

Investigating the Role of Genetic Polymorphism in the Exon 3 of ATG16L1 Gene in T2DM Patients: A Pilot Study

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ABSTRACT

OBJECTIVE Diabetes mellitus (DM) is a metabolic disease characterized by persistently high blood sugar levels and has several potential causes. We aimed to find out whether there are any single nucleotide polymorphisms in a particular region of the ATG16L1 gene which are linked to type 2 diabetes.

METHODS A total of forty participants were included in this study; 20 were part of the patient group and had type 2 diabetes mellitus (T2DM), while 20 were part of the healthy control group. High-resolution melt (HRM) analysis was used for genotyping along with sequencing for confirmation of the results.

RESULTS The melting behavior showed consistent DNA sequence in the exon 3 of the ATG16L1 gene in both the patient and the healthy control groups in the study.

CONCLUSIONS The ATG16L1 gene does not include any SNPs in the exon 3 region associated with type 2 diabetes. However, further studies are needed to determine the role of the autophagy gene in T2DM.

KEYWORDS ATG16L1, HRM, SNP, T2DM, autophagy

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a widespread metabolic disease characterized by the failure of insulin-responsive cells to respond to insulin or to the insufficient secretion of insulin by insulin-producing cells. During the disease, insulin secretion becomes inadequate for glucose regulation, resulting in hyperglycemia, the hallmark of T2DM (1). There is mounting evidence that genetic factors play a role in susceptibility to type 2 diabetes (2, 3). Autophagy is a cellular process that eliminates and recycles damaged cellular components. The ATG16L1 gene encodes the ATG16L1 protein, which is essential for this activity (4). Along with its function in autophagy,

ATG16L1 also controls the immunological response, intracellular trafficking, and inflammation (5).

The ATG16L1 gene is essential for the autophagic process in pancreatic β -cells, which is vital for insulin release and β -cell functionality. (6). People with diabetes, especially type 2 diabetes, commonly experience higher levels of oxidative stress and endoplasmic reticulum stress in their β -cells, which can be managed by autophagy, a defensive process that cells employ (7). Autophagy ensures the well-being of β -cells in diabetes by eliminating damaged organelles and misfolded proteins. However, β -cell failure and the advancement of diabetes are caused by dysregulated autophagy, which is observed in diabetic circum-

stances (8). Several autophagy proteins, including ATG16L1, govern autophagosome production, which degrades and recycles cellular components (9). β -cells in T2DM patients have impaired autophagy resulting in the accumulation of toxic proteins, which induces endoplasmic reticulum stress and diminishes β -cell function, thereby exacerbating insulin resistance and elevated blood glucose levels (10, 11). This study aimed to investigate specific genetic markers in the selected region on the ATG16L1 gene that may be associated with type 2 diabetes.

METHODS

Subjects. The pilot study consisted of 40 participants in total, 20 individuals with T2DM in the patient group, and the same number in the healthy control group. Diabetic status was determined using standards set by the World Health Organization (12, 13) and based on reports by the patients' endocrinologists. Patients with either type 1 diabetes or other chronic diseases were excluded from the study. The control group included healthy individuals with no history of diabetes or related metabolic disorders. They were recruited from Al-Sader Teaching Hospital, Najaf, Iraq. Before blood collection, we ensured that patients' informed consent was obtained.

Ethical approval for conducting this study was obtained from the College of Medical Technology, the Islamic University in Najaf (No. 40, 1/6/2024).

The study was carried out in the molecular laboratories at the Al-Furat Al-Awsat Technical University and the Islamic University, Najaf, Iraq. Both groups underwent similar testing procedures, including the collection of blood samples for genetic and biochemical analysis.

Pre-genotyping. DNA was extracted from whole blood samples following the manufacturer's protocol (iNtRON Biotechnology, Seongnam, Korea) as in our previous studies (12, 14). The primers for PCR were prepared using the stand-

ard method for dilution of stock primers to concentrations of 10 μ M.

Genotyping. The Rotor-Gene Q RT-PCR system (Qiagen, Hilden, Germany) was employed to carry out high-resolution melt (HRM) analysis using real-time PCR. Following the instructions of the kit, 5 μ L of DNA was mixed with 10 μ L of GoTaq® qPCR master mix (Promega), and one μ L each of forward and reverse primers (10 μ M) was subsequently introduced into the mixture. The final volume was then adjusted to 20 μ L. The PCR program was conducted as follows. The mixture was preheated to 95°C. Then 40 cycles of the run, each consisting of three steps (denaturation at 95 °C/25 S, annealing at 58 °C/40 S, and elongation at 72 °C/35 S), were conducted. Primer sequences are listed in Table 1. After amplification, the PCR products were subjected to gradual melting from 60° to 95 °C at a ramp rate of 0.1 °C per second as described in our previous study (14). The melting curves were analyzed using the Rotor-Gene Q-Pure Detection software (Qiagen, Hilden, Germany).

