Contents available at ScienceDirect

Diabetes Research and Clinical Practice

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

International Diabetes Federation

Serotonin receptor 3B polymorphisms are associated with type 2 diabetes: The Korean Genome and Epidemiology Study

Yu-Jin Kwon [a,b,1], Kyung-Won Hong [c,1], Byung Jin Park [a], Dong-Hyuk Jung [a,*]

[a] Department of Family Medicine, Yonsei University College of Medicine, Yong-in Severance Hospital, Gyeonggi-do, Republic of Korea
[b] Department of Medicine, Graduate School of Yonsei University College of Medicine, Seoul, Republic of Korea
[c] TheragenEtex BioInstitue Co., Ltd., Suwon, Gyeonggi-do, Republic of Korea

ARTICLE INFO

Article history:
Received 10 April 2019
Received in revised form
13 May 2019
Accepted 23 May 2019
Available online 29 May 2019

Keywords:
Type 2 diabetes
Serotonin
5-hydroxytryptamine receptor 3B
Polymorphism

ABSTRACT

Aims: Serotonin, or 5-hydroxytryptamine (5-HT), and serotonin receptor (HTR) subtypes contribute to controlling energy homeostasis. We investigated the association of polymorphisms of serotonin related genes with type 2 diabetes in Korean adults using a community-based prospective cohort study.

Methods: A total of 8840 participants (4205 Ansung, 4635 Ansan) from the Korean Genome and Epidemiology Study (KoGES)-Ansan and Ansung were included. The mean follow-up duration was 7.6 years, and the Ansan and Ansung cohorts were treated as independent replicates. Individuals with existing and new-onset type 2 diabetes were identified at baseline and follow-up evaluations, respectively. Logistic regression analysis was used to evaluate the association of 3402 single nucleotide polymorphisms (SNPs) in serotonin related genes with type 2 diabetes after adjusting for baseline age, sex, body mass index, drinking status, and smoking status.

Results: The baseline case-control comparison revealed significant association of 26 SNPs in HTR3B and HTR2A with type 2 diabetes. Interestingly, HTR3B SNP rs1176744, which is involved in behavioral disorders, was associated with type 2 diabetes (p-value = 0.0002). Furthermore, HTR3B polymorphisms that significantly associated with type 2 diabetes were located in the 3′ downstream region. The new-onset type 2 diabetes case-control study revealed significant association of 3 additional SNPs of the HTR4.

Conclusions: We found that rs1176744 in HTR3B was associated with type 2 diabetes. Additionally, our study suggests that polymorphisms in the downstream region of HTR3B may contribute to the development of type 2 diabetes.

* Corresponding author at: Department of Family Medicine, Yonsei University College of Medicine, Yong-in Severance Hospital, 225 Geumhakno, Cheoin-gu, Yongin-si, Gyeonggi-do 17046, Republic of Korea.
E-mail address: balsan2@yuhs.ac (D.-H. Jung).

[1] Y.-J. Kwon and K.-W. Hong are co-first authors who equally contributed to this work.

https://doi.org/10.1016/j.diabres.2019.05.032
0168-8227/

1. Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), functions in both the central nervous system (CNS) and peripheral nervous system (PNS) [1]. 5-HT is derived from the dietary amino acid L-tryptophan through the action of tryptophan 5-hydroxylase (TPH) and aromatic amino acid decarboxylase (DDC) [2]. Once 5-HT is released, signaling is initiated by 5-HT receptor (HTR) binding and is terminated by 5-HT transporters (SERT and SLC6A4) [1]. Because peripheral 5-HT cannot pass the blood-brain barrier, there are physiologically distinct CNS and PNS pools of 5-HT [3]. Centrally, 5-HT is synthesized by raphe nuclei in the hindbrain and acts as a neurotransmitter to regulate neuropsychological processes (i.e., mood, food intake, and sleep-awake cycle) [4]. Almost 90% of 5-HT is produced outside the CNS, primarily in the enterochromaffin cells of the gut and other peripheral organs such as liver, muscle, adipose tissue, and pancreas [5]. Both central and peripheral 5-HT play important roles in controlling energy homeostasis.

Type 2 diabetes and its complications have contributed greatly to mortality and disability worldwide [6]. In 2017, the International Diabetes Federation (IDF) estimated that 451 million adults aged 20–79 years had diabetes mellitus globally [7]. This estimate is projected to rise to 693 million by 2045 [7]. There are multiple reasons for the increasing incidence of diabetes including not only ageing, urbanization, and sedentary lifestyles but also genetic factors [7]. Type 2 diabetes is a metabolic disorder characterized by relative insulin deficiency caused by pancreatic β-cell dysfunction and insulin resistance [8]. A growing body of evidence suggests that 5-HT may be an important regulator of β-cell proliferation and insulin secretion [9,10]. Although several HTR polymorphisms are known to be associated with metabolic disorders [11,12], there is still little evidence to support an association between serotonin, HTR, and type 2 diabetes. To our knowledge, no prospective community-based cohort study has been conducted to examine the effects of serotonin related gene polymorphisms on new-onset type 2 diabetes. Genome-wide association studies (GWAS) using the data from the Korean Genome Epidemiology Study (KoGES) have investigated the genetic variants and susceptibility of type 2 diabetes in Korean adults [13,14]. Traditionally, GWAS report only genes with high p-values ($<5 \times 10^{-8}$); however, genes with lower p-values may still functionally influence diabetes. Therefore, the purpose of this study was to identify serotonin related genes that are important factors in insulin secretion and the incidence of type 2 diabetes. Additionally, we investigated the impact of polymorphisms of serotonin related gene on type 2 diabetes and new-onset type 2 diabetes in Korean adults using a community-based prospective cohort study.

