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ORIGINAL ARTICLE

Associations between GRM7 polymorphisms and obesity in patients selected for sleeve gastrectomy

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Abstract
Obesity is a worldwide problem in which genetic factors have a prominent role. We have selected two single nucleotide polymorphisms (SNPs) within *glutamate metabotropic receptor 7* (*GRM7*) gene, namely rs6782011 and rs779867 to weigh their association with obesity in an Iranian cohort. The distribution of rs6782011 alleles was significantly different in the obese patients from normal controls ($P < 0.0001$; 434 obese patients vs. 297 normal controls). Distribution of alleles was also measured between sex-based groups of obese patients and controls. We detected remarkable differences between female obese cases and female control subjects ($P < 0.0001$; 374 female obese cases vs. 216 female normal controls); nevertheless, the difference in allele distribution was not significant for male cases compared with corresponding normal controls ($p = 0.47$; 60 male patients vs. 81 normal males). Contrariwise, distribution of rs779867 alleles was not significantly different between total obese patients compared with normal controls ($P = 0.21$; 434 obese patients vs. 297 normal BMI controls). There was also no significant difference for female and male obese patients compared with female and male normal BMI controls. Thus, *GRM7* can be considered as a risk locus for obesity.

Keywords GRM7 · rs6782011 · rs779867 · Obesity

Introduction

Obesity is a worldwide problem with increasing prevalence (Organization 2019). This trait is a multifactorial condition, governed by both genetic and environmental elements and interplay between these two sets of factors. Tens of candidate genes have been found to be associated with obesity (Jiao et al., 2008). In fact, food intake, energy expenditure and adiposeness regulate body weight (Fawcett and Barroso 2010). Genetic factors contribute to obesity through affecting the activity of metabolism-related pathways and modulating neuron pathways and appetite axes. Moreover, genetic factors can affect insulin resistance, lipid metabolism, inflammatory responses, blood pressure, and ectopic fat deposition-particularly in the liver (Sanghera et al., 2019). Genetic obesity is classified to the following subtypes: monogenic obesity, polygenic obesity and syndromic obesity. However, in the most cases, obesity is related to polymorphisms within several loci, each of them having a small contribution to this condition. Genome wide association studies were used to recognize some genetic loci being associated with body weight (Wang et al., 2011). Another

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approach for identification of risk loci for this condition is candidate gene approach. We have designated two single nucleotide polymorphisms (SNPs) in the *glutamate metabotropic receptor 7* (*GRM7*) gene, namely rs6782011 and rs779867 to weigh their association with obesity in an Iranian cohort. This receptor is activated by L-glutamate, a crucial excitatory neurotransmitter in the central nervous system. Glutamatergic neurotransmission contributes to the majority of normal brain functions and is impaired in several neuropathologic conditions. *GRM7* has been among obesity candidate genes that might participate in the etiology of highly penetrant kinds of familial obesity (Serra-Juhé et al., 2017). Pathogenic copy number variants within *GRM7* have been found to be involved in inherited forms of obesity (Serra-Juhé et al., 2017). Besides, co-expression analyses have shown relation between GRM7 expression pattern and expressions of leptin-melanocortin pathway genes, as a pathway that is significantly associated with obesity (Serra-Juhé et al., 2017). The rs6782011 and rs779867 polymorphisms in *GRM7* have been suggested to contribute to the risk of neuropsychiatric conditions (Noroozi et al., 2019). We aimed at identification of the association between these polymorphisms and obesity in Iranian population in order to find putative underlying mechanisms for obesity and identify at-risk persons.

