Biomedicine & Pharmacotherapy 103 (2018) 429–434

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Association of melatonin & *MTNR1B* variants with type 2 diabetes in Gujarat population

Roma Patel[a], Nirali Rathwa[a], Sayantani Pramanik Palit[a], A.V. Ramachandran[b], Rasheedunnisa Begum[a,*]

[a] Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, 390002, Gujarat, India
[b] Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, 390002, Gujarat, India

ARTICLE INFO

Keywords:
Single nucleotide polymorphism
Fasting blood glucose
Lipids

ABSTRACT

Aim/hypothesis: Melatonin is a circadian rhythm regulator and any imbalance in its levels can be related to various metabolic disorders. Melatonin and the genetic variants of Melatonin Receptor 1B (*MTNR1B*) are reported to be associated with Type 2 Diabetes (T2D) susceptibility. The aim of the present study was to investigate i) plasma melatonin levels ii) Single Nucleotide Polymorphisms (SNPs) of *MTNR1B* and iii) Genotype-phenotype correlation analysis in T2D patients.

Methods: Plasma and PBMCs were separated from venous blood of 478 diabetes patients and 502 controls. Genomic DNA was isolated from PBMCs. PCR-RFLP was used for genotyping. Melatonin was estimated from plasma samples by ELISA.

Results: Our study suggests: i) decreased plasma melatonin levels in T2D patients and, ii) association of *MTNR1B* rs10830963 GG genotype with increased Fasting Blood Glucose (FBG).

Conclusion: It can be concluded that reduced titer of melatonin along with altered FBG due to *MTNR1B* genetic variant could act as a potent risk factor towards T2D in Gujarat population.

1. Introduction

A disturbed circadian rhythm is strongly related to Type 2 Diabetes (T2D) and insulin resistance in recent years [1]. Melatonin, a pineal hormone, is known to regulate circadian rhythm and sleep [2]. Melatonin mediates its action through two receptors; MT1 (*MTNR1A*) and MT2 (*MTNR1B*) present in various tissues including pancreatic islets [3,4]. The finding that insulin secretion and plasma melatonin levels are inversely correlated suggests a possible association between melatonin and T2D [5].

It is well-known that T2D is a multifactorial and polygenic metabolic disorder [6]. Substantial variation between different ethnic populations has been reported with regard to the genetic architecture underlying T2D [7,8]. *MTNR1B* (13.16 kb) comprises of two exons, one intron, and 5′- and 3′-flanking regions [9]. Recent studies have identified genetic polymorphisms within *MTNR1B* i.e. rs4753426, rs10830962, and rs10830963 (−1193 C/T, 5′ UTR G/C and intron C/G respectively) to be associated with higher fasting glucose levels, impaired insulin secretion, increased risk of T2D and gestational diabetes in different ethnicities [10,11]. Reduced melatonin levels and an increased melatonin signaling are known to be the risk factors for T2D [12,13].

The aim of this study was to examine whether i) plasma melatonin and polymorphisms in *MTNR1B* (rs4753426 C/T, rs10830962 G/C, and rs10830963 C/G) are associated with T2D in Gujarat population and, ii) the genotype-phenotype correlation of the above-mentioned Single Nucleotide Polymorphisms (SNPs) and plasma melatonin with the metabolic profile are associated with T2D. This is the first genetic association study of *MTNR1B* variants with T2D and metabolic profile in Gujarat population.

2. Materials and methods

2.1. Study subjects

This study was conducted according to the declaration of Helsinki and was approved by the Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao

Abbreviations: MTNR1A, Melatonin Receptor 1A; MTNR1B, Melatonin Receptor 1B; TC, Total Cholesterol; TG, Triglycerides; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; BMI, Body Mass Index; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

* Corresponding author.
E-mail addresses: rasheedunnisab@yahoo.co.in, begum.rasheedunnisa-biochem@msubaroda.ac.in (R. Begum).

https://doi.org/10.1016/j.biopha.2018.04.058
Received 8 January 2018; Received in revised form 7 April 2018; Accepted 9 April 2018

University of Baroda, Vadodara, Gujarat, India (FS/IECHR/2013/1). The importance of the study was explained to all the participants and a written consent was obtained from all diabetes patients and control subjects. The study group included 478 T2D patients (213 males and 265 females) and 502 control subjects (251 males and 251 females). Further, the T2D subjects recruited for the study displayed fasting blood glucose (FBG) levels > 125 mg/dL. BMI (weight kg/height m^2) was calculated by recording height and weight.

2.2. Blood collection, DNA extraction, and lipid profiling

Three ml venous blood was drawn from diabetes patients and ethnically matched control subjects between 8:00 AM to 10:00 AM and collected in K_3EDTA coated tubes (Greiner Bio-One, North America Inc., North Carolina, USA). Plasma was separated and stored at −20 °C for evaluation of lipid profile and assay of melatonin. FBG, Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein (HDL) were estimated by using appropriate commercial kits (Reckon Diagnostics P. Ltd, Vadodara, India). Low-Density Lipoprotein (LDL) was calculated by using Friedewald's (1972) formula. DNA was extracted by phenol-chloroform method and the DNA content and purity were determined spectrophotometrically by 260/280 absorbance ratio. The integrity of DNA was checked electrophoretically on 0.8% agarose gel. The DNA was normalized and stored at 4 °C until further analysis.

