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Potential Genetic Association of ITPR3 SNP rs999943 with Type 2 Diabetes in Kuwait: Case-Control Pilot Study

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ABSTRACT

Background: Prevalence of diabetes has markedly increased over the past three decades in Kuwait with approximately one quarter of its population suffering from Type 2 diabetes (T2D). A combination of genetic and environmental factors impacted by regions socioeconomic status have been widely associated with the onset of the disease. Genetic predisposition to T2D has been proved by monozygotic twin and case control association studies. ITPR3 plays a prominent role in intracellular calcium signalling influencing wide range of cellular and metabolic activities.

Aim: to explore the genetic association and contribution of ITPR3 SNP rs999943 with the development of T2D and related metabolic traits such as body mass index (BMI), plasma glucose (PG), low density lipoprotein (LDL), triglyceride (TGL), high density lipoprotein (HDL), total cholesterol (TC), systolic and diastolic blood pressure (SBP/DBP) in Kuwaiti population.

Method: A subset of 223 Kuwaiti natives (120 T2D and 103 non-diabetic) from a large cohort collected for genome- wide association study on obesity traits were included in this study. Genotyping of ITPR3 A/G SNP rs999413 was carried out using the TaqMan® SNP Genotyping assay on ABI PRISM 7500 Real-Time PCR System. Statistical analysis was carried out using SNPassoc and Genetics software package.

Results: Screening region of interest revealed significant association of rs999943 A/G SNP with susceptibility to T2D. The frequency of the G allele was higher in T2D subjects (0.30) than that in non-diabetic (0.17), with an OR of 2.02 at 95% CI 1.27-3.18 for the G allele. Heterozygous AG genotype conferred susceptibility to T2D with an OR of 2.13 at 95% CI 1.17-3.39. Stratified analysis of rs999943 SNP revealed significant association of -AG genotype with BMI ($p = 0.004$), T.CHOL ($p = 0.0009$), LDL ($p = 0.003$), TGL ($p = 0.005$), AHDL ($p = 0.0042$), SBP ($p = 0.0024$) and DBP ($p = 0.0035$). Individuals carrying rs999413-AG genotype, showed a negative correlation between HDL levels and systolic blood pressure ($r2 = 0.30$ and $p = 0.039$. Likewise, a negative correlation was also observed between HDL levels and diastolic blood pressure ($r2 = 0.31$ and $p = 0.009$) in individuals carrying rs999413-AG and –GG genotypes. Interestingly, a gender based quantitative trait analysis indicates the significance of ITPR3 rs999943 SNP as a strong genetic determinant of type 2 diabetes and its related metabolic trait in Kuwait women. Analysis indicates a very strong association of rs999413 SNP with BMI, total cholesterol, LDL, triglyceride, AHDL, SBP, DBP and cholesterol in females by all three tested models ($p < 0.001$).

Discussion: Our finding indicates significance of rs999413 SNP in T2D among Kuwaiti natives and highlights the gender based overriding effect of rs999413 SNP with extreme metabolic phenotypes, driving towards in-depth population based representative studies.

KEYWORDS

SNP polymorphism, Genetic Association, SNP Genotyping, T2D

INTRODUCTION

Diabetes prevalence in recent years has risen substantially worldwide compromising a global epidemic [1, 2]. Most dominant feature of this rapid increase is the emergence of type 2 diabetes (T2D) that is defined as inflammatory complex disorder triggered by impaired metabolism [3]; which along with its major risk mediators such as obesity, insulin resistance and destruction of β-cell lead to deficiency of insulin secretion [4,5]. Kuwait was marked as one of the prevalent countries of T2D, with approximately one quarter of its population suffering from diabetes over the past three-decades [6]. Prevalence of diabetes in Kuwait was reported to be 15.8% in 2017 (% of population ages 20 to 79) and overall prevalence of 34.9 per 100,000 in children [7]. Genetic association to T2D has been evaluated across different studies worldwide [8]. A genome-wide association studies (GWASs) identified common susceptibility genes for T2D suggested associated with defects in insulin section and thereby risk of T2D [9, 10]. While, ongoing epigenome-wide association studies (EWASs) have recently defined epigenetic markers and their potential role in the pathogenesis of T2D [11-3]. Systematic assessment by GWAS studies for the relationship between genetic variants and disease susceptibility revealed 120 T2D susceptibility genetic loci [14]; among which number of pleiotropic variants were reported as obesity-mediated T2D risk [15]. However, the proportion of overall trait variance explained by these associated loci is modest (~5–10% for T2D, ~2% for body mass index (BMI) [16]. Of which, ITPR3 gene variants rs2229634 and rs2296336 which were reported to be associated with Type1 diabetes (T1D) [17], and ITPR3 gene variant rs999943 was reported associated with extreme obesity in different populations worldwide [18]. Furthermore, GWAS study from Kuwaiti admixture population revealed a possible risk association of rs999943 variant to T2D as well [19]. Therefore, we aimed to investigate the putative role of ITPR3 gene variant rs999943 with T2D in a case-control pilot study setting.