2. Research design and methods

2.1. Study population

This study used data from the KoGES-Ansan and Ansung conducted by the National Research Institute of Health (NIH), Centers for Disease Control and Prevention, and the Ministry of Health and Welfare of Korea. The KoGES aims to investigate the genetic and environmental etiology of non-communicable chronic diseases such as type 2 diabetes, obesity, and cardiovascular diseases. A total of 10,030 participants between the ages of 40 and 69 years were recruited through two population-based prospective cohort studies conducted in the Ansung (n = 5018) and Ansan (n = 5020) regions of South Korea. Of the 10,030 participants, genotype data was available for 8840 participants. After excluding participants with a missing value (n = 147), the number of participants included in this study totaled 8693. The KoGES is ongoing, with biannual repeated surveys beginning in 2001–2002. Data from the baseline study to the fifth examination, in 2011–2012, were used in the current study. Detailed information about the KoGES is described in a previous report [15]. This study was approved by the institutional review boards of Yong-In Severance Hospital (IRB No: 9-2016-0020).

2.2. Data collection

Baseline and follow-up recruitment and assessment were conducted after obtaining written informed consent. Study participants were interviewed by trained medical staff and underwent physical examination according to standard protocol [15]. Smoking status was classified into four categories: non-smokers, ex-smokers (those who stopped smoking at least 1 month prior to survey), light smokers (those who sometimes smoked cigarettes), and heavy smokers (those who smoked cigarettes habitually). Drinking status was classified into three groups: non-drinkers (those who drink less than 12 times a year, where one drink does not exceed one cup), ex-drinkers, and current drinkers. At baseline survey, the level of physical activity was not assessed. Anthropometric measurements were collected while participants were wearing light clothing and bare footed. Weight and height measurements were rounded to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood samples were drawn after overnight fasting, and venous blood for fasting and oral glucose tolerance test (OGTT) was collected in a plain tube. Biochemical parameters, including fasting serum glucose, hemoglobin A1c (HbA1c), OGTT 60 min, and 120 min serum glucose, were measured with an autoanalyzer (ADVIA 1650, Siemens, Tarrytown. NY, USA).

2.3. Genotypes

The genotypes were obtained from the Korea National Institute of Health, and the detailed experiments and quality control parameters have been previously described [16]. Briefly, the single nucleotide polymorphism (SNP) genotypes of participants were extracted from the Affymetrix 5.0 SNP microarray. SNPs with a missing genotype call rate >0.1, minor allele frequency <0.01, or Hardy-Weinberg equilibrium (HWE) $p < 1 \times 10^{-6}$ were excluded. Ultimately, 333,651 experimen-

tally determined SNPs and 1,000,000 SNPs computationally imputed based on the 1000 Genome haplotype phase of the Asian panel or the Korean HapMap were used in this study [17,18]. Among the 333,651 SNPs, we focused on 19 serotonin related gene regions outlined in Table 2. These genes included 16 HTR genes, two TPH genes, and one serotonin transporter (SLC6A4) gene. The number of SNPs in these gene regions totaled 3402, and each gene region contained between 43 and 630 SNPs.

2.4. *Assessment of type 2 diabetes and new-onset type 2 diabetes*

Individuals were considered to have type 2 diabetes when any of the following criteria were met [19]: (1) fasting blood glucose $\geq$ 126 mg/dl, (2) HbA1c $\geq$ 6.5% (48 mmol/mol), (3) 2 h plasma glucose level of 200 mg/dl during a 75 g OGTT, (4) currently taking an anti-diabetic drug or insulin, or (5) diagnosed by a physician. Participants who met these type 2 diabetes criteria during follow-up evaluations were considered to have new-onset type 2 diabetes.

2.5. *Statistical analysis*

Here, we tested two case-control studies: "baseline type 2 diabetes and baseline control" and "new-onset type 2 diabetes and follow-up control" (Table 1). The Ansan and Ansung cohorts were treated as independent replicates. Using the 3402 SNPs in serotonin related gene regions, the effect of genotype was determined by logistic regression analysis between baseline type 2 diabetes cases and controls after adjusting for baseline age, sex, BMI, drinking status, and smoking status in the Ansung cohort, Ansan cohort, and total population. The effect of genotype was also determined by logistic regression analysis between new-onset type 2 diabetes cases and follow-up controls after adjusting for age (onset age for diabetes cases and age at final follow-up for controls), sex, BMI, drinking status, and smoking status. PLINK (ver. 1.07) was used for all statistical tests [20]. All tests were based on an adjusted model. Significant associations were defined as a p-value < 0.05 in both the Ansung and Ansan cohorts and a p-value < 0.01 in the total population.

3. Results

3.1. *Baseline characteristics of the study population*

We analyzed the genetic association between serotonin related genes and type 2 diabetes patients in two large cohorts. The participants of this study included 4205 individuals of the rural community-based Ansung cohort and 4635 individuals of the urban community-based Ansan cohort in the Gyeonggi-do province of South Korea. The mean follow-up duration of the current study was 7.6 years. The characteristics of the study population are detailed in Table 1. The proportion of men was 43.0% in the Ansung cohort and 51.0% in the Ansan cohort. At baseline, there were 731 (17.9%) individuals with type 2 diabetes in the Ansung cohort and 694 (15.0%) individuals with type 2 diabetes in the Ansan cohort. During follow-up, 460 (14.7%) and 547 (16.0%) new-onset type 2 diabetes cases developed in the Ansung and Ansan cohorts, respectively. The mean age ± standard deviation (SD) was 55.7 ± 8.7 in the Ansung cohort and 49.1 ± 7.6 in the Ansan

Table 1 – Baseline characteristics.