Materials and methods

Obese patients and normal weight subjects

Blood samples were obtained from patients underwent sleeve gastrectomy. The inclusion criteria were: BMI ≥ 40 kg/m2 without simultaneous medical problems, BMI ≥ 35 kg/m2 and 1 or more serious obesity-related conditions (Mancini 2014). Samples were taken from patients referred to Erfan-Niayesh Hospital, Tehran, Iran, during 2021–2022. Controls were healthy individuals with BMI ≤ 25. Informed consent form was obtained from obese patients and healthy subjects. The study procedure was permitted by ethical committee of Shahid Beheshti University of Medical Sciences (Ethical approval code: IR.SBMU.RETECH.REC.1401.23, Approval Date: 2022-07-24).

Table 1 Demographic data of cases and controls

Parameters	Cases	Controls
Male, n	60	81
Female, n	374	216
Female: Male ratio	6.23	2.6
Age, mean ± SE (y)	37.42 ± 22.16	40.58 ± 19.34
BMI	mean ± SE: 41.13 ± 5.88	Range: 18.5–24.9

Genotyping

rs6782011 and rs779867 were genotyped using ARMS-PCR method. PCR program included an initial denaturing at 94 °C for 6 min, 35 cycles at 94 °C for 45 s, specific annealing temperatures (57 °C for rs6782011 and 53 °C for rs779867) for 45 s, extension temperature at 72 °C for 50 s; and a final extension at 72 °C for 10 min. Table S1 shows nucleotide sequences of primer pairs and PCR protocols.

Statistical methods

SPSS v.22.0 (SPSS Inc., Chicago, IL) and SNP Analyzer 2.0 were used for analyses. Chi-square test ($\chi 2$) was used to determine the differences between genotypic and allelic frequencies in the obese cases and normal BMI controls. The comparison was performed for subgroups (male and female obese cases with relevant control subgroups) and for total study groups (total obese cases with total normal BMI controls). Hardy-Weinberg equilibrium analysis for each SNP was calculated by the SNP analyzer 2.0 using chi-square test to determine if observed data is equivalent to expected data. P value > 0.05 was considered to be consistent with Hardy-Weinberg equilibrium. Odds ratios (OR) for effect alleles and genotypes were calculated by logistic regression. Adjusted Odds ratios were calculated with gender as a covariate. Associations between genomic variants and obesity were judged in four models. The result of association analyses was designated as Odds ratios (OR) and 95% confidence interval of OR (95% CI), P-value and FDR adjusted q-values. The FDR adjusted q-values were obtained *via* assessment of a stack of p values in column analysis. P values < 0.05 were considered significant. Haplotypes and linkage disequilibrium (LD) blocking were analyzed by SNP Analyzer 2.0.

Association of haplotypes with risk of obesity was examined using a haplotype-specific test with one degree-of-freedom. D′ and r values were measured for valuation of linkage between rs779867 and rs6782011 variants.

Results

Demographic facts of obese cases and controls are summarized in Table 1.

Figure 1. rs779867 and rs6782011 in the *GRM7* gene. The rs779867 is located in the intron 5 at position 586,670, and the rs6782011 is located in the intron 6, at position 601,847 of the *GRM7* gene on chr 3.

