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ORIGINAL ARTICLE

The Effects of Green Tea Consumption on KCNJ11 Gene Expression in Patients with Type 2 Diabetes

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ABSTRACT
The KCNJ11 *gene provides instructions to make parts (subunits) of the ATP-sensitive potassium (K-ATP) channel. Each K-ATP channel contains eight subunits. Four subunits are produced from the* KCNJ11 *gene, and four are produced from another gene called* ABCC8.*K-ATP channels are within beta cells, which are cells in the pancreas that secrete the hormone insulin. And as so, it will be interesting to compare the total amount of KCNJ11and its gene expression level between diabetic and normal mice. NMRI female mice (6 weeks old) were randomly divided in to three groups (n=8).The first group were normal mice that didn't undergo any treatment. The next group were normal mice which received extract of green tea extract (0.2 mg). The 3rd group was mice with diabetes that received extracts of green tea extract (0.2 mg). The experiment duration was 8 weeks, and glucose was weekly measured. Following this period, mice were sacrificed, pancreas beta cell tissues were isolated and KCNJ11 expression of the four groups was measured. To the end, after RNA isolation and cDNA synthesis, real time PCR was finished with specific primer for the KCNJ11 gene. KCNJ11 expression in the pancreas of untreated mice increased compared to normal control mice and this increase was statistically significant (P value = 0.01). The typical KCNJ11 gene expression changes in the pancreas of treated mice was reduced in comparison to untreated mice, and this reduction was statistically significant (P value = 0.04). Therefore, we could claim that green tea extract consumption during treatment has an impact on KCNJ11 gene expression.*
Keywords: *Green tea, Diabetes, KCNJ11 gene*

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INTRODUCTION

Diabetes is just a multifactorial disease due to both genetic and environmental factors and their complex interaction. Environmental risk factors include obesity, physical inactivity, hypertension, abnormal cholesterol levels, age, and smoking [1].

Diabetes is classified into various types, which type 2 (T2DM) occurs most frequently. Approximately 5%–10% of patients with DM are influenced by type 1 (T1DM) and significantly more than 90% by T2DM. T1DM (formerly insulin-dependent diabetes, or juvenile diabetes) results from the autoimmune destruction of the insulin-producing beta cells in the pancreas [2]. T2DM (formerly noninsulin-dependent DM) is really a metabolic disorder as a result of hyperglycemia in the context of insulin resistance and relative not enough insulin. That is in comparison to T1DM, where there's complete insufficient insulin because of the breakdown of islet cells in the pancreas [3].

Multiple genes are associated with Diabetes. The ones that have garnered probably the most attention would be the ATP-binding cassette transporter subfamily C member 8 (ABCC8) gene; the KCNJ11 gene;

and the peroxisome proliferator-activated receptor-gamma (PPARG) gene. These types of genes are involved with insulin action/glucose metabolism, pancreatic beta cell function and other metabolic conditions (e.g., energy intake/expenditure, lipid metabolism) [4]. Mutations in genes such as for example ABCC8 and KCNJ11 can disrupt the potentiation activity of the KATP channel and have thus been connected with permanent neonatal Diabetes [5]. The PPARG gene is implicated in adipogenesis and the development of insulin resistance. Deleterious mutations in this gene impair insulin resistance and could cause not enough a reaction to insulin [6].

The KCNJ11 gene provides instructions in making parts (subunits) of the ATP-sensitive potassium (K-ATP) channel. Each K-ATP channel contains eight subunits. Four subunits are produced from the KCNJ11 gene, and four are produced from another gene called *ABCC8* [6].

K-ATP channels are within beta cells, which are cells in the pancreas that secrete the hormone insulin. The K-ATP channels are embedded in cell membranes, where they open and close in a reaction to the total amount of glucose in the bloodstream [7, 8]. Glucose is really a simple sugar and the principal energy source for some cells in the body. Closure of the K-ATP channels in a reaction to increased glucose triggers the release of insulin out of beta cells and in to the bloodstream, which supports control blood sugar levels.

Mutations in the KCNJ11 (MIM# 600937), and ABCC8 (MIM# 600509) genes encoding both protein subunits (kir6.2 and SUR1) of the ATP-sensitive potassium channel are one of the very most common causes of both permanent and transient neonatal diabetes mellitus [9, 10].

The KCNJ11 gene, a member of the potassium channel gene family, is found at 11p15.1 and does not have any intron. This gene encodes an inward-rectifier potassium ion channel (Kir6.2). The Kir6.2 protein, alongside the high-affinity sulfonylurea receptor 1 (SUR1), forms the KATP channel. SUR1 is encoded by the ABCC8 gene located close to the KCNJ11 gene. The Kir6.2 protein is really a 390-amino acid protein with two transmembrane domains (M1 and M2) and intracellular N- and C-terminals. Structurally, Kir6.2 tetramers form the pore and four high-affinity SUR1 subunits surround the pore of the KATP channel located at the plasma membrane of pancreatic beta cells. This channel modulates insulin production and secretion through glucose metabolism [11].