Variables	Ansung	Ansan	Total
Number	4205	4635	8840
Sex			
Male, n (%)	1809 (43.0)	2373 (51.0)	4182 (47.0)
Female, n (%)	2396 (57.0)	2232 (49.0)	4658 (53.0)
Age	55.7 ± 8.7	49.1 ± 7.6	52.2 ± 8.9
Body mass index (kg/m^2)	24.5 ± 3.3	24.7 ± 3.0	24.6 ± 3.1
Drink			
Never, n (%)	2068 (50.0)	1995 (43.1)	4062 (46.4)
Past, n (%)	320 (7.7)	243 (5.3)	563 (6.4)
Current, n (%)	1749 (42.3)	2388 (51.6)	4137 (47.2)
Smoking status			
Never, n (%)	2493 (60.7)	2650 (57.4)	5143 (58.4)
Past, n (%)	512 (12.5)	842 (18.2)	1435 (16.3)
Light, n (%)	153 (3.7)	103 (2.2)	256 (2.9)
Heavy, n (%)	950 (23.1)	1019 (22.1)	1969 (22.4)
Baseline diabetes			
Yes, n (%)	731 (17.9)	694 (15.0)	1425 (16.4)
No, n (%)	3349 (82.1)	3918 (85.0)	7267 (73.6)
New onset diabetes			
Yes, n (%)	460 (14.7)	547 (16.0)	1007 (15.4)
No, n (%)	2668 (85.3)	2878 (84.0)	5546 (84.6)

Table 2 – Target genes for the association analysis of serotonin related genes.

Genes	Description	Chromosome	Position (hg19)		Analysis Region (hg19) Position ± 10 kbp		Origin + Impute
			Start	End	Start	End	SNP No.
HTR1A	5-hydroxytryptamine receptor 1A	5	63,255,875	63,258,119	63,245,875	63,268,119	54
HTR1B	5-hydroxytryptamine receptor 1B	6	78,150,565	78,173,739	78,140,565	78,183,739	93
HTR1D	5-hydroxytryptamine receptor 1D	1	23,518,388	23,521,222	23,508,388	23,531,222	75
HTR1E	5-hydroxytryptamine receptor 1E	6	87,647,024	87,726,397	87,637,024	87,736,397	264
HTR1F	5-hydroxytryptamine receptor 1F	3	87,841,917	88,042,981	87,831,917	88,052,981	630
HTR2A	5-hydroxytryptamine receptor 2A	13	47,405,677	47,471,211	47,395,677	47,481,211	224
HTR2B	5-hydroxytryptamine receptor 2B	2	231,972,947	231,989,824	231,962,947	231,999,824	43
HTR3A	5-hydroxytryptamine receptor 3A	11	113,845,797	113,861,034	113,835,797	113,871,034	59
HTR3B	5-hydroxytryptamine receptor 3B	11	113,775,518	113,817,283	113,765,518	113,827,283	118
HTR3C	5-hydroxytryptamine receptor 3C	3	183,770,835	183,778,461	183,760,835	183,788,461	94
HTR3D	5-hydroxytryptamine receptor 3D	3	183,749,332	183,757,157	183,739,332	183,767,157	112
HTR3E	5-hydroxytryptamine receptor 3E	3	183,812,906	183,822,966	183,802,906	183,832,966	81
HTR4	5-hydroxytryptamine receptor 4	5	147,830,595	148,016,624	147,820,595	148,026,624	462
HTR5A	5-hydroxytryptamine receptor 5A	7	154,862,034	154,879,102	154,852,034	154,889,102	151
HTR6	5-hydroxytryptamine receptor 6	1	19,991,780	20,007,459	19,981,780	20,017,459	100
HTR7	5-hydroxytryptamine receptor 7	10	92,500,576	92,617,671	92,490,576	92,627,671	311
TPH1	Tryptophan hydroxylase 1	11	18,042,084	18,062,335	18,032,084	18,072,335	83
TPH2	Tryptophan hydroxylase 2	12	72,332,626	72,426,221	72,322,626	72,436,221	388
SLC6A4	Solute carrier family 6 member 4	17	28,521,337	28,562,986	28,511,337	28,572,986	60

Table 3 – Adjusted association of selected SNPs for type 2 diabetes.

