The polymorphism Arg585Gln in the gene of the sterol regulatory element binding protein-1 (SREBP-1) is not a determinant of ketosis prone type 2 diabetes (KPD) in Africans


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Abstract

Aim. – Ketosis prone type 2 diabetes (KPD) is an atypical form of diabetes described mainly in people of sub-Saharan African origin. Its pathogenesis is unknown, although we have previously described a high prevalence of glucose-6-phosphate-dehydrogenase (G6PD) deficiency in patients with KPD. However, 50% of these deficient patients lacked the G6PD gene mutation. The isoforms of the transcription factor sterol regulatory element binding protein 1 (SREBP-1) are known to stimulate G6PD gene expression, and some polymorphisms in the SREBP-1 gene (SREBF-1) have been described only in Africans. We investigated one of these, the Arg585Gln polymorphism, in a candidate gene approach for KPD.

Methods. – We examined the presence of the Arg585Gln polymorphism in SREBF-1 in 217 consecutive unrelated Africans [73 patients with KPD, 80 with classical type 2 diabetes (T2D) and 64 nondiabetic subjects]. Patients underwent clinical and biochemical evaluations, and were assessed for G6PD activity and insulin secretion (glucagon test).

Results. – There were no differences in frequency of the Arg585Gln polymorphism and the 585Gln allele among the three groups (allele frequency: KPD: 0.089, T2D: 0.031, nondiabetic group: 0.070; \( P = 0.1 \)).

Conclusion. – The results of this exploratory study show that the polymorphism Arg585Gln in SREBF-1 is not associated with the KPD phenotype. Further studies in larger populations are needed to confirm our findings.

Résumé

Le polymorphisme Arg585Gln du gène du facteur de transcription SREBF-1 n’est pas un déterminant du diabète de type 2 cétoasique de l’Africain.

But. – Le diabète de type 2 cétoasique (DT2C) a été décrit majoritairement chez les Africains sub-sahariens. Sa pathogénie n’est pas connue, bien que nous ayons décrit antérieurement une forte prévalence du déficit en G6PD chez des patients atteints de DT2C. Cependant, 50% de ces patients déficients n’avaient pas de mutation du gène de la G6PD. Les facteurs de transcription SREBP-1 stimulent l’expression du gène de la G6PD et certains polymorphismes de leur gène SREBF-1 ont été décrits exclusivement chez des Africains. Nous avons étudié l’un de ces polymorphismes, Arg585Gln dans une approche gène candidat du DT2C.

Méthodes. – La présence du polymorphisme Arg585Gln a été étudiée chez 217 Africains non apparentés (73 DT2C, 80 [DT2] classique, 64 non diabétiques). Une évaluation clinique et biochimique a été réalisée chez les diabétiques, ainsi que la mesure de l’activité de la G6PD et de l’insulinosécrétion (test au glucagon).

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DT2C. Des études à plus grande échelle sont requises pour confirmer ces résultats.

Conclusion. – Les résultats de cette étude exploratoire montrent que le polymorphisme Arg585Gln de SREBF-1 n’est pas associé au phénotype DT2C. Des études à plus grande échelle sont requises pour confirmer ces résultats.

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Keywords: Ketosis prone type 2 diabetes; SREBF-1; Arg585Gln; G6PD; Insulin secretion

Mots clés : Diabète de type 2 cétosique ; SREBF-1 ; Arg585Gln ; G6PD ; Insulinosécrétion

1. Introduction

Ketosis prone type 2 diabetes (KPD) is an atypical form of diabetes mellitus described mainly in people of sub-Saharan African origin. Its onset is clinically similar to that of type 1 diabetes, with an acute sustained hyperglycaemia, ketosis or ketoacidosis demanding insulin therapy, although there is no evidence of autoimmune destruction of islet beta cells [1–3]. However, its later course is closer to that of type 2 diabetes (T2D) and is characterized by insulin free remission in more than 50% of cases [1]. The pathogenesis of KPD remains unknown, hence its classification as a subset of type 1 diabetes (idiopathic type 1 or type 1B) [4] despite major efforts to characterize it over the past two decades [1,2,5–9]. The partially reversible insulin secretory defect that characterizes KPD at onset may be related to glucotoxicity as a consequence of oxidative stress [5,10]. Regarding that hypothesis, we recently found a 42.3% prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency, a key enzyme in the fight against oxidative stress, and a positive correlation between insulin secretion and residual erythrocyte G6PD activity in KPD [11]. However, common mutations of the gene coding for G6PD were absent in half of the G6PD-deficient KPD cases, suggesting that a factor regulating the expression of the G6PD gene may also be involved.