Statistical analysis. The GraphPad Prism program version 9 for Mac (San Diego, CA, USA) was used to perform the statistical analysis, using chi-square and student's t-test. Qualitative data are shown as frequencies. The alignment of the sequencing data was performed using Geneious Prime software (Biomatters Ltd., Auckland, New Zealand). The analysis of the melting curves was carried out with the assistance of the Rotor-Gene Q-Pure Detection program.

RESULTS

The characteristics of the participants in the study are summarized in Table 2. The mean age of the control group was 42.75 and of the study group was 54.90. The mean duration of diabetes was 6.733 years and the average body mass index was 25.95 Kg/m² in the control and 29.71 Kg/m² in the patient group. Random blood sugar (RBS)

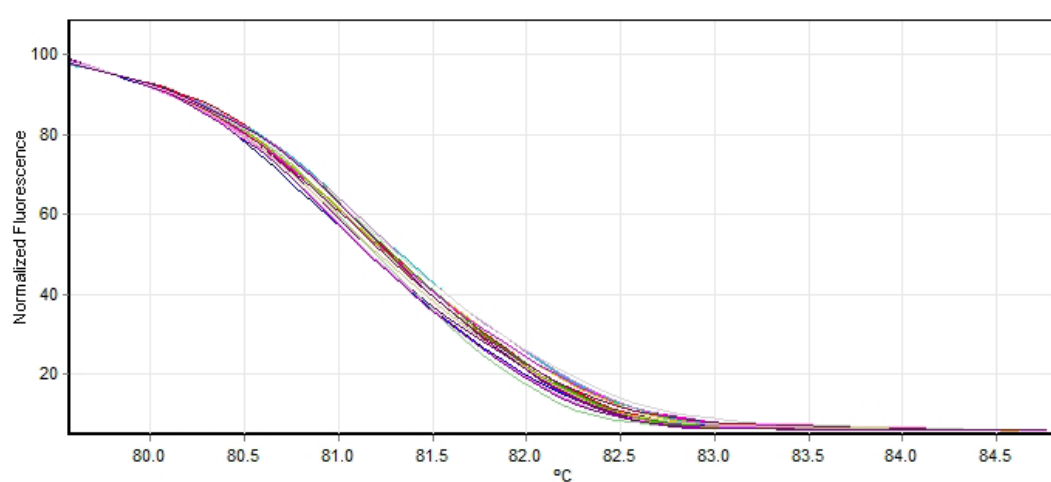
Table 1. Primer sequences of exon 3 of the ATG16L1 gene

Primer direction	Primer sequence	Tm	Annealing temperature	Amplicon (bp)
Forward	CTTCATCTGCCTGGTTTGCC	60	58 °C	135
Reverse	ACCTCCCCACGTTTCTTGTTG	60		

Table 2. Characteristics of patient and control groups in the study

Characteristics	Control (n=20)	Patient (n= 20)	p-value
Age	42.75 (13.67)	54.90 (13.11)	0.0067
Sex; N (%)			
Male	10 (50.00)	11 (55.00)	0.7520
Female	10 (50.00)	9 (45.00)	
Duration of diabetes (years)		6.733 (7.35)	
Height (m)	174.4 (10.09)	170.1 (11.55)	0.1040
Weight (Kg)	75.40 (14.98)	80.50 (20.63)	0.3770
BMI (Kg/m ²)	25.95 (4.65)	29.71 (6.31)	0.0386
RBS (mg/dL)	99.80 (16.90)	183.0 (64.64)	< 0.0001
HbA1c (%)	5.11 (0.58)	8.00 (2.253)	< 0.0001

BMI, body mass index; RBS, random blood sugar

**Figure 1.** Normalized HRM PCR plot

and HbA1c levels were significantly elevated in the patient group. The mean RBS in the patient group was 183.0 mg/dL (SD 64.64) compared to 99.80 mg/dL (SD 16.90) in the control group ($p < 0.0001$). Similarly, HbA1c levels were significantly higher in the patient group, with a mean of 8.00% (SD 2.253) compared to 5.11% (SD 0.58) in the control group ($p < 0.0001$).

