population are presented in Table 1. There were no difference in sex distribution, age, BMI, diastolic blood pressure, atherogenic index, and triglycerides between the KPD group and the normoglycemic healthy control group (all p<0.05). Waist-to-hip ratio, fat mass and fasting plasma glucose were higher in KPD group than in the control group ([median 0.90 (IQR 0.90-1.00) vs. 0.90 (0.08-0.90); p<0.0001] for Waist-to-hip ratio) and [median 28.05 (IQR 20.15-32.30) % vs. 33.65 (24.43-39.74) %; p=0.0395 for fat mass] and [median 3.92 (IQR 3.11-4.71) g/l vs. 0.94 (0.85-1.02) g/l; p<0.0001 for fasting plasma glucose]). Total cholesterol, HDL-cholesterol and LDL-cholesterol were higher in KPD patient group than in the control group ([median 4.23 (IQR 3.67-5.30) g/l vs. 1.90 (1.74-2.13) g/l; p<0.0001 for Total cholesterol] and [median 0.98 (IQR 0.79-1.21) g/l vs. 0.50 (0.45-0.54) g/l; p<0.0001 for HDL-cholesterol] and [median 2.67 (IQR 1.91-3.47) g/l vs. 1.09 (0.94-1.42) g/l; p<0.0001 for LDL-cholesterol]).

Genetic variants and ketosis-prone diabetes

The participants with KPD and the normoglycemic controls were positively genotyped for the KLF14 rs4731702 (C/T) gene polymorphism. After amplification, the product was characterized on agarose gel by one band of 347bp (Figure 1), the genotyping was characterized on agarose gel by two bands of 214bp and 133bp for the wild type homozygote CC, one band of 347bp for the mutant homozygote TT , and three bands of 347pb, 214pb and 133bp for the mutant heterozygote CT (Figure 2). The KLF14 rs4731702 (C/T) gene polymorphism was associated with KPD; OR=5.345 (95 % CI [2.843- 10.05]; p<0.0001) for the T allele and OR=0.150 (95 % CI [0.057–0.392]; p<0.0001) for the C allele. The CC genotype was the protective genotype (20.59% KPD vs. 63.38% for healthy control participants, OR=0.187 [p<0.0001]). The CC genotype was the protective genotype (20.59% KPD vs. 63.38% for healthy control participants, p<0.0001) against the disease.
Discussion

T2D, and related morbidity and mortality, is highly prevalent in Cameroon [2].

Several authors have described KPD, mainly in Africans and African Americans, as a form of diabetes diagnosed with ketosis or ketoacidosis, resembling autoimmune T1D but without markers of specific autoimmunity [28,29].

However, the data on genetic abnormalities that can account for KPD are very little and not sufficient. In this study, we investigated the association of KLF14 rs4731702(C/T) gene polymorphism with KPD in a Cameroonian population. This is the first study that examines the association of this variant of the KLF14 gene with KPD in Africa and the world at large. The results obtained demonstrate that this KLF14 gene is an interesting determinant of KPD risk in the Cameroonian population (Table 2).

The frequency of the mutated T was found to be 43.81%, which is more elevated than that observed in Chinese (36.7%), Japanese (30.0%) and Yoruba (23.3%) populations. It is approximately similar to the frequency observed in the European population (45.0%), but lower than that observed in Icelanders without T2D (56.1%) [30].

In addition, our study revealed, for the first time, that the T allele was strongly implicated in the susceptibility to KPD with an OR of 5.345 (95% CI 2.843 – 10.05, p<0.0001). Recently, in a Chinese population, the KLF14 gene’s rs972283 polymorphism was studied in relation to T2D, rather than the rs4731702 polymorphism in the current investigation [31].