Sterol regulatory element binding proteins (SREBP) are transcription factors that play a major role in the regulation of carbohydrate and lipid metabolism [12,13]. The isoforms 1a and 1c, produced from the single gene SREBF-1 [14], also increase G6PD gene expression [15,16]. Polymorphisms of SREBF-1 have been associated with T2D in various Caucasian cohorts [17,18], and previous screening for SREBF-1 has identified some single nucleotide polymorphisms (SNP) exclusively found in Afro-Caribbeans [17]. For this reason, we targeted SREBF-1 as a candidate gene for KPD, and hypothesized that one of these polymorphisms—namely, SNP11 (Arg585Gln, exon 10)—might be associated with KPD in Africans as the factor responsible for G6PD deficiency in the absence of the G6PD gene mutation previously described in KPD. The main objective of the present study was to compare the 585Gln allelic frequency in patients with KPD compared with T2D patients and control subjects.

2. Material and methods

In this cross-sectional study, consecutive unrelated patients of sub-Saharan African origin admitted in our department were considered for inclusion. Exclusion criteria were positive determinations of antiglutamic acid decarboxylase 65 (GAD65) or antityrosine phosphatase, or anti-islet cell antibodies. We enrolled 153 patients between 2004 and 2006, among whom 73 patients with KPD and 80 patients with classical T2D. Patients were classified as KPD or T2D as previously described [1], and were compared to 64 nondiabetic control subjects of the same geographical origins living in Paris (France), recruited by public advertisement over the same time period. Control subjects were matched to patients by age at diagnosis of diabetes and region of origin in sub-Saharan Africa, and had no history of T2D in a first-degree relative. Age, gender and year of diabetes diagnosis were recorded for each patient through a structured questionnaire.

Anthropometric parameters were measured in patients using validated methods and included: weight to the nearest 0.5 kg; height to the nearest 0.5 cm; and body mass index (BMI) calculated as weight in kilogram divided by the square of height in metre. Fasting plasma glucose, triglycerides, and total and HDL cholesterol were measured in patients using standard laboratory methods, and LDL cholesterol was calculated by the Friedewald formula. The protocol was approved by the local ethics committee, and all participants gave their written informed consent.

To determine whether or not the Arg585Gln polymorphism was associated with KPD, we tested patients with KPD, using those with T2D and nondiabetics as two distinct control groups. DNA was extracted from peripheral blood mononuclear cells obtained from a venous blood sample, and the coding region of exon 10 of the SREBF-1 was amplified by polymerase chain reaction (PCR). The search for the presence or absence of SNP was performed by allelic discrimination (Assay-by-design; Applied Biosystems, Foster City, CA, USA) using exon specific fluorescent probes. The presence of the mutation was confirmed by sequencing, using an ABI PRISM® 310 automated DNA sequencer (Applied Biosystems). The sequencing reaction was performed with the BigDye® Terminator kit (Applied Biosystems), and sequences were analyzed using Sequence Navigator (Applied Biosystems).

To confirm whether or not the presence of the polymorphism influenced G6PD activity, we measured erythrocyte G6PD activity using spectrophotometry. Erythrocyte homogenates from patients were saturated with G6P and Nicotinamide Adenine Dinucleotide Phosphate (NADP), followed by spectrophotometric detection of Nicotinamide Adenine Dinucleotide Phosphate-Hydrogen (NADPH) appearance at
an absorbance of 340 nm in kinetic mode. The results were calculated by evaluating the increase in optical density per minute (slope) for unknowns against the slope for a standard with known G6PD activity. Total erythrocyte enzyme activity was determined (U/g Hb), and the residual erythrocyte enzyme activity (REA) was calculated by dividing the observed value for each patient by the value of the standard with known normal G6PD activity. G6PD deficiency was defined as an REA inferior to 40% of the normal value. The presence of G6PD deficiency was confirmed by a second measurement [11].

We also tested whether the polymorphism could be involved in either insulin secretion or insulin sensitivity dysfunction. Insulin secretion was assessed in response to intravenous glucagon as previously described [1,2]. C-peptide was measured by immunoradiometric assay (IRMAC–C-PEP; CIS International, Gif-sur-Yvette, France) with an intra-assay coefficient of variation (CV) of 3.7–6.6% and an interassay CV of 4.4–8.0%. Insulin sensitivity was measured at least 24 h later, using a short insulin tolerance test (ITT) as previously described [19]. Both tests were performed after a 12 h overnight fast at least 48 h after the resolution of ketosis, if any was present, and provided that morning fasting plasma glucose levels less than 10 mmol/L were achieved prior to the test.