							Combined results					Ansung					Ansan				
Gene	Chr	SNP	BP	A1	A2	MAF	Total	OR	L95	U95	P	Total	OR	L95	U95	P	Total	OR	L95	U95	P
Baseline DM																					
HTR3B	11	rs4938057	113,789,292	A	T	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	rs1176756	113,789,299	A	G	0.232	8692	1.2	1.091	1.319	2.E−04	4080	1.226	1.075	1.398	2.E−03	4612	1.17	1.019	1.344	3.E−02
HTR3B	11	rs4938058	113,789,380	G	A	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	11:113789384:A_AT	113,789,384	I	R	0.24	8692	1.186	1.079	1.303	4.E−04	4080	1.197	1.05	1.364	7.E−03	4612	1.174	1.024	1.346	2.E−02
HTR3B	11	rs7103572	113,790,099	T	C	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	rs12271442	113,790,240	A	T	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	rs11214769	113,790,668	G	A	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	11:113791109:G_GT	113,791,109	I	R	0.232	8692	1.2	1.091	1.32	2.E−04	4080	1.226	1.075	1.398	2.E−03	4612	1.171	1.02	1.344	3.E−02
HTR3B	11	rs12273321	113,791,278	A	T	0.231	8692	1.194	1.086	1.314	3.E−04	4080	1.216	1.067	1.388	4.E−03	4612	1.17	1.019	1.343	3.E−02
HTR3B	11	rs4020502	113,791,716	C	G	0.231	8692	1.198	1.089	1.318	2.E−04	4080	1.22	1.07	1.391	3.E−03	4612	1.174	1.023	1.347	2.E−02
HTR3B	11	rs12270070	113,799,047	C	G	0.231	8692	1.193	1.084	1.312	3.E−04	4080	1.213	1.064	1.384	4.E−03	4612	1.17	1.019	1.343	3.E−02
HTR3B	11	rs1176744	113,803,028	C	A	0.233	8692	1.201	1.093	1.321	2.E−04	4080	1.227	1.076	1.399	2.E−03	4612	1.172	1.022	1.345	2.E−02
HTR3B	11	rs2276307	113,803,887	G	A	0.232	8692	1.195	1.087	1.314	2.E−04	4080	1.211	1.061	1.381	5.E−03	4612	1.177	1.026	1.351	2.E−02
HTR3B	11	rs2276308	113,803,976	G	C	0.232	8692	1.195	1.087	1.314	2.E−04	4080	1.211	1.061	1.381	5.E−03	4612	1.177	1.026	1.351	2.E−02
HTR3B	11	rs11605098	113,812,819	T	C	0.238	8692	1.202	1.094	1.321	1.E−04	4080	1.228	1.078	1.399	2.E−03	4612	1.174	1.024	1.345	2.E−02
HTR3B	11	rs11605102	113,812,895	A	C	0.232	8692	1.191	1.083	1.311	3.E−04	4080	1.204	1.055	1.374	6.E−03	4612	1.176	1.024	1.35	2.E−02
HTR3B	11	rs4936285	113,817,579	C	T	0.246	8692	1.193	1.087	1.31	2.E−04	4080	1.201	1.055	1.368	6.E−03	4612	1.184	1.036	1.355	1.E−02
HTR3B	11	rs12795805	113,817,680	C	T	0.242	8692	1.202	1.095	1.32	1.E−04	4080	1.221	1.072	1.39	3.E−03	4612	1.182	1.033	1.354	2.E−02
HTR3B	11	rs4936286	113,818,271	C	T	0.246	8692	1.196	1.089	1.313	2.E−04	4080	1.204	1.057	1.371	5.E−03	4612	1.187	1.038	1.357	1.E−02
HTR3B	11	rs4938059	113,818,329	T	C	0.246	8692	1.196	1.089	1.313	2.E−04	4080	1.204	1.057	1.371	5.E−03	4612	1.187	1.038	1.357	1.E−02
HTR3B	11	rs11214780	113,819,452	A	G	0.246	8692	1.196	1.089	1.313	2.E−04	4080	1.204	1.057	1.371	5.E−03	4612	1.187	1.038	1.357	1.E−02
HTR3B	11	rs12291118	113,820,652	T	G	0.246	8692	1.197	1.091	1.314	2.E−04	4080	1.204	1.057	1.371	5.E−03	4612	1.19	1.04	1.36	1.E−02
HTR3B	11	rs1177066	113,822,262	T	G	0.246	8692	1.195	1.089	1.312	2.E−04	4080	1.201	1.055	1.367	6.E−03	4612	1.189	1.04	1.359	1.E−02
HTR2A	13	rs2070040	47,467,626	A	G	0.425	8692	1.145	1.053	1.244	1.E−03	4080	1.125	1.001	1.264	5.E−02	4612	1.161	1.031	1.309	1.E−02
HTR2A	13	rs9534510	47,468,309	T	G	0.424	8692	1.15	1.058	1.25	1.E−03	4080	1.125	1.002	1.264	5.E−02	4612	1.172	1.04	1.32	9.E−03
HTR2A	13	rs9534511	47,468,580	T	C	0.425	8692	1.147	1.055	1.246	1.E−03	4080	1.124	1.001	1.263	5.E−02	4612	1.167	1.035	1.314	1.E−02
New onset DM																					
HTR4	5	rs10223307	147,996,087	T	C	0.293	6553	0.839	0.7522	0.935	2.E−03	3128	0.839	0.715	0.984	3.E−02	3425	0.841	0.724	0.9756	2.E−02
HTR4	5	rs7701432	147,998,579	G	T	0.294	6553	0.834	0.7479	0.929	1.E−03	3128	0.826	0.704	0.969	2.E−02	3425	0.843	0.726	0.9774	2.E−02
HTR4	5	rs5028114	148,011,739	T	C	0.291	17,680	0.833	0.747	0.929	1.E−03	3128	0.825	0.703	0.969	2.E−02	3425	0.841	0.725	0.9769	2.E−02

Chr, chromosome; SNP, single nucleotide polymorphism; A1, minor allele; A2, major allele; MAF, minor allele frequency; L95, lower 95% confidence interval; U95, upper 95% confidence interval; P, p-value.

