sciendo

Short Communication

DOI:10.2478/rrlm-2019-0033

Is there a correlation between *GAD2* gene -243 A>G polymorphism and obesity?

Camelia Alkhzouz[1], Diana Miclea[1*], Marius Farcas[2], Simona Bucerzan[1], Georgiana Cabau[2], Radu Anghel Popp[2]

1. "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania
2. "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca

Abstract

Introduction: *GAD2 gene encodes the glutamate decarboxylase enzyme which catalyses the transformation of glutamate into γ-aminobutyric acid, GABA. It is suggested that some polymorphic alleles of GAD2 gene, such as -243A>G, have an increased transcriptional effect compared with the wild type, which results in an increase of GABA in the hypothalamus with the subsequent increase of the neuropeptide Y, thus exacerbating the hunger centre and the appetite. The aim of this study was to observe an association between the -243A>G polymorphism with obesity, comparatively studying a group of obese patients and a group of patients with normal weight.*

Patients and method: *127 patients were clinically evaluated in the Genetic and Endocrine Department of Children's Emergency Clinical Hospital, Cluj. The patients were included in two study groups, case group, with obesity (BMI higher than 97 kg/m2) and control group, with normal weight (BMI less than 97 kg/m2). Genotyping for GAD2 -243A>G polymorphism was performed using PCR-RFLP technique, the two groups being compared regarding the genotypes and phenotypes.*

Results and conclusions*: In the obesity group, there is a statistically significant difference in BMI (kg/m2) between the subgroups with different genotypes (p=0.01), the AA genotype being less severely affected than AG and GG genotypes. In the normal weight group there is no association between BMI and different genotypes (AA, AG or GG). Also, there is a greater distribution of GG genotypes and G allele in the obesity group compared with the control group, with an odds ratio which suggest that -243A>G polymorphism is a risk factor in obesity development (GG genotype OR=3.76, G allele OR=1.73, p=0.04).*

The finding of our study is important in explaining the multifactorial model of obesity, our research demonstrating that the GAD2 -243 A> G variant could be a risk factor that added to other obesogenic factors would potentiate their effect.

Keywords: obesity, GAD2 gene, polymorphism

Received: 4th May 2019; Accepted: 16th September 2019; Published: 6th October 2019

***Corresponding author**: Diana Miclea, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania. E-mail: bolca12diana@yahoo.com

Introduction

GAD2 gene (glutamate decarboxylase 2), located on the short arm of chromosome 10 (10p12.1) encodes the glutamate decarboxylase enzyme, which catalyses the transformation of glutamate into γ-aminobutyric acid (GABA). GABA acts on the neuropeptide Y, in the arcuate nucleus of the hypothalamus, neuropeptide Y being considered the most potent stimulator of the hunger centre (1). Glutamate decarboxylase has two isoforms, encoded by two different genes, one of 67kDa (*GAD1* gene) and the other of 65kDa (*GAD2* gene). Glutamate decarboxylase is an enzyme which is particularly studied because of its ability to become an autoantigen in 2 autoimmune diseases, type 1 diabetes mellitus and *stiff man syndrome* (2-3) *GAD2* gene (10p12.1, OMIM 138275) is very structurally similar to *GAD1* gene (2q31, OMIM 605363), but *GAD1* gene is expressed only in the central nervous system (CNS), while *GAD2* gene is expressed at both pancreatic and CNS level (2-7). Cerebral and insular autoimmune damage will lead to *stiff man syndrome*, respectively type 1 diabetes. Autoantigenicity of GAD2 appears to be due to molecular mimicry, with similarities to a viral polypeptide (possibly the P2-C protein of the Coxsackie virus) (8). On the other hand, these anti-GAD2 autoantibodies were also observed in a subgroup of patients with type 2 diabetes (9), this condition being known as LADA (latent autoimmune diabetes in adults).