METHODS

Study Participants

A total of 223 Kuwaiti subjects (120 T2D and 103 non-diabetic subjects, ≥ 18 yrs old), were recruited in this observational pilot case-control study conducted at Genetic Clinic at Dasman Diabetes Institute (DDI). Subjects were categorized based on T2D status into 120 patients versus 103 non-diabetic controls, all Kuwaitis. Clinical evaluation for diabetes and obesity common parameters were performed that are, plasma glucose (PG), total cholesterol (T. CHOl), Low density lipoprotein (LDL), Triglyceride (TGL), High density Lipoprotein (HDL), Diastolic blood pressure (DBP) and systolic blood pressure (SBP). The average of 3 BP readings were recorded by a registered nurse. Anthropometric measures such as age, Body Mass Index (BMI), gender was collected. BMI was calculated using the standard formula: body weight (kilograms) /height (meters square). Both patients and controls medical history were thoroughly investigated. Written informed consent was obtained from each participant under the protocols approved by the Ethical Review Committee at DDI. Blood samples were collected from all subjects in EDTA treated and plain tubes. DNA was isolated from peripheral blood using Gentra (Minneapolis, USA) following manufacturer's instructions. Sera and Plasma were stored at -20°C for up to 1 month or at -80°C for long-term storage following standard operating procedures (SOPs).

Sample Genotyping

SNP genotyping of ITPR3 rs999943 SNP was carried out using TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) and ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Each PCR reaction contained 10 ng of genomic DNA, 5x FIREPol® Master mix (Solis BioDyne, Tartu, Estonia, Europe) and 1 µl of 20x TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, USA). Thermal cycle conditions were 60°C for 1 minutes, 95°C for 15 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The genotypes ascribed by Real-time PCR were confirmed by direct sequencing of the PCR products for selected cases of homozygotes and heterozygotes. Sequencing reaction was performed using the BigDyeTM terminator cycle sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommendations on an ABI- PRISM 3130 genetic analyzer (Applied Biosystems, USA).

RESULTS

A total of 223 Kuwaiti subjects (120 cases with diabetes and 103 non-diabetic cases, ≥ 18 yrs old) were tested for the association with T2D and its related metabolic traits. Gender based clinical characteristics differed significantly between case and controls (Table 1). Significant differences in the measures of T. CHOL, LDL, SBP and DBP were observed specifically in males, while females showed significant differences in BMI, T. CHOL, LDL and SBP ($p < 0.05$, Table 1). No significant deviation from HWE was observed for the tested marker ($p > 0.05$). Screening region of interest revealed significant association of rs999943 A/G SNP with susceptibility to T2D. The frequency of the G allele was higher in T2D subjects (0.30) than that in non-diabetic (0.17), with a significant $\chi 2$ of 9.47 ($p = 0.002$) and an OR 2.02 at 95% CI, 1.27-3.18 for the G allele. Similarly, comparison of genotype frequencies between the studied groups showed significant association with both AA ($p = 0.002$, OR = 0.412, 95% CI=0.24 -0.73) and AG genotype ($p = 0.012$, OR = 2.13, 95% CI=1.17-3.388) with T2D. Heterozygous AG genotype confers susceptibility to T2D with an OR of 2.13 (Table 2). We further assessed the association of rs999943 SNP with T2D while adjusting for confounding factors such as age, gender and medication (Table 3). Univariate logistic regression analysis indicates that association improves significantly after step-wise correction for age, gender and medication (Table 3). The rs999943.

