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Original research

E23K variant in *KCNJ11* gene is associated with susceptibility to type 2 diabetes in the Mauritanian population

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ABSTRACT

Aims: Many genetic association studies reported the contribution of *KCNJ11* gene to type 2 diabetes susceptibility in different populations. We aimed to evaluate the association between E23K variant of *KCNJ11* and type 2 diabetes in the Mauritanian population.
Materials and methods: We performed a case-control association study including 135 type 2 diabetes Mauritanian patients and 135 controls. Genotyping for the E23K variant was performed using a TaqMan allelic discrimination assay.
Results: We found significant association between *KCNJ11* E23K variant and type 2 diabetes (Global model, OR = 2.08, 95% CI = 1.09–3.97, $p = 0.026$). In the Moor ethnic group, E23K was also associated with type 2 diabetes in the general model (OR = 2.08, 95% CI = 1.09–3.97, $p = 0.026$) and under the dominant model (OR = 2.49, 95% CI = 1.12–5.55, $p = 0.026$). In the Mauritanians of African descent, KK genotype was not found. Besides, E23K variant was not associated with type 2 diabetes (OR = 0.69, 95% CI = 0.04–11.32, $p = 0.793$).
Conclusions: Our results revealed the risk of type 2 diabetes conferred by *KCNJ11* E23K gene variant in the Mauritanian population.

1. Introduction

Various mitochondrial and nuclear DNA sequences were identified to contribute in determining type 2 diabetes complex phenotypes. A mtDNA poly-C tract (T16189C variant) located between nucleotide positions 16184 and 16193 (near the origin of replication) was thus found to be associated with type 2 diabetes related traits such as obesity, insulin resistance and thinness at birth [1]. Candidate gene approach

Abbreviations: SNP, single nucleotide polymorphism; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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also revealed several nuclear sequences linked with diabetes.

Although the effect of most of these sequences, e.g. CDKAL1, CDKN2B and IGF2BP2 remained modest or not replicated in other ethnic groups (ABCC8), consistent association has been reported between variants of genes such as KCNQ1 and KCNJ11 and type 2 diabetes [2–4].

KCNJ11 gene known as "potassium inwardly-rectifying channel, subfamily J member 11" encodes Kir6.2, the pore forming subunit of the ATP-sensitive potassium (K_{ATP}) channels. These channels are embedded in the ß-cells membranes where they function to link insulin release by the pancreatic cells in response to increased glucose blood concentration [5]. E23K variant, resulting in substitution of glutamic acid (E) by lysine (K) at position 23 of the *KCNJ11* gene, has been associated with type 2 diabetes in European, Asian and Arab populations [4,6,7]. Mutations in this gene were also the most common cause of PNDM (permanent neonatal diabetes mellitus) [8].

In Mauritania, the increase of diabetes prevalence was attributed mainly to the profound change in lifestyle which accompanied the shift from a longstanding nomadic-pastoralist system to a settled urban society over the past decades [9,10]. As in the whole Africa continent, genetic susceptibility to diabetes remained barely investigated. In a recent study, we showed that 27% of surveyed type 2 diabetes patients reported at least one relative with diabetes. The prevalences of affected first and second degree relatives were also consistent with a familial aggregation of this disease in the Mauritanian population [11].

While family history of diabetes was shown to increase the likelihood of diabetes, no specific gene has been directly correlated with this factor. We previously found no significant association between mtDNA 16184–16193 poly-C tract and type 2 diabetes [12].

In this study, to further investigate the genetic contribution to diabetes in the Mauritanian population, we explored the influence of E23K variant of *KCNJ11* gene on this disease in a representative group of type 2 diabetes Mauritanian patients.

2. Subjects and methods

2.1. Subjects

Participants comprised 135 unrelated type 2 diabetes patients (44 men and 91women; average age at recruitment 55 ± 8.02 years, average age at type 2 diabetes diagnosis 51.16 ± 8.26) under follow up by an endocrinologist in the diabetes center of Nouakchott (the capital city) and 135 controls (82 men and 53 women average age at recruitment 38.61 ± 9.61 years), all Mauritanians. The two ethnic groups in the country, i.e. the Moors (whites and black) and the black Africans (Pulhars, Soninkes and Wolof) were included in the study. Ethnic origin was defined from a brief questionnaire recording the full name, native language and geographic origin of each participant, filled in after his informed consent. The main anthropometric and clinical characteristics (BMI, total cholesterol, triglycerides HDL-cholesterol and LDL-cholesterol and fasting plasma glucose) were also determined. Type 2 diabetes diagnosis was based on WHO criteria [13]. Controls were identified by a fasting plasma glucose level <6.1 mmol/l, a blood HbA1c value of <39.89 mmol/mol (5.8%) and no antidiabetes medication. This protocol was approved by the national ethic committee.

2.2. Polymorphism genotyping

DNA of type 2 diabetes patients and controls was extracted from peripheral blood leucocytes by salting out method [14]. Genotyping of E23K SNP was carried out using a TaqMan allelic discrimination assay kit (Applied Biosystem/ABI, Foster City, CA, USA) supplied with two allele-specific TaqMan fluorescent MGB probes specific of the region CGCTGGCGGGCACGGTACCTGGGCT[C/T]GGCAGGGTCCTCTGCCAGGCGTGTC and two forward/reverse primers designed to amplify the polymorphic DNA sequence of interest (ID number: C__11654065_10). PCR reaction mix contained SNP genotyping assay, TaqMan genotyping Master mix, AmpliTaq Gold DNA polymerase (ABI, Foster City, CA, USA) and genomic DNA template in a final reaction volume of 20 ul per well. Amplification was performed on an ABI Prism 7500 sequence detection System (Applied Biosystems, Foster City, CA, USA) according to the temperature and cycles conditions given by manufacturer: 10 min at 95 °C for AmpliTaq Gold Enzyme activation followed by 50 cycles of 15 s at 92 °C for denaturation and 90 s at 60 °C for annealing/extension. The fluorescence intensity in each well was then read by ABI PRISM instruments and analyzed for genotype determination using specific software both from Applied Biosystems. Twenty seven DNA samples (10% of the total) were randomly re-tested with the same method to confirm genotype accuracy which showed a 100% concordance rate.

2.3. Statistical analysis

Hardy–Weinberg equilibrium analyses were performed on R using the function "hwexact" [15]. E23K allelic and genotypic association with T2DM risk was tested using multivariate logistic regression to adjust for age and gender. Odd ratios (Ors) with 95% Confidence Interval (95% CI) were assessed for the risk allele. Genotypic association was also tested under additives (EE vs. EK and EE vs. KK), dominant [EE vs. (EK + KK)] and recessive [KK vs. (EE + EK)] genetic models. Allelic exact test was computed on R using allelic package. Statistical analyses were performed using Stata 11 software (StataCorp, College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

3. Results

The genotype distribution of E23K SNP was in Hardy–Weinberg equilibrium both in patients and controls groups (Table 1). No significant allelic association was observed with type 2 diabetes before correction (OR = 1.51, 95% CI = 0.98–2.34, $p = 0.10$). After adjusting for age and gender by multivariate logistic regression analysis, E23K polymorphism was found to be associated with type 2 diabetes under both general (OR = 2.08, 95% CI = 1.09–3.97, $p = 0.026$) and dominant models (OR = 2.49, 95% CI = 1.12–5.55, $p = 0.026$) in the global population (Table 1).