2.3. Genotyping of MTNR1B SNPs by PCR-RFLP

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to genotype the three *MTNR1B* polymorphisms. The primers used for genotyping are mentioned in Table S1. The reaction mixture (20 µL) included 3.0 µL (150 ng) of genomic DNA, 11.0 µL nuclease-free water, 2.0 µL 10X PCR buffer, 2.0 µL 25 mM dNTPs (Puregene, Genetix Biotech), 1.0 µL of 10 mM corresponding forward and reverse primers (Eurofins, Bangalore, India), and 0.2 µL (5 U/µL) Taq Polymerase (Puregene, Genetix Biotech). DNA amplification was performed using an Eppendorf Mastercycler gradient (USA Scientific, Inc., Florida, USA). The protocol followed was: initial denaturation at 95 °C for 10 min. followed by 39 cycles of 95 °C for 30 s. (denaturation), primer-dependent annealing (Table S1) for 30 s., extension at 72 °C for 30 s and the final extension at 72 °C for 10 min. 5 µL of the amplified product was checked by electrophoresis on a 2% agarose gel stained with ethidium bromide. Details of the restriction enzymes (Fermentas, Thermo Fisher Scientific Inc., USA) and digested products are mentioned in Table S1. 15 µl of the amplified product was digested with 1U of the corresponding restriction enzyme in a total reaction volume of 20 µl as per the manufacturer's instruction. The digestion products with 50 base pair DNA ladder (Genei Bangalore, India) were resolved on 3.5% agarose gels or 15% polyacrylamide gels stained with ethidium bromide and visualized under UV transilluminator. More than 10% of the samples were randomly selected for confirmation and the results were 100% concordant (analysis of the chosen samples was repeated by two researchers independently) and, further confirmed by sequencing.

2.4. Estimation of plasma melatonin

Plasma levels of melatonin in age and sex-matched patients and controls were measured using human melatonin ELISA Kit (Glory Science Co., Ltd, TX, USA) as per the manufacturer's protocol. The melatonin levels were estimated in the study subjects between the ages of 35–50 years only as the melatonin levels are known to decrease significantly after the age of 50 years.

2.5. Statistical analyses

Evaluation of the Hardy-Weinberg equilibrium (HWE) was performed for all the polymorphisms in patients and controls by comparing the observed and expected frequencies of the genotypes using chi-squared analysis. The distribution of the genotypes and allele frequencies of *MTNR1B* polymorphisms for patients and control subjects were compared using the chi-squared test with 2 × 2 contingency tables respectively using Prism 5 software (GraphPad software Inc; San Diego CA, USA). P values less than 0.017 for genotype and allele distribution were considered as statistically significant due to Bonferroni's correction for multiple testing. Odds ratio (OR) with respective confidence interval (95% CI) for disease susceptibility was also calculated. Haplotypes and linkage disequilibrium (LD) coefficients D' = D/Dmax and r^2values for the pair of the most common alleles at each site were obtained using http://analysis.bio-x.cn/myAnalysis.php [14]. Plasma melatonin levels in patient and control groups were plotted and analyzed by unpaired t-test using Prism 5 software. Association studies of polymorphisms with other parameters were performed using analysis of variance (ANOVA) and Kruskal-Wallis test while correlation analysis was performed using multiple linear regression and spearman's correlation analysis in Prism 5 software after adjusting confounding variables like age and sex. P values less than 0.05 were considered significant for all the association analysis. The statistical power of detection of the association with the disease at the 0.017 level of significance was determined by using the G* Power software.

3. Results

Clinical parameters differed significantly between controls and patients (Table 1). Patients had a significantly higher FBG ($p < 0.0001$). Moreover, obesity factors like BMI, TC, and TG were significantly elevated ($p < 0.0001$, $p = 0.0420$, $p = 0.001$ respectively) while HDL was significantly decreased ($p < 0.0001$) in patients as compared to controls. However, LDL did not differ in the study groups ($p = 0.9322$).

3.1. Association of MTNR1B polymorphisms with T2D

The genotype and allele frequencies of the investigated *MTNR1B* polymorphisms (rs4753426 C/T, rs10830962 G/C, and rs10830963 C/G) are summarized in Table 2 while the representative gel images for PCR-RFLP analysis of *MTNR1B* polymorphisms are shown in Fig. S1. The distribution of genotype frequencies for all the polymorphisms investigated was consistent with Hardy-Weinberg expectations in both patient and control groups ($p > 0.05$).

Furthermore, genotype and allelic frequencies of *MTNR1B* polymorphisms were found to be statistically indifferent ($p > 0.017$) with

Table 1
Baseline characteristics of diabetes patients and controls from Gujarat population.

	Controls (Mean ± SD)	Patients (Mean ± SD)	P value
	(n = 502)	(n = 478)	
Age	39.64 ± 16.35 yr	55.99 ± 10.42 yr	–
Sex: Male	251(50)%	213 (44.5%)	–
Female	251 (50%)	265 (55.5%)	–
Fasting blood glucose (mg/dL)	100.1 ± 7.32	155.3 ± 62.09	< **0.0001**
BMI (Kg/m^2)	24.24 ± 5.2	27.04 ± 5.1	< **0.0001**
Total Cholesterol (mg/dL)	160.9 ± 42.2	166.2 ± 39.68	**0.0420**
Triglycerides (mg/dL)	111.7 ± 60.90	164.5 ± 111.1	< **0.001**
HDL (mg/dL)	42.79 ± 15.94	38.2 ± 12.6	< **0.0001**
LDL (mg/dL)	95.32 ± 41.79	95.10 ± 37.52	0.9322
Onset age (Years)	NA	50.65 ± 10.10	–
Duration of disease (Years)	NA	.06 ± 7.3	–
Family history	NA	64 (14%)	–

Data are presented as Mean ± SD. Statistical significance was considered at $p < 0.05$.