SNP also showed significant association with quantitative traits such as BMI, total cholesterol, LDL, triglyceride, AHDL, SBP and DBP in all three models (co-dominant/ dominant/ log additive) with a $p < 0.05$ (Table 4). Association remained significant after correction for covariates such as gender and medication (Table 3). Stratified analysis of rs999943 SNP with metabolic traits revealed significant association of AG genotype with BMI ($p = 0.004$), T.CHOL ($p = 0.0009$), LDL ($p = 0.003$), TGL ($p = 0.005$), AHDL ($p = 0.0042$), SBP ($p = 0.0024$) and DBP ($p = 0.0035$), when individually compared to reference AA genotype. Subjects carrying GG failed to show any significant association with a $p > 0.05$. We also observed significant correlation between ITPR3 rs999943 SNP and blood pressure. Individuals carrying rs999943 AG genotype showed a negative correlation between HDL levels and systolic blood pressure ($r2 = 0.25$ and $p = 0.039$, Figure 1). Likewise, individuals carrying rs999413 AG and GG genotypes, showed a negative correlation between HDL levels and diastolic blood pressure ($r2 = 0.311$ and $p = 0.009$, Figure 2). We further analysed the influence of gender on association of ITPR3 rs999943 SNP with T2D. Both male and female showed significant association with T2D after correction for age and medication by dominant and log additive models; while test for association with co-dominant model failed to show any statistically significant association. Interestingly, gender based quantitative trait analysis indicates the significance of ITPR3 rs999943 SNP as a strong genetic determinant of type 2 diabetes and its related metabolic trait in Kuwait women. Analysis indicates very strong association of Kuwaiti females with BMI, total cholesterol, LDL, triglyceride, AHDL, SBP and DBP by all three models.

DISCUSSION

Knockout studies in mice suggested that type 2 and type 3 inositol 1,4,5-trisphosphate receptors encoded by (ITPR3; formerly IP3R3) gene contains an intracellular calcium channel that play a key role in exocrine secretion underlying energy metabolism and growth [20]. Genetic association studies reported three key SNPs located on chromosome 6 of the ITPR3 gene were found associated with diabetes mellitus and extreme obesity. SNP rs943466 and rs999943 were reported as risk alleles associated with BMI and extreme obesity, respectively [18,21]. While SNP rs4713646 reported as risk allele for type 1 diabetes in the general population [17,22]. In addition to

the previously reported association of ITPR2 with T1D, our results showed that Kuwaitis carrying rs999943-AG genotype showed two-fold increased susceptibility to T2D. Gender based quantitative trait analysis indicates overriding effect of rs999943 SNP with extreme metabolic phenotypes in Arab women, driving towards in-depth population based representative studies. To explain this, we sought to look at the concept of SNPs crosstalk. Recently more accumulative evidence is being reported for a complex crosstalk among genetic variants affecting epigenetic patterns and disease susceptibility [23]. We then examined the location of rs999943 SNP (6:33656956) and found it was adjacent to SNP rs4713646 (6:33638746) associated with T2D. We therefore, speculated that both SNPs might have crosstalk reaction which was reflected on T2D susceptibility. However, these findings open the door for further research on the functional aspects of SNP rs999943 and testing the allele-specific DNA methylation patterns as well as environmental factors for increased risk of T2D in Kuwaiti population.

Statistical Analysis and Power of Calculation

Statistical analysis was carried out using SNPassoc and genetics analysis software. Continuous variables are presented as Mean ± Standard deviation. Comparisons of means between the diabetic and non-diabetic subjects were assessed by the Student t test. The gene variant was further investigated for deviation from the Hardy–Weinberg Equilibrium (HWE). Differences in allele and genotypic frequencies were assessed by Chi-square p value test and odds ratio (OR) and 95% confidence interval (CI) were calculated. Logistic regression analysis was carried out to measure the potential risk factor using individuals homozygous for the non-susceptible allele as the reference. Phenotype–genotype correlations were assessed using the Pearson correlation test. Association analysis of the tested SNP with BMI, T. CHOL, LDL, HDL, SBP and DBP were performed using multivariate logistic regression analysis under co-dominant, dominant and log additive models, after adjustment for confounding factors such as age, gender and medication. A P-value of ≤0.05 was considered as statistically significant. Author's Contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Maisa Kamkar, Sriraman Devarajan, Ajmal Dalwai and Sameer Hassan. DNA Extraction and processing was performed by Aditi Mathur. The first draft of the manuscript was written by Maisa Kamkar and Sameer Hassan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interests to disclose.

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DECLARATION

This study has been conducted following Dasman Diabetes Institute Ethical Committee Rules and Regulations.

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