Statistical analyses were performed using the SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). Data are presented as proportions or means ± standard deviation unless otherwise stated. Comparisons of categorical data were performed using Fisher’s exact test. Three different sets of analyses were used for comparisons of the frequency of SNP between groups and were based on:

- a hypothesis of co-dominance in which homozygous were dissociated from heterozygous participants;
- an hypothesis of rare allele dominance in which homozygous and heterozygous participants were pooled;
- allele frequency.

As only a few patients displayed the 585Gln allele, we pooled them into two groups (carriers and noncarriers), regardless of the type of diabetes, to compare metabolic parameters. Quantitative variables were compared using analysis of variance (Anova) tests, and a *P*-value inferior to 0.05 was considered significant.

### Table 1
Characteristics of patients at the time of study inclusion.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>KPD (n = 73)</th>
<th>T2D (n = 81)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 ± 11.0</td>
<td>49.0 ± 11.7</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>89.0</td>
<td>62.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>2.0 ± 3.2</td>
<td>6.1 ± 7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 4.0</td>
<td>25.3 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0 ± 1.1</td>
<td>5.2 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.2 ± 1.0</td>
<td>3.3 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 ± 1.5</td>
<td>1.2 ± 0.7</td>
<td>0.045</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>11.5 ± 3.6</td>
<td>10.2 ± 3.2</td>
<td>0.032</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>7.1 ± 2.1</td>
<td>7.4 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/mL)</td>
<td>1.19 ± 0.96</td>
<td>1.52 ± 0.96</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide increment (ng/mL)</td>
<td>1.36 ± 1.02</td>
<td>1.73 ± 1.31</td>
<td>NS</td>
</tr>
<tr>
<td>KITT (%/min)</td>
<td>1.46 ± 0.98</td>
<td>1.64 ± 1.19</td>
<td>NS</td>
</tr>
</tbody>
</table>

KPD: ketosis prone type 2 diabetes; T2D: type 2 diabetes; BMI: body mass index; NS: not significant.

#### 3. Results

The mean age at inclusion was 46.4 ± 11.0 years for KPD patients, 49.0 ± 11.7 for T2D patients and 43.8 ± 10.7 for control subjects (P = 0.03). However, the mean age of patients with KPD (44.4 ± 10.3 years) or T2D (42.8 ± 10.6 years) at the time of diagnosis was similar to that of the nondiabetic controls (43.8 ± 10.7 years) (P = 0.6). Men represented 62.5% of patients with T2D (n = 50) and of the controls (n = 40), and 89.0% (n = 65) of those with KPD (P < 0.001). The characteristics of these patients are presented in Table 1. Compared with patients with KPD, patients with T2D had higher HDL and lower triglyceride levels, and were better controlled. Fifty nine percent (n = 43) of the KPD patients had experienced at least one prolonged insulin free remission (HbA1c ≤ 6.5% for at least 3 months in the absence of insulin treatment) in the course of their disease. A total of 35 (47.9%) patients with KPD vs 32 (42.5%) with T2D were taking insulin treatment at the time of inclusion in the study (P = 0.3).

Table 2 shows the genotype and allele frequencies among the three study groups. Overall, there was no significant difference in either genotype or allele frequency between groups. One patient with KPD was homozygous, but had no outlying characteristics compared with the other patients. When the homozygous and heterozygous KPD patients were pooled (hypothesis of dominance of a rare allele), the genotype frequency was higher when KPD patients were compared separately with T2D.

### Table 2
Frequency of Arg585Gln polymorphism in SREBF-1 across the study groups.

<table>
<thead>
<tr>
<th>Genotype frequency, n (%)</th>
<th>KPD (n = 73)</th>
<th>T2D (n = 80)</th>
<th>Nondiabetic (n = 64)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>KPD vs T2D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>61 (83.5)</td>
<td>75 (93.7)</td>
<td>55 (85.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>11 (15.1)</td>
<td>5 (6.3)</td>
<td>9 (14.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>585Gln</td>
<td>0.089</td>
<td>0.031</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td>0.032</td>
</tr>
</tbody>
</table>

KPD: ketosis prone type 2 diabetes; T2D: type 2 diabetes.
patients ($P = 0.045$). Likewise, allele frequency was higher in KPD patients when compared separately with T2D patients ($P = 0.032$; Table 2).