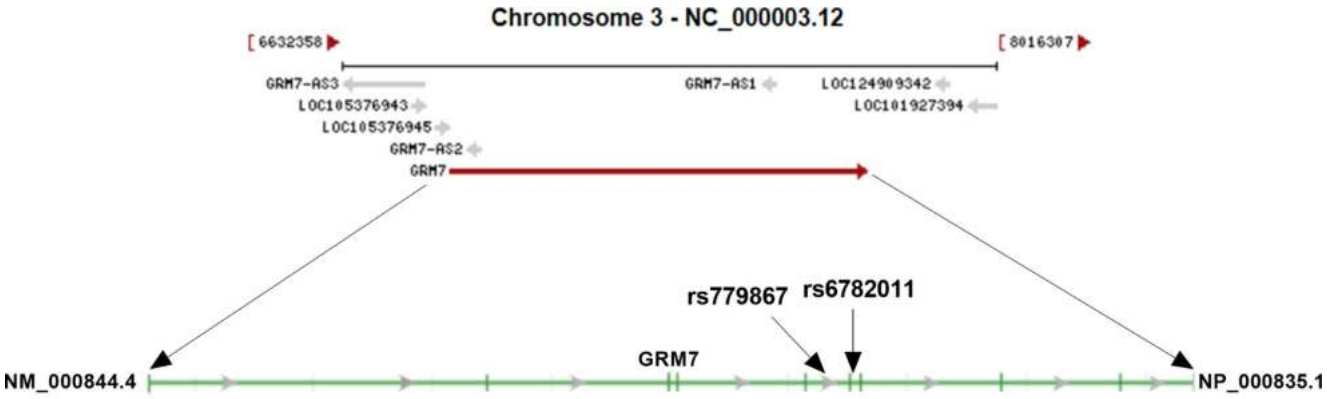

Fig. 1 shows the position of assessed SNPs in the *GRM7* gene

Association studies

The distribution of rs6782011 alleles was significantly different in the obese patients from normal controls ($P < 0.0001$; 434 obese patients vs. 297 normal controls). Distribution of alleles was also assessed at gender level between obese patients and normal controls by chi-square test. There was a significant difference between female obese cases and normal weight females ($P < 0.0001$; 374 female patients vs. 216 female normal controls); nevertheless, the difference in allele distribution was not significant for male cases compared with male normal controls ($p = 0.47$; 60 male patients vs. 81 male normal control). Contrariwise, distribution of rs779867 alleles was not significantly different