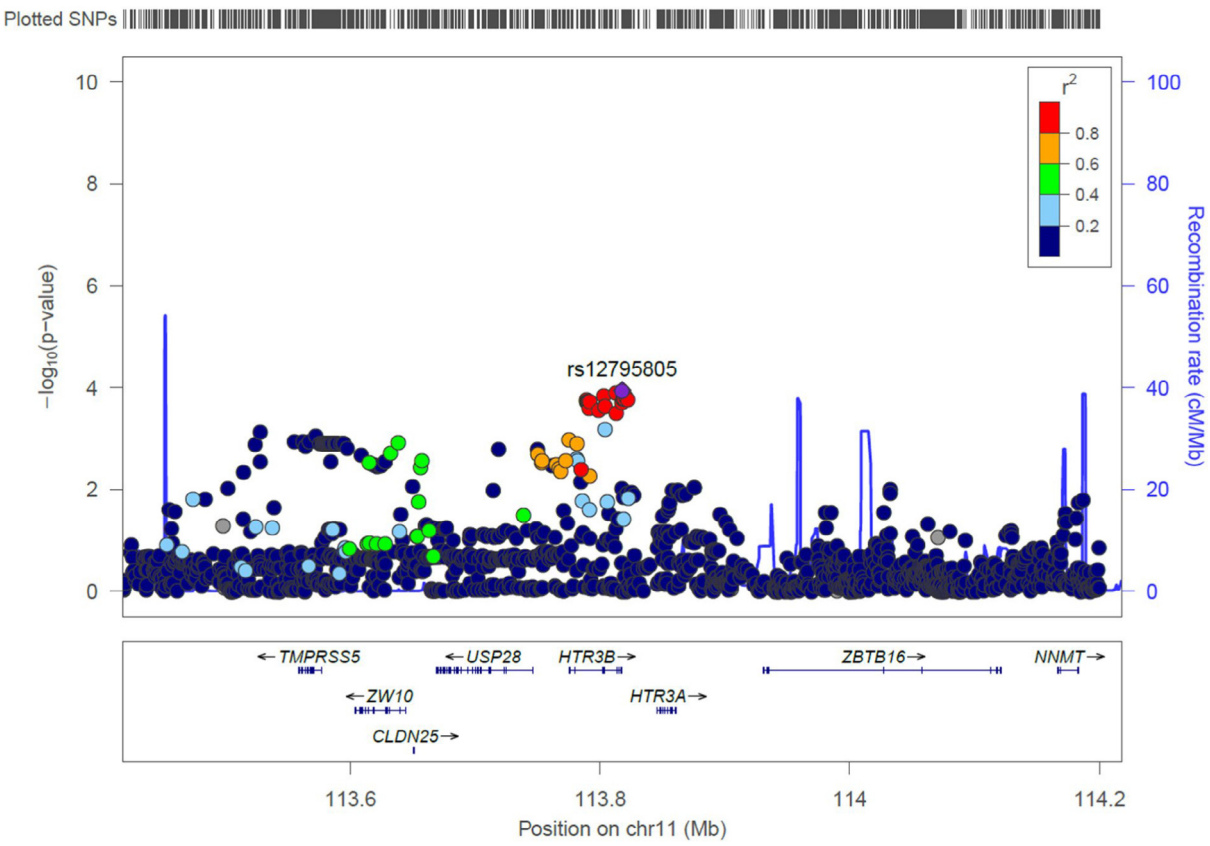

Fig. 1 – Significant SNP associations with baseline type 2 diabetes.

cohort. The mean BMI ± SD was 24.5 ± 3.3 in the Ansung cohort and 24.7 ± 3.0 in the Ansan cohort.

3.2. *Association between selected SNPs and type 2 diabetes*

To test the association between serotonin related genes and type 2 diabetes, we analyzed SNPs in 19 gene regions in Table 2. The resulting significant SNP associations are detailed in Table 3. In the baseline case-control study, we found significant association between type 2 diabetes cases and 26 SNPs in the HTR3B and HTR2A genes. Fig. 1 depicts the signal plot of the SNPs in HTR3B associated with type 2 diabetes. Among these SNPs, rs12795805 resulted in the most significant p-values. Moreover, rs1176744, another HTR3B SNP associated with type 2 diabetes, is known to enhance 5-HT response. Interestingly, in the new-onset type 2 diabetes case-control study, 3 different SNPs in the HTR4 genes were significantly associated with new-onset type 2 diabetes. In silico annotation of the position of the SNPs in the gene regions is described in Table 4. This analysis revealed several interesting findings. First, SNP rs1176744 was located in exon 5 of HTR3B, resulting in a tyrosine/serine substitution in HTR3B. Further, many of the remaining polymorphisms in HTR3B that were significantly associated with type 2 diabetes were located in the 3′ downstream region. The most significant SNP (rs1279805) was also located in the 3′ downstream region. The HTR2A SNPs were all located in intron 2. In new-onset type 2 diabetes, a total of 3 SNPs in HTR4 were located in intron 1.

4. Discussion

This is the first study to show an association of rs1176744 in HTR3B with type 2 diabetes. Additionally, our study suggests that polymorphisms in the downstream region of the HTR3 gene could contribute to the development of type 2 diabetes. Several lines of evidence support these findings. First, many previous studies have shown that serotonin and serotonergic receptor subtypes have a pivotal function in feeding behavior through the CNS motivational circuitry [4]. As a result, polymorphism of serotonin receptors and the impaired activity of related genes contributes to hyperphagia [21] and obesity [11,12], which lead to peripheral insulin resistance, altered glucose homeostasis, and subsequent development of new-onset type 2 diabetes. Further, the rs1176744 polymorphism results in a tyrosine/serine substitution in HTR3B and consequently leads to increased 5-HT response [22]. Moreover, rs1176744 is associated with eating disorders, alcohol dependence, and major depression [21–24]. Serotonin and dopamine signaling has also been suggested as a mechanistic link between rs1176744 and psychiatric disorders [24]. Building upon this existing evidence, we now show significant

Table 4 – In silico annotation of the significant SNPs.