On comparing carriers with noncarriers of the 585Gln allele, there were no differences in G6PD activity (carriers 86.7 ± 42.6%, noncarriers 76.8 ± 28.9%; $P = 0.3$), C-peptide response to intravenous glucagon (fasting C-peptide: carriers 1.11 ± 0.98 ng/mL, noncarriers 1.38 ± 0.97 ng/mL; $P = 0.4$; C-peptide increment: carriers 1.06 ± 0.55 ng/mL, noncarriers 1.59 ± 1.22 ng/mL; $P = 0.15$) or insulin sensitivity ($\text{K}_\text{ITT}$; carriers 1.55 ± 1.37% per minute, noncarriers 1.55 ± 1.07% per minute; $P = 0.99$). Carriers had lower total and LDL cholesterol levels (2.6 ± 0.6 mmol/L vs 3.3 ± 1.0 mmol/L; $P = 0.025$), which accounted for their lower total cholesterol level (4.2 ± 0.8 mmol/L vs 5.2 ± 1.1 mmol/L; $P = 0.006$).

4. Discussion

The vast majority of descriptions of KPD cohorts have involved people of sub-Saharan African descent. This ethnic specificity, added to the high frequency of a family history of diabetes, strongly suggests a genetic predisposition to the disease. In metabolic studies, insulin secretion impairment has been found to be the major determinant of ketosis onset, the hallmark of KPD [1,3,20,21]. This deficit is characterized by a loss of acute phase insulin release in response to glucose or glucagon. Our team and others have investigated the implication of the ethnic-specific mutations/polymorphisms of some of the genes involved in insulin secretion or maintenance of the beta cell mass, including PAX4 [22] and HNF-1-alpha [23,24], but found no strong associations. However, because insulin secretion impairment can be due to glucotoxicity as a consequence of oxidative stress [5,10], and because G6PD is a major enzyme in the fight against oxidative stress, we tested the implication of the G6PD deficiency frequently found in Black Africans due to a mutation of the G6PD gene (A- variant) in these populations. We tested the role of G6PD and found an association between KPD and G6PD activity deficiency that could not be explained in half of the cases by the well-known gene mutation [11]. In the present study, we have investigated the role of an ethnic-specific SNP of SREBF-1, a gene coding for transcription factors that stimulate G6PD gene expression [15,16].

Overall, the results of our study show no association between the Arg585Gln polymorphism of SREBF-1 and the KPD phenotype. Also, no relationship was found between G6PD activity and insulin secretion, measured by the C-peptide response to glucagon. This suggests that the studied polymorphism probably does not play a major role in KPD. However, due to the rarity of this polymorphism, the small sample size and the consequential lack of statistical power, further studies with larger cohorts are needed to draw any definite conclusions. In fact, when a separate comparison was made between the KPD and T2D groups, the 585Gln allele frequency was significantly higher in the KPD patients. Although this may be a chance finding, we cannot exclude the possibility that it may suggest a possible relationship between the 585Gln allele and KPD phenotype. For instance, it might interact with other, as yet unidentified factors in people predisposed to develop T2D to convert it to the ketosis prone phenotype. This suggests that if individuals at risk of developing T2D also happen to carry the 585Gln allele, some other unknown add-on factors may be involved, resulting in the development of the KPD phenotype, thereby explaining the lower allele frequency in T2D patients compared with controls.

Another noteworthy finding is the lower total and LDL cholesterol levels in carriers compared with noncarriers. The primary role of the SREBP-1a and -1c transcription factors coded by SREBF-1 is the regulation of carbohydrate and lipid metabolism [12,13]. Both SREBP-1a and the isofrom SREBP-2, coded by a different gene, are known to be the most efficient activators of the cholesterogenic genes [25]. To the best of our knowledge, similar observations of such a lipid profile have not been previously reported. However, given the small number of carriers compared with noncarriers, this observation may be another chance finding. Nevertheless, it is possible that carrying the 585Gln allele may lead to a better lipid profile through mechanisms that are yet to be elucidated.

In addition to the small sample size, our study was also limited by the fact that patients were not gender-matched because of the well-recognized high male predominance in patients with KPD vs classical T2D [5,6]. Furthermore, our candidate gene approach was limited to only one polymorphism. Screening for other polymorphisms located on the gene may have strengthened the findings.

In conclusion, the results of this exploratory study suggest that the polymorphism Arg585Gln does not determine G6PD activity and is not associated with KPD in Africans. Further studies with larger cohorts are required to draw definite conclusions regarding this issue.

Conflicts of interest

There are none to declare.

Acknowledgements

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