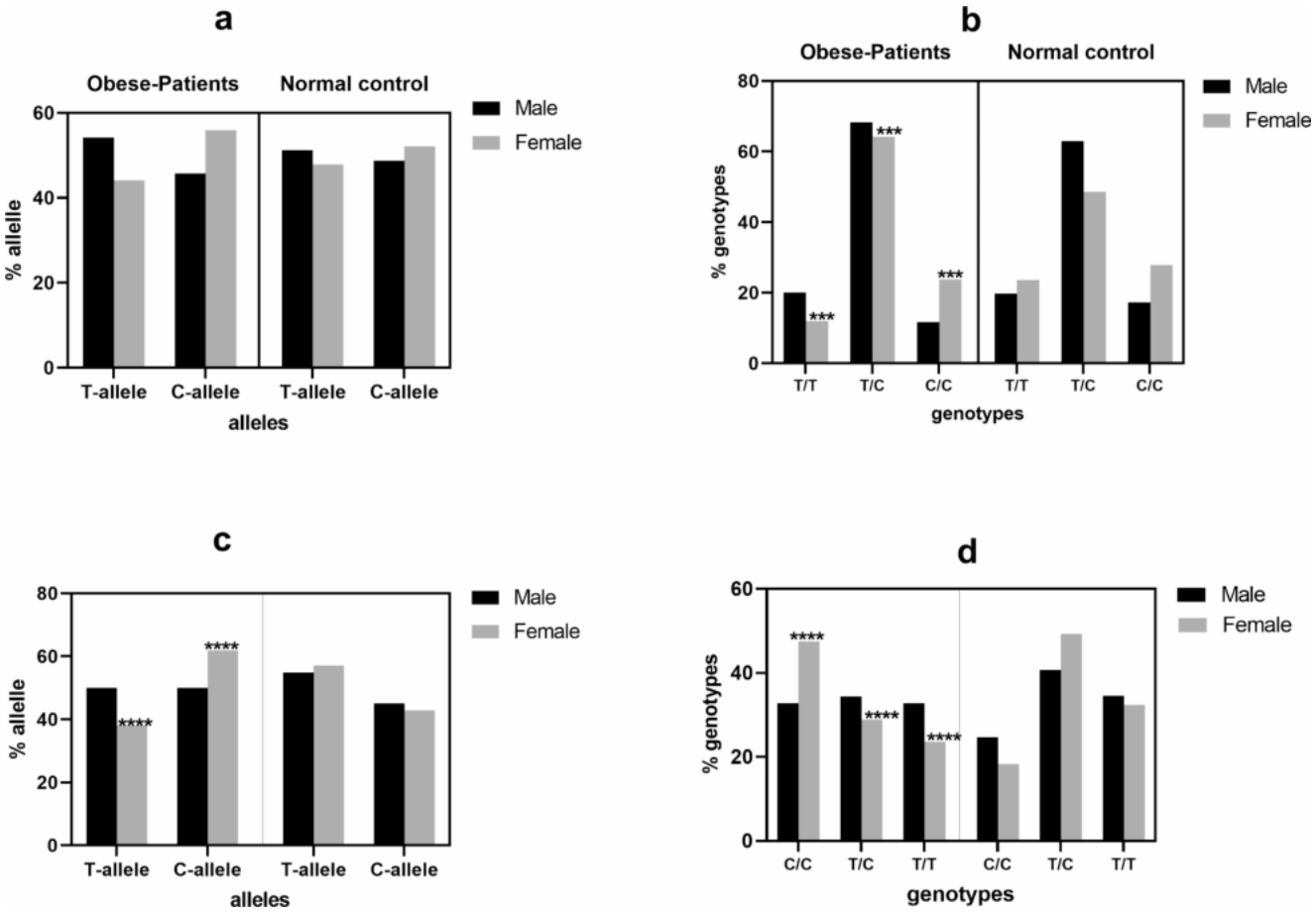

Fig. 2 Alleles and genotypes distribution of rs779867 (**a**, **b**) and rs6782011 (**c**, **d**) variants in the *GRM7* gene between obese patients and normal controls and subgroups at gender level. Significant differences in genotype and allele distribution in study subgroups vs. respective normal controls are shown with stars

Table 2 Genotype and allele frequencies of two *GRM7* SNPs in study groups by gender (n, %)

Locus	Allele	Cases						Controls					
		Allele Frequency	Genotype	Genotype Frequency	Observed Count	Expected Count	Chi Square P value	Allele Frequency	Genotype	Genotype Frequency	Observed Count	Expected Count	Chi Square P value
rs779867	C	0.545	CC	0.221	96	128.9	40.049	0.512	CC	0.249	74	77.9	0.77
	T	0.455	CT	0.647	281	215.2	**< 0.0001**	0.488	CT	0.525	156	148.4	0.37
			TT	0.131	57	89.8			TT	0.226	67	70.7	
rs6782011	C	0.603	CC	0.455	199	159.3	63.32	0.435	CC	0.2	60	56.8	0.57
	T	0.397	CT	0.297	130	209.7	**< 0.0001**	0.565	CT	0.47	141	147.5	0.44
			TT	0.249	109	69			TT	0.33	99	95.8	

The wild alleles for rs779867 and rs6782011 are T and C, respectively; yet, the allele T was the minor allele for both SNPs in this study (total population) and was considered as effect allele

T/T, homozygous reference; T/C, heterozygous and C/C, homozygous mutant for rs779867 SNP (based on SNP database)

C/C homozygous reference; T/C, heterozygous and T/T, homozygous mutant for rs6782011 SNPs (based on SNP database)

The Hardy-Weinberg equilibrium analysis for each SNP was calculated by the SNP analyzer 2.0 using chi-square test. P value > 0.05 was considered to be consistent with Hardy-Weinberg equilibrium

between total obese cases compared with normal controls (P = 0.21; 434 obese patients vs. 297 normal BMI controls). There was also no significant difference for female and male obese patients compared with female and male normal BMI controls.