Gene	CHR	SNP	Genome position	HGVS description
Baseline DM				
HTR3B	11	rs4938057	intron 2	NM_006028.4:c.213 + 9115 T > A
HTR3B	11	rs1176756	intron 2	NM_006028.4:c.213 + 9122G > A
HTR3B	11	rs4938058	intron 2	NM_006028.4:c.213 + 9203A > G
HTR3B	11	11:113789384:A_AT	intron 2	NM_006028.4:c.213 + 9207A->AT
HTR3B	11	rs7103572	intron 2	NM_006028.4:c.213 + 9922C > T
HTR3B	11	rs12271442	intron 2	NM_006028.4:c.213 + 10063 T > A
HTR3B	11	rs11214769	intron 2	NM_006028.4:c.213 + 10491A > G
HTR3B	11	11:113791109:G_GT	intron 2	NM_006028.4:c.213 + 10932G->GT
HTR3B	11	rs12273321	intron 2	NM_006028.4:c.214–10828 T > A
HTR3B	11	rs4020502	intron 2	NM_006028.4:c.214-10390G > C
HTR3B	11	rs12270070	intron 2	NM_006028.4:c.214-3059G > A
HTR3B	**11**	**rs1176744**	**Exon 5**	**NM_006028.4:c.386A > C (p.Tyr118Ser)**
HTR3B	11	rs2276307	Intron 6	NM_006028.4:c.696 + 72A > G
HTR3B	11	rs2276308	Intron 6	NM_006028.4:c.696 + 161C > G
HTR3B	11	rs11605098	Intron 6	NM_006028.4:c.697-885C > T
HTR3B	11	rs11605102	Intron 6	NM_006028.4:c.697-809C > A
HTR3B	11	rs4936285	3′ downstream	NM_006028.4:c.*720 T > C
HTR3B	11	rs12795805	3′ downstream	NM_006028.4:c.*821 T > C
HTR3B	11	rs4936286	3′ downstream	XM_017018552.1:c.*1412 T > C
HTR3B	11	rs4938059	3′ downstream	XM_017018552.1:c.*1470C > T
HTR3B	11	rs11214780	3′ downstream	XM_017018552.1:c.*2593G > A
HTR3B	11	rs12291118	3′ downstream	XM_017018552.1:c.*3793G > T
HTR3B	11	rs1177066	3′ downstream	NC_000011.10:g.113951540 T > G
HTR2A	13	rs2070040	Intron 2	NM_000621.4:c.413-901C > T
HTR2A	13	rs9534510	Intron 2	NM_000621.4:c.412 + 1321A > C
HTR2A	13	rs9534511	Intron 2	NM_000621.4:c.412 + 1050A > G
New onset DM				
HTR4	5	rs10223307	Intron 1	NM_000870.6:c.26 + 20465A > G
HTR4	5	rs7701432	Intron 1	NM_000870.6:c.26 + 17973C > A
HTR4	5	rs5028114	Intron 1	NM_000870.6:c.26 + 4813A > G

Chr, chromosome; SNP, single nucleotide polymorphism; HGVS, Human Genome Variation Society.

association of rs1176744 with type 2 diabetes in two community-based Korean cohorts.

A meta-analysis established that adults with depression have a 37% increased risk of type 2 diabetes [25]. Recent studies also reported that major depressive disorder patients who have an impaired serotonergic system were 25% more likely to develop type 2 diabetes irrespective of body mass [26]. Therefore, the increased risk of type 2 diabetes related to serotonin cannot be attributed solely to the effects of increased body weight. On the other hand, the majority of serotonin is produced in peripheral tissues, primarily by the enterochromaffin cells of the gut [5]. Moreover, other cells and organs, such as β-cells of the endocrine pancreas, have their own serotonin metabolic system. This peripheral serotonin system synthesizes, secretes, and responds to cues from the extracellular environment, such as altered glucose, through cell surface receptor subtypes [1]. Based on the roles of peripheral serotonin in energy homeostasis, the serotonergic system in peripheral tissues could contribute to insulin resistance and development of type 2 diabetes.

Due to their role in filtering environmental cues and initiating intracellular signaling, serotonin receptors are promising targets for many diseases.

Unlike other G-protein-coupled HTRs in the serotonergic receptor family, HTR3 subtypes are ligand-gated ion channels [27]. HTR3 channels allow a serotonin-mediated influx of cations that subsequently depolarize resting membrane potential and lower the threshold for glucose induced insulin exocytosis in mice [10]. Although there may be differences between species, insulin secretion is affected by HTR3 in mice. This finding implies that polymorphism of serotonin receptors could affect insulin secretion and glucose metabolism in humans.

In the current study, HTR2A is also associated with type 2 diabetes. Several GWAS have shown that HTR2A polymorphism is closely associated with obesity [28]. Additionally, 5-HT regulates lipogenesis and lipolysis through HTR2A activation. Kitajima and colleagues [29] showed that HTR2A is highly expressed in hypertrophic 3T3-L1 adipocytes. Oh and colleagues [30] also reported that treatment with an HTR2A antagonist reduced lipid accumulation in 3T3-L1 adipocytes. Additionally, they found that 5-HT regulates thermogenesis in brown adipose tissue through HTR3 [30]. Therefore, 5-HT influences energy homeostasis in brown and white adipose tissue via HTR3 and HTR2A.