The region of exon 3 of the ATG16L1 gene was explored to pinpoint specific SNPs or mutations that may play a role in T2DM susceptibility. The results of HRM show the melting curves that represent the dissociation of double-stranded DNA as the temperature increases. The consistent and smooth downward trend across the samples reflects a similar melting behavior, with melting transitions occurring between approximately 80.5 °C and 84.5 °C (Figure 1). The close pattern of curves in melting profiles between the different samples indicates there was no change in the sequence of this region e.g., no single nucleotide polymorphisms (SNPs) or other small genetic

variations within exon 3 of the ATG16L1 gene. The absence of major shifts or distinct outliers in the melting patterns further emphasizes that exon 3 contains no changes in its sequence that might influence its function. The lack of deviations in the melting temperature in this region was further confirmed by the sequencing technique (Figure 2). The alignment of the sequencing data was compared with the reference sequence of the ATG16L1 gene and the analysis confirmed that the selected region appeared to have remained largely conserved in the T2DM cohort. This consistency is consistent with the overlapping patterns of the HRM melt curves, suggesting that the ATG16L1 gene function in type 2 diabetes may be influenced by factors other than exon 3, or by more systemic genetics.

DISCUSSION

The ATG16L1 gene plays a fundamental role in the process of autophagy, a process which is critical for maintaining cellular homeostasis. To our

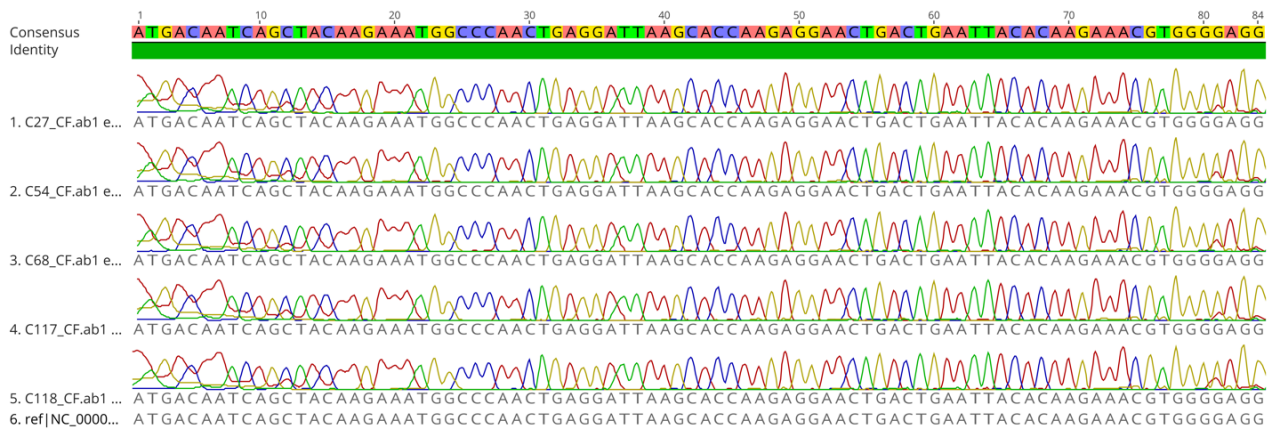


Figure 2. Sequence alignment results for exon 3 of the ATG16L1 gene

knowledge there have been no previous studies investigating the relationship between ATG16L1 gene polymorphisms and diabetes patients, and few studies on the relationship of ATG16L1 gene polymorphisms and other diseases. We conducted this study to determine whether any of the expected SNPs were associated with T2DM to advance a step in the understanding of the genetic factors that increase T2DM susceptibility and to help other researchers in their investigations.

ATG16L1 represents a critical node in various physiological and pathological processes, one of which is diabetes. Due to the fact that autophagy protects cells from oxidative stress, a possible reason for hyperglycemia might be the failure of the autophagic mechanism in beta cells which exacerbates beta cells activity (6). According to Gao et al., the ATG16L1 gene may play a significant role as a biomarker in the onset and progression of diabetic retinopathy (15). That suggests a crucial role of autophagy and, indeed, of the ATG16L1 gene in diabetes. A previous study revealed that type 2 diabetes patients' beta cells have abnormalities in autophagy (16). We were, however, unable to identify any genetic polymorphism in our population of T2DM. This study has some limitations: the small sample size and that the region of the ATG16L1 gene under investigation was limited to exon 3.

CONCLUSION

Our work is among the initial investigations exploring the correlation between the ATG16L1 gene polymorphisms and T2DM. Our results indicate that the ATG16L1 gene remains a wild sequence and contains no SNP in the exon 3 region that is linked to T2DM patients. Further studies

with larger sample size on different exons of the ATG16L1 gene are needed to determine if any SNP is associated with T2DM.

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CONFLICT OF INTEREST

No conflicts of interest.

ADDITIONAL INFORMATION

Data availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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