Recently, green tea extract (*Camellia sinensis*) has received a lot of attention especially due to its content of polyphenols. Catechins, including epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epicatechin (EC), are major polyphenols present in green tea extract and possess a wide variety of health promoting effects [12, 13].

Several animals and human studies suggested that green tea extract consumption is beneficial in lowering blood glucose in diabetes population [14, 15].

Green tea extract is sparking tremendous excitement as new health applications keep being discovered [16, 17].The most active green tea extract constituent is known as epigallocatechin gallate. This excellent flavonoid favorably alters pathways underlying pathological processes such as for instance cancer, cardiovascular disease, diabetes, obesity, and Alzheimer's and Parkinson's diseases [18, 19]. This points to green tea extract as a broad-spectrum nutrient which could promote endurance [20, 21].Green tea is consumed routinely in Asian populations, who've greater longevity and lower mortality rates for diseases which can be prevalent in Western society [22].

MATERIAL AND METHODS

Animals and treatment

White male NMRI mice (20-25g) were obtained from Kharazmi University and housed at 28±2 in humidity-controlled (30-70 %) facilities, on a 12 h light/dark cycle with usage of mouse pellet food. Mice were divided in to two groups of six mousses. Control group was fed only by normal mouse pellet diet for 8 weeks whilst the case group received a extract of green tea and following ingredients: 150 g of grind laboratory pellet food, 20 g of roasted sesame, 100 g of milk chocolate, 50 g of creamy biscuits, and100 g peanut. These components were powdered by grinding and prepared in the shape of pellets. Food was stored at ~4°Ctoprevent oxidation of the fat components.

Mice were weighed weekly for 8 weeks. By the end of the period, the mice were anaesthetized with chloroform and exsanguinated through cardiac puncture. 2ml blood samples were taken from both groups for further biochemical analyses. The pancreas was removed and washed bycoldPBS1xin order to eradicate RBCs and debris, from then on 500mg pancreas tissue was homogenized in 1ml lysis buffer (20m MTris-HCl, pH 7.5, 0.3m M-phenylmethylsulfonyl fluoride (PMSF) and 0.1m M-benzamidine chloride). The resulting homogenate was centrifuged at 1500rpm for 20 minutes. Supernatant (pancreas extract) was filtered through a 0.45 micron filter and stored at -80°C.A percentage of the pancreas tissue was utilized in 5ml culture media containing antibiotic/antimycotic for preparing beta cell culture. The

experimental protocol was performed in respect with the international guidelines put down in the Guide forthe Care and Utilization of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and approved by the Research and Ethics Committee of The Endocrinology and Metabolism Research Institute (EMRI) (EC-00247).

RNA isolation and relative quanti cation by real-time PCR

Total RNA was purified from hepatocytes using Trizol according to standard protocol (GIBCO-USA).250ng aliquot of total RNA from each sample was reverse transcribed to cDNA using random hexanucleotide primers and Revert AID First Strand cDNA Synthesis kit (Fermantas). (Table1)(Table 2)The resulting cDNA from each sample was subjected to quantitative real-time PCR. Hypoxanthine-guanine phosphoribosyl transferase (HPRT) housekeeping gene was used as internal control for normalizationThe primer pairs sequences are as follow: 5´-GTG GTT AGC GTT GAT CAA CCA G-3 ', 5´- GCA AGT CTT TCA GTC CTG TCC-3´ for HPRT and 5´-AAGATCTCCTACTTAAAGAACGGG-3´,5´-ACCCGTGTGATTCCATAAGG-3´ for KCNJ11 gene.(Table 3)

Each quantitative real-time PCR reaction was performed in duplicate assays. A20µl reaction was set by mixing the following components: 30ng cDNA, 10 µl RT2 Real-Time™ SYBR Green/ROX PCR Master, primer pairs and nuclease-free water to 20 µl. Thermal condition was used for 40 cycles consisting of initial polymerase activation step at 95˚C for 10 min, followed by cycles of denaturationat95˚C for 5 s, and finally annealing and extension at 61˚C for 40s.Subsequently for melting curve analysis, the PCR was continued by further steps including: 95°C for 15 s, 61°C for 15 s, and 95°C for 15 s on an ABI step One™ quantitative PCR system (Applied Biosystems, CA, USA).