Also, total distribution of rs779867 and rs6782011 genotypes in the obese patients significantly differed from normal controls (P = 0.001 and P < 0.0001, respectively). There were substantial differences between female obese patients and female normal BMI controls for both rs779867 and rs6782011 genotypes (P = 0.00013 and P < 0.0001, respectively; 374 female patients vs. 216 female normal controls); however, the genotype distribution for rs779867 and rs6782011 was not significantly different between male obese patients and normal males (P = 0.64 and P < 0.54, respectively; 60 male patients vs. 81 male normal controls).

Alleles and genotypes distribution is presented in Table 2; Fig. 2.

Distribution of genotypes was in conformity with Hardy-Weinberg equilibrium in controls, but not in cases (Table 3).

The rs6782011 was associated with obesity in allelic model (T vs. C: OR (95% CI) = 0.5 (0.41–0.62), P value < 0.0001). Moreover, this SNP was associated with the mentioned trait in all assessed inheritance models, including co-dominant (for both TT vs. CC and TC vs. CC comparisons), dominant (TT + TC vs. CC), recessive (TT vs. TC + CC) and over-dominant (TT + CC vs. TC) models. Based on these comparisons, the C allele and the CC genotypes conferred risk of obesity (Table 4). In over-dominant model, the OR (95% CI) for TT + CC vs. CT genotypes was 2.1 (1.54–2.85).

The rs779867 was associated with obesity in recessive and over-dominant models. In recessive model, the OR (95% CI) for TT vs. TC + CC genotypes was 0.52 (0.35–0.76) (P value = 0.001). In over-dominant model, the OR (95% CI) for TT + CC vs. TC was 0.6 (0.44–0.81) (P value = 0.001).

Figure 3 shows the results of comparisons in allelic models.

Figure 4 shows the result of risk associations for rs6782011 and rs779867 genotypes in dominant, recessive and over-dominant models.

D and r values showed that two polymorphisms within *GRM7* gene were not in the linkage disequilibrium (LD) in total study population. These SNPs demonstrated D' = 0.02, r2 = 0.0004 (P value = 0.44).

The CC haplotype conferred risk of obesity (OR (95% CI) = 2.41 (1.83–3.18), P < 0.0001). On the other hand, TC and TT haplotypes had protective effects against obesity (OR (95% CI) = 0.6 (0.47–0.75) and 0.66 (0.5–0.87), P values < 0.0001 and = 0.004, respectively) (Table 5).

Table 3 Assessment of Hardy-Weinberg equilibrium (P values and genotypes distribution are presented)

	rs779867				rs6782011			
	CC	TC	TT	Hardy-Weinberg P-value	CC	TC	TT	Hardy-Weinberg P-value
Obese group	96	281	57	< 0.00001	199	130	109	< 0.00001
Normal controls	74	156	67	0.37	60	141	99	0.44

Table 4 Association of rs779867 and rs6782011 variants with obesity in different models (allelic and genotypes). Unadjusted odds ratios (plus confidence intervals) and adjusted odds ratios by gender are reported for effect alleles and genotypes

ID	Models	OR (95% CI) (1)	p-Value (1)	FDR q-Value (1)	OR (95% CI) (2)	p-Value (2)	FDR q-Value (2)
rs6782011	Allele model T vs. C	0.5 (0.41–0.62)	< 0.0001	< 0.0001	0.51 (0.42–0.64)	< 0.0001	< 0.0001
	Co-dominant TT vs. CC	0.33 (0.22–0.49)	< 0.0001	< 0.0001	0.35 (0.23–0.52)	< 0.0001	< 0.0001
	TC vs. CC	0.27 (0.19–0.4)	< 0.0001	< 0.0001	0.28 (0.19–0.41)	< 0.0001	< 0.0001
	Dominant TT + TC vs. CC	(0.22–0.43)0.3	< 0.0001	< 0.0001	0.3 (0.21–0.43)	< 0.0001	< 0.0001
	Recessive TT vs. TC + CC	0.67 (0.48–0.92)	0.016	0.016	0.69 (0.5–0.96)	0.032	0.033
	Over dominant TT + CC vs. TC	2.1 (1.54–2.85)	< 0.0001	< 0.0001	2.11 (1.55–2.88)	< 0.0001	< 0.0001
rs779867	Allele T vs. C	0.87 (0.71–1.08)	0.21	0.176400	0.9 (0.73–1.12)	0.36	0.189000
	Co-dominant TT vs. CC	0.65 (0.41–1.05)	0.07	0.078750	0.68 (0.42–1.1)	0.11	0.086625
	TC vs. CC	1.38 (0.96–1.99)	0.075	0.078750	1.55 (11.07–2.24)	0.02	0.021000
	Dominant TT + TC vs. CC	1.16 (0.82–1.65)	0.38	0.266000	1.27 (0.89–1.81)	0.18	0.113400
	Recessive TT vs. TC + CC	0.52 (0.35–0.76)	0.001	0.002100	0.51 (0.34–0.76)	0.001	0.001575
	Over dominant TT + CC vs. TC	0.6 (0.44–0.81)	0.001	0.002100	0.56 (0.41–0.76)	0.0002	0.000630