Since GAD2 enzyme is required for GABA synthesis, animal studies suggest that the *GAD2* gene could play a central role in influencing GABAergic neurons linked to sensitive terminations and thus would influence presynaptic inhibition (10). On the other hand, increasing GABA amount in the hypothalamus causes a subsequent increase in the neuropeptide Y, thus excessively stimulating the hunger centre and appetite (11-13). Animal model studies have also demonstrated that the *GAD2* gene polymorphism causes an increase in GABA in the β cells, thus altering the first phase of insulin secretion and increasing glycemia (1,14).

Several single nucleotide polymorphism (SNPs) were identified in *GAD2* gene in correlation with obesity, some of them located in the promoter region: -243 A>G, c.61450C> A, c.83897T> A, c.8473A> C (15).

Wild allele has a protective role in obesity. Polymorphic alleles have an increased transcriptional effect compared with the wild type, which results in an increase of GABA in the hypothalamus with subsequent increase of neuropeptide Y, thus exacerbating the activation of the hunger centre and appetite (16). The -243 A>G polymorphism in the promoter region was studied in a French cohort and an association with obesity and possibly lower birth weight was revealed (17). Also, polymorphism was related to some metabolic risk factors and possibly to a certain eating behaviour in correlation with the effect of insulin (17). However, few studies, sometimes contradictory, have evaluated the link between genetic variants in *GAD2* and obesity (16-19).

The aim of this study is to observe an association of the -243 A>G polymorphism with obesity, by comparing a group of obese patients with a group of patients with normal weight.

Patients and method

This study was observational, analytical, prospective, case-control.

The main inclusion criterion in case group was the presence of obesity, according to the definition of World Health Organisation (WHO) (20). Therefore, in case group the inclusion criterion was the weight for height greater than 3 standard deviation (SD) above the WHO Child Growth Standards median for the patients under 5 years of age and a body mass index (BMI) greater than 2 SD above WHO Growth Reference median for

the patients over 5 years of age. For the control group, the inclusion criterion in patients under 5 years of age was a weight for height less than 3SD below the WHO Child Growth Standards median and in children above 5 years of age, a BMI less than 2SD below WHO Growth Reference median. Exclusion criteria were: absence of the inclusion criteria, absence of biological material for genetic testing or absence of informed consent.

For each patient, an informed consent from the parents was obtained to participate in the study. The study was approved by the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca.

One hundred twenty-seven patients were evaluated in the Genetic and Endocrine Department of Children's Emergency Clinical Hospital, Cluj, between 1st October 2013 and 1st October 2017. The age of these patients was between 1 and 18 years. Sixty patients were diagnosed with obesity, they were included in the case group, and 67 patients were of normal weight, being included in the control group. Patients in the case group were evaluated, clinically and by laboratory investigations, the phenotype data being evaluated and submitted in a database by the investigator physician. The evaluated phenotype included the auxology. Z score for weight, height and BMI was calculated according to WHO growth standards and reference for age and sex.

An amount of 3 mL of blood in a vacutainer containing EDTA was collected for each of these patients. DNA extraction was performed using the DNA extraction kit (*Wizard Genomic DNA Purification Kit, Promega, Madison, WI, USA*). Genotyping for *GAD2* -243 A>G polymorphism was performed using PCR-RFLP technique, as previously described. Briefly, performing PCR, a 203 bp amplicon was obtained, using the primers: *GAD2*-243-F: 5′-AGCTCCCTCCCTCTCTCGTGTTT-3′ and *GAD2*-243-R: 5′-TATGCGAGCTGGAGACAGGGTTTA-3′. Enzymatic digestion with DraI led, in the case of A allele, to 2 fragments of 144 bp and 59 bp, and in case of G allele, an undigested amplicon, of 203 bp.