In our population, the general genotypic distribution was 49.52% (52/105) for the CC genotype, 13.33% (14/105) for the CT genotype and 37.14% (39/105) for the TT genotype. These observation, when compared to frequencies calculated by Hardy-Weinberg (H-W) formulas, show that H-W equilibrium was not reached (χ²=32.24; P<0.0001) in the general population. This deviation from the H-W equilibrium was probably due to our small sample size [32].

### Table 2. Case-control association analysis of KLF14 rs4731702(C/T).

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Ketosis-prone diabetic patients</th>
<th>Healthy controls</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>20 (29.41)</td>
<td>98 (69.01)</td>
<td>0.187 (0.099 – 0.352)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T</td>
<td>48 (70.59)</td>
<td>44 (30.99)</td>
<td>5.345 (2.843 – 10.05)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total (2n)</td>
<td>68 (100)</td>
<td>142 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>07 (20.59)</td>
<td>45 (63.38)</td>
<td>0.150 (0.057 – 0.392)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C/T</td>
<td>06 (17.65)</td>
<td>08 (11.27)</td>
<td>1.688 (0.535 – 5.322)</td>
<td>0.3737</td>
</tr>
<tr>
<td>T/T</td>
<td>21 (61.76)</td>
<td>18 (25.35)</td>
<td>4.756 (1.984 – 11.40)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Total (n)</td>
<td>34 (100)</td>
<td>71 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results are given as n (%); KLF14: Kruppel Like Factor 14; OR: Odds Ratio; CI: Confidence Interval

Conclusion

Our present study has demonstrated, for the first time, that the KLF14 gene, previously identified as a master switch gene for T2D and obesity through a Genome-Wide Significant Trans (GWST) association, driven by rs4731702(C/T) in European populations, is strongly associated with an increased risk for KPD in a Cameroonian population. Nevertheless, the functional implications of this genetic association remain to be elucidated in order to further contribute to understanding the physiopathology of this disease.

Acknowledgements

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Abbreviations

T2D: Type 2 Diabetes;  
KPD: Ketosis-prone diabetes;  
KLF14: Krüppel-Like Factor 14;  
APH1B: APH1B gamma secretase subunit;  
ARSD: Arylsulfatase D;  
CBSorf2: Chromosome 8 open reading frame 82;  
GNB1: Guanine nucleotide binding protein (G protein), beta polypeptide 1;  
KLF13: Krüppel-like factor 13;  
MYL5: Myosin, light chain 5, regulatory;  
NINJ2: Ninjurin 2;  
PRMT2: Protein arginine methyltransferase 2;  
SLC7A10: Solute carrier family 7 (neutral amino acid transporter light chain, asc system), member 10;  
TPMT: Thiopurine S-methyltransferase;  
GWAS: Genome Wide Association Studies;  
SNP: Single Nucleotide Polymorphism;  
BMI: Body Mass Index;  
WHR: Waist Hip Ratio;  
DBP: Diastolic Blood Pressure;  
SBP: Systolic Blood Pressure;  
HDL-C: High-Density Lipoprotein-Cholesterol;  
LDL-C: Low-Density Lipoprotein-Cholesterol;  
HOMA: Homeostasis Model Assessment;  
GWST: Genome-Wide Significant Trans;  
PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism;

Disclosure of Interest

The authors declare that they have no conflicts of interest concerning this article.

Authors’ Contributions

Conception and design of experiments: M.G-F., B.A-T., E.S., J-C.M., W.F.M.  
Data collection and biochemical analysis: M.G-F., E.L-Y.  
Gene amplification, molecular genotyping and statistical analysis: M.G-F.  
Data interpretation: M.G-F., B.A-T., E.L-Y., E.S., J-C.M., W.F.M.  
Preparation of the first draft of the manuscript with the guidance of W.F.M.: M.G-F.  
Critical discussion and manuscript revision: M.G-F., B.A-T., E.L-Y., E.S., J-C.M., W.F.M.  
Final editing and approval of the manuscript: B.A-T., E.S., J-C.M., W.F.M.

All the authors read and approved the final version of the manuscript.

References