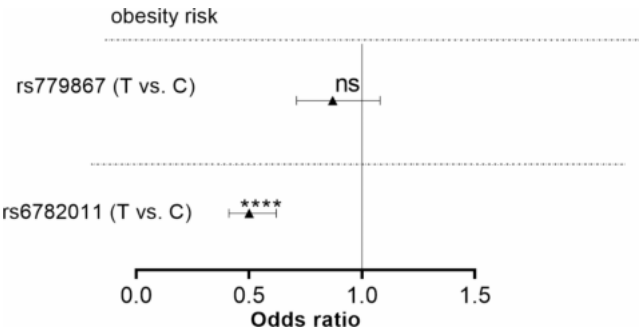

Fig. 3 Results of risk association for rs6782011 and rs779867 alleles. Values on the right of Y axis show causative effect toward the risk and values on the left show protective effect. The effective alleles (T) were examined against C alleles. The odds ratio and CI values are shown on the X axis in a linear scale. The T allele of rs6782011 showed a significant protective effect against the risk for obesity. (****P<0.0001)

Discussion

Identification of risk loci for obesity has implications in the management of obese patients and their family members to prevent obesity-related metabolic and physical complications. We assessed association between two *GRM7* genotypes and obesity in Iranian population. Glutamate receptors have been found to be involved in body weight. For instance, Santos et al. have shown that the absence of mGluR5 reduces weight increase and visceral adiposity in a mouse model. Moreover, absence of this receptor has led to reduction of inflammatory responses in the visceral adipose tissues indicating the role of mGluR5 in the modulation of adiposity (Santos et al., 2022). Oliveira et al. have shown that negative regulation of mGluR5 decreases binge-like eating, the most frequent kind of eating disorder. Based on their experiments, they have suggested mGluR5 as a probable target for treatment of obesity (Oliveira et al., 2021). Another study has shown that infusion of a mGluR2/3 agonist into the dorsomedial striatum can amend obesogenic diet-associated defects in the goal-directed control (Shipman and Corbit 2022). Moreover, the predisposition to diet-induced obesity has been shown to be linked with compulsive-like eating of appetizing food and is associated with 'addiction-like' deregulation of glutamatergic pathway in the nucleus accumbens (Sketriene et al., 2022).