Statistical analysis was performed using the IBM SPSS Statistics software (*IBM Corp., Armonk, NY, USA*). Quantitative variables were described as mean and standard deviations, qualitative variables were described as frequencies. To compare the quantitative variables, *Student (t)* test was used. For the verification of the Hardy-Weinberg equilibrium with respect to the allelic frequency, *Chi square test* ($\chi 2$) was used. To compare the frequency of genotypes and alleles between groups, *Chi square test* ($\chi 2$) was used. We estimated the odds of the association calculating the odds ratio (OR) at a 95% confidence interval (CI). P value under 0.05 was considered statistically significant.

Results

The demographic and auxological characteristics of the two studied groups are described in Table 1. In terms of age and sex, the two groups are comparable.

The correlation study between genotypes and auxological traits for obesity group is presented in Tables 2.

In the obesity group, there is a statistically significant difference in BMI (Z score) between the subgroups with different genotypes ($p = 0.04$) if the dominant model is considered, AG and GG genotypes being more severely affected than AA genotype. A recessive model does not exceed a significant statistical threshold ($p = 0.36$). In order to observe a more important correlation in case group between the auxology and the genotype, we selected two subgroups of patients, according to age. One, group A, including patients from obesity group with age between 5 and 10 years, and the other, group B, including patients with age over 10 years. No important statistical

Table 1. Characteristics of patients with obesity vs controls

Variable	Obesity group n (%) 60 (47.2%)	Control group n (%) 67 (52.8%)	p
Age (years)	11.46±3.39	11.12±4.62	0.66
BMI (kg/m2)	29.03±5.56	20.39±4.21	<0.001
BMI (Z score) Total group	2.23±0.61	0.14±2.3	<0.001
A Group(>5 yrs and ≤10yrs)	2.55±1.01		0.61*
B Group(>10yrs)	2.18±0.23		
Weight (Z score)	2.31±0.62	0.12±2.04	<0.001
A Group(>5 yrs and ≤10yrs)	2.66±0.90		0.07*
B Group(>10yrs)	2.35±0.45		
Height (Z score)	0.8±1.50	-0.5±1.82	<0.001

*A group with B group were compared

Table 2. Characteristics of the patients correlated with genotypes in obesity group

Variable	AA Total Group n=33(55%) A Group n=11 B Group n=22	AG Total Group n=19(31.7%) A group n=4 B group n=13	GG Total Group n=8(13.3%) A group n=2 B group n=6	p (dominant model)
BMI(kg/m2)	27.78±4.58	31.02±6.85	29.46±5.09	0.05
BMI (Z score)	2.12±0.24	2.52±1.02	2.24±0.17	0.04
Weight (Z score)	2.23±0.43	2.52±0.93	2.16±0.32	0.36
Height (Z score)	0,9±1.63	0.85±1,18	0.26±1,70	0.58
Age (years)	10.89±2.64	11.71±4.36	13.18±3.34	0.15
BMI(kg/m2)				
A group(>5 yrs and ≤10yrs)	24.51	27.05	29.55	0.12
B group(>10yrs)	29.42	32.85	29.43	0.16
BMI (Z score) Obesity Group				
A group(>5 yrs and ≤10yrs)	2.2	3.09	2.4	0.09
B group(>10yrs)	2.09	2.14	2.11	0.56
Weight (Z score) Obesity Group				
A group(>5 yrs and ≤10yrs)	2.35	3.06	2.01	0.47
B group(>10yrs)	2.17	2.27	2.31	0.26
Height (Z score) Obesity Group				
A group(>5 yrs and ≤10yrs)	1.22	1.33	-1.58	0.51
B group(>10yrs)	0.73	0.64	0.87	0.95
Age (years) Obesity Group				
A group(>5 yrs and ≤10yrs)	7.92	6.45	9.85	0.44
B group(>10yrs)	12.38	14.14	14.3	0.01

significance in either of these groups regarding the correlation between the auxological traits and the genotype (table II) was noted.