Table 1: Material used for RTPCR

Material	Volume	Final Volume
Taq DNA Polymerase Master Mix RED(Amplicon)	5 µL	**Cat.No:180301**
Forward Primer	0.5 µL	**0.05µM**
Reverse Primer	0.5 µL	**0.05µM**
c DNA(1/10 dill)	7.5 µL	**100 ng/µl**
13.5 µL	Total	

Table 2: Reverse and forward primer that used for RTPCR

	Primer sequence	Amplicon length
Forward	5´- AAGATGTCCTACTTAAAGAACTGG -3´	197 bp
Reverse	5´- ACCCGTGTGAAACCATAAGG -3´	

Table 3: Reverse and forward primer that used for Real Time PCR

Gene	Primer pair sequences	Amplicon length
HPRT F	5´-GTG ATT AGC GAT GAT GAA CCA G-3´	125 bp
HPRT R	5´- GCA AGT CTT TCA GTC CTG TCC-3´	
KCNJ11 F	5´-AAGATGTCCTACTTAAAGAACTGG-3´	197bp
KCNJ11 R	5´-ACCCGTGTGAAACCATAAGG-3´	

Statistical analysis and data normalization

Pancreas KCNJ11 gene expression was quantified by normalization against HPRT as reference gene. 2^{-CT} method was employed for performing data analysis. The significant difference for gene expression profile between case and control groups was assayed student t-test. All statistical analyses were carried out by SPSS version 18.P value under 0.05 was considered significant. Statistical tests for serum biochemical analyses were done by Mann-Whitney and Independent-Samples T Test.

RESULTS

Glucose changes in mice were measured at the conclusion of every week. As shown in table 1, the green tea diet could result in decrease weight in the treated group also, compared with controls. By the end of 8th week, mean glucose changes were significant in case and in control subjects.

Pancreas *KCNJ11* gene expression in mice pancreas extract

An important increase in pancreas *KCNJ11* gene expression was observed in of mice which were fed by high green tea diet compared with control mice. Mean changes in gene expression were 1.03±0.28in normal mice and2.61±0.63 (P = 0.03) in control (Fig. 1).

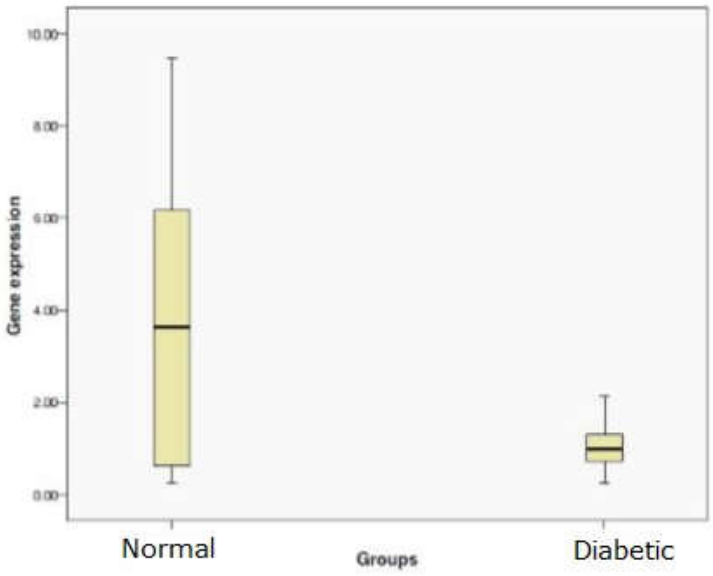

Fig 1: *KCNJ11* gene expression in control and normal mice

DISCUSSION

KCNJ11 gene expression levels may be afflicted with several factors, as an example consequently of corresponding tissues being damaged (e.g. pancreas. Increased levels of KCNJ11 gene expression have previously been correlated with various conditions such as for example as an example psychosocial stress and physical stressors like cold exposure and exercises. Interestingly, there's been reports on higher levels of KCNJ11 gene expression in patients with diabetes disorders .Changes in KCNJ11 gene expression activity have now been observed in response to hormones, and adrenaline decreasing and insulin increasing pancreas activity.

On the basis of the outcomes of the current study, the average degree of pancreas KCNJ11gene expression high-green tea-diet-fed mice was approximately doubled compared with this of normal-diet-fed mice. This increased gene expression was detected at RNA level by real-time PCR and at protein level by Bernfield and ELISA method. Accordingly, KCNJ11 gene expression levels showed significant escalation in diabetes mice compared on track controls. These results declare that increased expression of pancreas KCNJ11 expression might be indicative of initial stages of diabetes and associated with previous studies on its association with diabetes. In summary, detailed investigation of KCNJ11 gene expression activity in a variety of stages of diabetes, with the utilization of larger samples, may further confirm the use of pancreas KCNJ11gene expression as a marker of diabetes.

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