Notably, polymorphisms of serotonergic genes associated with baseline type 2 diabetes were different from the polymorphisms identified in new-onset type 2 diabetes in this study. In monozygotic twin pairs, only 50% of co-twins develop diabetes, indicating that there are both genetic and non-genetic mechanisms leading to development of diabetes [31]. In addition, environmental factors, such as exercise, diet, and stress, can alter epigenetic states and contribute to gene expression changes irrespective of genotype. For instance, participation in an exercise program for more than 3 months altered DNA methylation and gene expression in skeletal muscle and adipose tissue of type 2 diabetes patients [32,33]. Overall, the interaction between genes, epigenetics, and the environment is complex and influences gene expression. Accordingly, environmental factors and byproducts of metabolism, such as reactive oxygen species and inflammatory cytokines, can influence genetic susceptibility to the development of diabetes, which varies across stages of life. Thus, we hypothesize that the association of serotonergic gene polymorphisms differed between baseline and new-onset type 2 diabetes due to environmental influences on gene expression. Additionally, enrolled participants were adults aged 40–69 years at baseline evaluation. Six follow-up evaluations were then performed every 2 years for up to 12 years. Therefore, individuals with new-onset type 2 diabetes were substantially older than those with type 2 diabetes at baseline (age: 58.4 ± 9.0 for new-onset type 2 diabetes vs. 55.7 ± 9.0 for baseline type 2 diabetes, p-value < 0.001) (Appendix Table A1).

In the current study, HTR4 were significantly associated with new-onset type 2 diabetes. Although little is known about the role of HTR4 in metabolic function, HTR4 has been widely investigated with regard to gastrointestinal motility, secretory, and sensory function [34]. HTR4 antagonist treatment improves irritable bowel syndrome [34]. Further, recent studies found that the gut microbiome plays an important role in the pathophysiology of type 2 diabetes [35].

Our study suggests that polymorphism of serotonin related genes in both the brain and peripheral tissues leads to impaired glucose metabolism driven by changes in serotonin-mediated regulation of energy metabolism and insulin sensitivity. This study has several strengths. First, the study was conducted in a large population-based cohort. Moreover, our findings were produced in two replicate sample cohorts (Ansan and Ansung) to further confirm the association of relevant SNPs with type 2 diabetes. Replication is thought to be the gold standard for reducing false positive errors in genotype–phenotype association studies [36]. Second, it is difficult to investigate the direct role of serotonin in type 2 diabetes in humans due to the inaccessibility of the serotonin system. We were able to identify potential effects of the serotonin system on development of type 2 diabetes through this genetic study. Finally, inclusion of the OGTT in the definition criteria for type 2 diabetes reduced the chances of undiagnosed type 2 diabetes cases. Our study also has limitations. First, the study was conducted in a Korean population and, thus, may not be applicable to non-Asian populations. Second, we could not directly investigate the association between serotonergic related genes and β-cell function or insulin sensitivity. Third, the baseline study in KoGES did not contain information on regular exercise. Therefore, we could not consider the exercise as confounding factors.

In summary, we found an association between rs1176744 in HTR3B and type 2 diabetes. Our study also revealed that polymorphisms in the downstream region of the HTR3B gene are associated with type 2 diabetes. Together, these findings suggest that the serotonin pathway plays a role in the development of diabetes. Further experimental studies are necessary to confirm whether polymorphism of genes involved in the serotonergic system result in alterations of the serotonin concentration and/or the number or affinity of HTRs in the CNS and PNS.

Acknowledgements

Data in this study were collected from the Korean Genome and Epidemiology Study (KoGES; 4851-302) conducted by the Korea National Research Institute of Health, Korea Centers for Disease Control and Prevention, and Ministry for Health and Welfare, Republic of Korea.

This study was supported by a 2018 Faculty Research Grant from Yonsei University College of Medicine (6-2018-0090) to Yu-Jin Kwon.

Declaration of interests

The authors declare no potential conflicts of interest.

Funding

None.

Appendix A

See Table A1.

Table A1 – Clinical characteristics of baseline diabetes and new-onset diabetes.

Variables	Baseline DM	New onset DM	P-value
N	1456	1021	
Age	55.7± 8.8	58.4 ± 9.0	<0.001
Sex			0.155
Men	745 (51.2)	552 (54.1)	
Women	711 (48.8)	469 (45.9)	
Area			0.006
Ansung	757 (48.0)	473 (46.3)	
Ansan	699 (52.0)	548 (53.7)	
Baseline BMI	25.5 ± 3.3	25.2 ± 3.2	0.037
Alcohol drinking			0.007
Never, n (%)	674 (46.8)	423 (41.7)	
Past, n (%)	118 (8.2)	70 (6.9)	
Current, n (%)	649 (45.0)	522 (51.4)	
Smoking status			0.392
Never, n (%)	785 (54.8)	526 (52.2)	
Past, n (%)	257 (17.9)	189 (18.8)	
Light, n (%)	54 (3.8)	32 (3.2)	
Heavy, n (%)	336 (23.5)	261 (25.9)	

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.05.032.