In the normal weight group, there is no association between BMI (Z score) and different genotypes, AA n=51(76.1%) (BMI, Zscore, -0.008±2.82),

AG n=14(20.9%) (BMI, Z score 0.38±1.13) and GG n=2(3%) (BMI, Z score 1.06±0.89) (p=0.58, dominant model) and also between weight (Z score) and genotypes (p=0.27, dominant model). Bringing together the patients of the two groups in a single large group, there is no statistically significant association between genotypes and BMI (Table 3).

Analysing the genotype and allelic distribution, there is statistically significant difference for GG genotypes distribution and G allele frequency between obesity group and control group with an odds ratio of 4.94 (p=0.01), respectively of 2.64 (p=0.001) (Table 4). We also observed a protective effect against obesity for AA genotype, compared with AG+GG genotype, odds ratio 0.38 (p=0.006). It was also observed that there was a difference regarding G allele frequency between A group - 8/34 alleles (23.5%) and B group – 25/60 (41.7%) (p=0.04), thus indicating a higher frequency of G alleles in obese children aged over 10 years.

Discussions

Our study has shown that *GAD2* -243 A>G is associated with weight gain and BMI in obese patients. G allele is more frequent in patients with obesity compared with those in the control group, an odds ratio of 2.64 indicating it as risk factor for obesity development. Also, it was observed that there was a greater G allele frequency over the age of 10 years, indicating the possibility that this factor could be a more important risk factor beginning with puberty towards the adulthood.

Hager et al. demonstrated for the first time in 1998, through genetic linkage studies, the existence of a major susceptibility locus on chro-

Table 3. Characteristics of the patients correlated with genotypes in all patients (obesity and normal weight group)

Variable	Total n=127	AA	AG	GG	p (dominant model)
		n=84(66.1%)	n=33(26%)	n=10(7.9%)	
BMI (kg/m2)	25.24±6.59	24.26±5.72	26.54±7.99	26.97±6.03	0.063
BMI (Z score)	1.32±1.93	1.09±2.24	1.6±1.51	1.82±0.76	0.131
Weight (Z score)	1.29±1.91	1.19±1.99	1.3±2.57	1.88±0.79	0.46
Height (Z score)	0.27±1.77	0.32±1.67	0.08±2.01	0.51±1.55	0.679
Age (years)	11.34±3.97	11.05±3.21	11.35±5.15	12.93±3.63	0.367

Table 4. Genotype distribution and alleles frequencies in *GAD2* gene in patients with obesity and controls

Variant	Obesity group n=60	Control group n=67	OR (95%CI)	p
Genotype AA *vs* AG+GG	33 (55%)	51 (76.1%)	0.386 (0.177-0.822)	0.006
Genotype AG				
***vs* AA+GG**	19 (31.7%)	14(20.9%)	1.747 (0.780-3.969)	0.087
***vs* AA**			2.084 (0.917-4.813)	0.039
Genotype GG				
***vs* AA+AG**	8 (13.3%)	2(3%)	4.942 (1.088-35.34)	0.018
***vs* AG**			3.248 (0.560-22.52)	0.113
G allele frequency	35 (29.2%)	18 (13.4%)	2.643 (1.409-5.069)	0.001
A allele frequency	85 (70.8%)	116 (86.6%)		

mosome 10, considered at the time very important for the development of obesity (21). In this region, *GAD2* gene was for the first time mentioned in association with obesity in 2003 by Boutin et al. (16), because, by its catalytic function in GABA formation, the latter interacts with the neuropeptide Y in the paraventricular nucleus and contributes to the regulation of food intake. Also, the level of anti-GAD2 autoantibodies correlates with islet cell function and insulin secretion. Thus, GABA is known to have an orexigenic effect, and genes involved in its metabolism can control food intake and are thus responsible for the development of obesity (16). Meyre et al. demonstrated on a cohort of children with severe obesity that *GAD2* is correlated with obesity and also asserts in the study the hypothesis of the insulin role on foetal growth and not that of the foetal programming (17,22).