GRM7 has been among obesity candidate genes that might participate in the etiology of highly penetrant kinds of familial obesity (Serra-Juhé et al., 2017). Gains of glutamate receptors (GRIK1, GRM7) have been among the

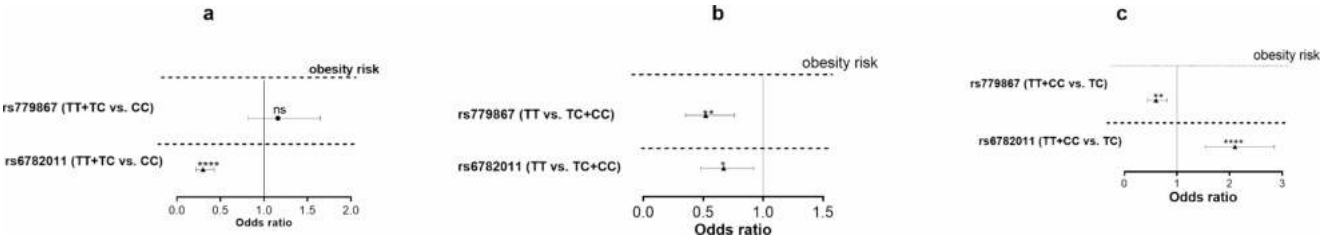

Fig. 4 Association between rs6782011 and rs779867 genotypes and obesity in dominant model (**a**), recessive model (**b**) and over-dominant model (**c**). Odds ratio and 95% CI values are shown on the X axis in a logarithmic scale. The effective genotypes TT + TC vs. CC for rs6782011 in dominant model (**a**) had a significant protective effect against obesity. In recessive model (**b**) tested genotypes showed likely significant protective effects against the obesity risk. In over-dominant model (**c**) tested genotypes for rs779867 showed protective effect against obesity, however, the tested genotypes for rs6782011 showed significant causative effects toward the obesity risk (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)

Table 5 The result of haplotype analyses in patients and control group

rs6782011	rs779867	Case	Control	Freq.	OR (95%CI)	P-value	Adjusted P-value
C	C	0.33	0.19	0.27	2.41 (1.83–3.18)	< 0.0001	< 0.0001
C	T	0.26	0.24	0.25	1.12 (0.89–1.4)	0.32	0.084
T	C	0.21	0.32	0.25	0.6 (0.47–0.75)	< 0.0001	< 0.0001
T	T	0.18	0.24	0.18	0.66 (0.5–0.87)	0.004	0.0014

likely pathogenic copy number variations detected in the inherited forms of obesity (Serra-Juhé et al., 2017).

We have shown association between the rs6782011 and risk of obesity in allelic model as well as all assessed inheritance models. Based on these comparisons, the C allele and the CC genotypes of this SNP conferred risk of obesity. A previous study has indicated association between the CC genotype of the rs6782011 and bipolar disorder type II in recessive model and with attention-deficit hyperactive disorder in dominant and co-dominant models (Noroozi et al., 2019). However, the impact of this SNP on expression or activity of the encoded protein is not clear. We also reported association between the rs779867 and obesity in recessive and over-dominant models. This polymorphism has been formerly revealed to be associated with autism spectrum disorder (Noroozi et al., 2016). Therefore, *GRM7* might be regarded as a shared locus for obesity and neuropsychiatric disorders, possibly explaining the observed associations between obesity and this kind of disorders (Rajan and Menon 2017). Notably, identification of risk alleles in patients may indicate their predisposition to psychiatric disorders necessitating especial attention for their treatment. Co-existence of obesity and a kind of neuropsychiatric disorder might change the treatment plan for obesity considering the fact that certain procedures are associated with subsequent risk of clinical depression (Kheirvari and Anbara 2021).

Haplotype analyses in the current study have confirmed the impact of mentioned SNPs on the obesity trait. Therefore, we suggest *GRM7* as a risk locus for obesity in Iranian population. Additional studies are desired to find the mechanism of participation of this locus in obesity. The current study has limitations in terms of sample size, lack of detailed demographic and clinical data and lack of expression/functional assays.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11011-023-01313-4.

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Authors' contributions SGF wrote the draft and revised it. MT and SA designed and supervised the study. SE analyzed the data. SAT and FR performed the experiment and data collection. All the authors read and approved the submitted version.

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Data Availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participant All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences.

Consent of publication Not applicable.

Competing interests The authors declare they have no conflict of interest.

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