REFERENCES

[1] Wyler SC, Lord CC, Lee S, Elmquist JK, Liu C. Serotonergic control of metabolic homeostasis. Front Cell Neurosci 2017;11:277.
[2] Grahame-Smith D. Serotonin (5-hydroxytryptamine, 5-HT). QJM: Int J Med 1988;67:459–66.
[3] Welford RW, Vercauteren M, Trebaul A, Cattaneo C, Eckert D, Garzotti M, et al. Serotonin biosynthesis as a predictive marker of serotonin pharmacodynamics and disease-induced dysregulation. Sci Rep 2016;6:30059.
[4] Muzerelle A, Scotto-Lomassese S, Bernard JF, Soiza-Reilly M, Gaspar P. Conditional anterograde tracing reveals distinct targeting of individual serotonin cell groups (B5–B9) to the forebrain and brainstem. Brain Struct Funct 2016;221:535–61.
[5] Namkung J, Kim H, Park S. Peripheral serotonin: a new player in systemic energy homeostasis. Mol Cells 2015;38:1023–8.
[6] Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1459–544.
[7] Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271–81.
[8] Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017;389:2239–51.
[9] Kim K, Oh CM, Ohara-Imaizumi M, Park S, Namkung J, Yadav VK, et al. Functional role of serotonin in insulin secretion in a diet-induced insulin-resistant state. Endocrinology 2015;156:444–52.
[10] Ohara-Imaizumi M, Kim H, Yoshida M, Fujiwara T, Aoyagi K, Toyofuku Y, et al. Serotonin regulates glucose-stimulated insulin secretion from pancreatic beta cells during pregnancy. Proc Natl Acad Sci USA 2013;110:19420–5.
[11] Kring SI, Werge T, Holst C, Toubro S, Astrup A, Hansen T, et al. Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. PLoS ONE 2009;4 e6696.
[12] Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5-HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia 2000;43:373–6.
[13] Hong KW, Kim SH, Zhang X, Park S. Interactions among the variants of insulin-related genes and nutrients increase the risk of type 2 diabetes. Nutr Res 2018;51:82–92.
[14] Park S, Liu M, Kang S. Alcohol intake interacts with CDKAL1, HHEX and OAS3 genetic variants, associated with insulin secretion, to increase the risk of type 2 diabetes in Korean adults. Alcohol Clin Exp Res 2018. https://doi.org/10.1111/acer.13888.
[15] Kim Y, Han BG. Cohort profile: the Korean genome and epidemiology study (KoGES) consortium. Int J Epidemiol 2017;46 e20.
[16] Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet 2009;41:527–34.
[17] Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56–65.
[18] Abdulla MA, Ahmed I, Assawamakin A, Bhak J, Brahmachari SK, Calacal GC, et al. Mapping human genetic diversity in Asia. Science 2009;326:1541–5.
[19] Association AD. 2. Classification and diagnosis of diabetes. Diabetes care 2015;38:S8–S16.
[20] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
[21] Hammer C, Kapeller J, Endele M, Fischer C, Hebebrand J, Hinney A, et al. Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. Pharmacogenet Genomics 2009;19:790–9.
[22] Walstab J, Hammer C, Bonisch H, Rappold G, Niesler B. Naturally occurring variants in the HTR3B gene significantly alter properties of human heteromeric 5-hydroxytryptamine-3A/B receptors. Pharmacogenet Genomics 2008;18:793–802.
[23] Enoch MA, Gorodetsky E, Hodgkinson C, Roy A, Goldman D. Functional genetic variants that increase synaptic serotonin and 5-HT3 receptor sensitivity predict alcohol and drug dependence. Mol Psychiatry 2011;16:1139–46.
[24] Yamada K, Hattori E, Iwayama Y, Ohnishi T, Ohba H, Toyota T, et al. Distinguishable haplotype blocks in the HTR3A and HTR3B region in the Japanese reveal evidence of association of HTR3B with female major depression. Biol Psychiatry 2006;60:192–201.
[25] Knol MJ, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F. Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. Diabetologia 2006;49:837–45.
[26] Pan A, Lucas M, Sun Q, van Dam RM, Franco OH, Manson JE, et al. Bidirectional association between depression and type 2 diabetes mellitus in women. Arch Intern Med 2010;170:1884–91.
[27] Thompson AJ, Lummis SC. 5-HT3 receptors. Curr Pharm Des 2006;12:3615–30.
[28] Halder I, Muldoon MF, Ferrell RE, Manuck SB. Serotonin receptor 2A (HTR2A) gene polymorphisms are associated with blood pressure, central adiposity, and the metabolic syndrome. Metab Syndr Relat Disord 2007;5:323–30.
[29] Uchida-Kitajima S, Yamauchi T, Takashina Y, Okada-Iwabu M, Iwabu M, Ueki K, et al. 5-Hydroxytryptamine 2A receptor signaling cascade modulates adiponectin and plasminogen activator inhibitor 1 expression in adipose tissue. FEBS Lett 2008;582:3037–44.
[30] Oh CM, Namkung J, Go Y, Shong KE, Kim K, Kim H, et al. Regulation of systemic energy homeostasis by serotonin in adipose tissues. Nat Commun 2015;6:6794.
[31] Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, et al. Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. Diabetologia 2001;44:354–62.
[32] Ronn T, Volkov P, Davegardh C, Dayeh T, Hall E, Olsson AH, et al. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet 2013;9 e1003572.
[33] Lindholm ME, Marabita F, Gomez-Cabrero D, Rundqvist H, Ekstrom TJ, Tegner J, et al. An integrative analysis reveals

coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. Epigenetics 2014;9:1557–69.

[34] Evans BW, Clark WK, Moore DJ, Whorwell PJ. Tegaserod for the treatment of irritable bowel syndrome and chronic constipation. Cochrane Database Syst Rev 2007. https://doi.org/10.1002/14651858.CD003960.pub3.Cd003960.

[35] Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 2014;383:1068–83.

[36] Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, et al. Replicating genotype-phenotype associations. Nature 2007;447:655–60.