The data obtained in our study were also observed in the recent study of Prakash et al (18), which also indicated the role of *GAD2* in the development of obesity. On the other hand, the study of Swarbrick et al. did not find an association between this polymorphism and morbid obesity (19).

However, additional data is needed to better understand the role of GAD2 both at pancreatic level and insulin secretion and also the GABA action at the level of the lateral hypothalamus and hunger centre. Very recent data further suggest the role of GAD2 in physical activity level programming, data currently observed in mice (23).

Our research brings important and necessary data to support the role of *GAD2*, our different approach, compared with other studies, being given by the case-control study type and also by the uniformly, monocentric research. Only few clinical trials were done in this field, not always concordant, indicating the need for future research. On the other hand, the limitation of this study could be due to the quite small number of analysed patients. Also, it must be taken into account that in obesity there are studies that indicate other genetic or epigenetic factors that isolated or associated are proved as risk factors for obesity (24,25).

In conclusion, the findings of our study are important in explaining the multifactorial model of obesity, our research demonstrating that the *GAD2* -243 A>G variant is a risk factor which added to other obesogenic factors could potentiate their effect.

Authors' contributions

A.C. and M.D. are co-first authors.

A.C., M.D. and P.R.A. contributed to conceptualisation, methodology, validation, investigation, manuscript writing, manuscript supervising.

F.M. contributed to manuscript writing, methodology of the study, validation, investigation.

B.S. and C.G. contributed to methodology of the study.

Conflict of Interest

The authors report no conflicts of interest in this work.

List of abbreviation

GAD - Glutamate Decarboxylase; GABA - γ-AminoButyric Acid; CNS - Central Nervous System; WHO - World Health Organisation; SD - Standard Deviation; BMI - Body Mass Index ; EDTA - Ethylenediaminetetraacetic Acid; PCR - Polymerase chain reaction; RFLP - Restriction Fragment Length Polymorphism; OR - Odds Ratio; CI - Confidence Interval

References

1. Choquette AC, Lemieux S, Tremblay A, Drapeau V, Bouchard C, Vohl M-C, et al. GAD2 gene sequence variations are associated with eating behaviors and weight gain in women from the Quebec family study. Physiol Behav. 2009 Oct;98(4):505-10. DOI: 10.1016/j.

physbeh.2009.08.004
2. Karlsen AE, Hagopian WA, Grubin CE, Dube S, Disteche CM, Adler DA, et al. Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci U S A. 1991 Oct;88(19):8337-41. DOI: 10.1073/pnas.88.19.8337
3. Grone BP, Maruska KP. Three Distinct Glutamate Decarboxylase Genes in Vertebrates. Sci Rep. 2016 Sept;6(1):30507. DOI: 10.1038/srep30507
4. Popp A, Urbach A, Witte OW, Frahm C. Adult and Embryonic GAD Transcripts Are Spatiotemporally Regulated during Postnatal Development in the Rat Brain. PLoS One. 2009 Feb;4(2):e4371. DOI: 10.1371/journal.pone.0004371
5. Trifonov S, Yamashita Y, Kase M, Maruyama M, Sugimoto T. Glutamic acid decarboxylase 1 alternative splicing isoforms: characterization, expression and quantification in the mouse brain. BMC Neurosci. 2014 Oct;15(1):114. DOI: 10.1186/1471-2202-15-114
6. Cram DS, Barnett LD, Joseph JL, Harrison LC. Cloning and partial nucleotide sequence of human glutamic acid decarboxylase cDNA from brain and pancreatic islets. Biochem Biophys Res Commun. 1991 May;176(3):1239-44. DOI: 10.1016/0006-291X(91)90418-7
7. Davis KN, Tao R, Li C, Gao Y, Gondré-Lewis MC, Lipska BK, et al. GAD2 Alternative Transcripts in the Human Prefrontal Cortex, and in Schizophrenia and Affective Disorders. PLoS One. 2016 Feb;11(2):e0148558. DOI: 10.1371/journal.pone.0148558
8. Albert LJ, Inman RD. Molecular Mimicry and Autoimmunity. N Engl J Med. 1999 Dec;341(27):2068-74. DOI: 10.1056/NEJM199912303412707
9. Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, et al. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes. 1999 Jan;48(1):150-7. DOI: 10.2337/diabetes.48.1.150
10. Fink AJP, Croce KR, Huang ZJ, Abbott LF, Jessell TM, Azim E. Presynaptic inhibition of spinal sensory feedback ensures smooth movement. Nature. 2014 May;509(7498):43-8. DOI: 10.1038/nature13276
11. Boloc D, Mas S, Rodriguez N, Ortiz AE, Morer A, Plana MT, et al. Genetic Associations of Serotoninergic and GABAergic Genes in an Extended Collection of Early-Onset Obsessive-Compulsive Disorder Trios. J Child Adolesc Psychopharmacol. 2019 Mar;29(2):152-7. DOI: 10.1089/cap.2018.0073
12. Lizarbe B, Soares AF, Larsson S, Duarte JMN. Neurochemical Modifications in the Hippocampus, Cortex and Hypothalamus of Mice Exposed to Long-Term High-Fat Diet. Front Neurosci. 2019 Jan;12:985. DOI: 10.3389/fnins.2018.00985
13. Jarvie BC, King CM, Hughes AR, Dicken MS, Dennison CS, Hentges ST. Caloric restriction selectively reduces the GABAergic phenotype of mouse hypothalamic proopiomelanocortin neurons. J Physiol. 2017 Jan;595(2):571-82. DOI: 10.1113/JP273020
14. Wang Q, Ren L, Wan Y, Prud'homme GJ. GABAergic regulation of pancreatic islet cells: Physiology and antidiabetic effects. J Cell Physiol. 2019 Jan;12:985.
15. Boutin P, Froguel P. GAD2: a polygenic contribution to genetic susceptibility for common obesity? Pathol Biol (Paris). 2005 Jul;53(6):305-7. DOI: 10.1016/j.patbio.2004.09.008
16. Boutin P, Dina C, Vasseur F, Dubois S, Corset L, Séron K, et al. GAD2 on Chromosome 10p12 Is a Candidate Gene for Human Obesity. John Bell, editor. PLoS Biol. 2003 Nov;1(3):e68. DOI: 10.1371/journal.pbio.0000068
17. Meyre D, Boutin P, Tounian A, Deweirder M, Aout M, Jouret B, et al. Is Glutamate Decarboxylase 2 (GAD2) a Genetic Link between Low Birth Weight and Subsequent Development of Obesity in Children? J Clin Endocrinol Metab. 2005 Apr;90(4):2384-90. DOI: 10.1210/jc.2004-1468
18. Prakash J, Mittal B, Awasthi S, Srivastava N. Association of the -243A>G, +61450C>A Polymorphisms of the Glutamate Decarboxylase 2 (GAD2) Gene with Obesity and Insulin Level in North Indian Population. Iran J Public Health. 2016 Apr;45(4):460-8.
19. Swarbrick MM, Waldenmaier B, Pennacchio LA, Lind DL, Cavazos MM, Geller F, et al. Lack of support for the association between GAD2 polymorphisms and severe human obesity. PLoS Biol. 2005 Sep;3(9):e315. DOI: 10.1371/journal.pbio.0030315
20. Obesity and overweight. 22 iulie 2019 [citat 22 iulie 2019]; Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
21. Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, et al. A genome-wide scan for human obesity genes reveals a major susceptibilitylocus on chromosome 10. Nat Genet. 1998 Nov;20(3):304-8. DOI: 10.1038/3123
22. Cinteza EE, Cinteza M. Biomarkers in Obesity. Rev Romana Med Lab. 2018;26(3):353-8. DOI: 10.2478/rrlm-2018-0027
23. Kosse C, Schöne C, Bracey E, Burdakov D. Orexin-driven GAD65 network of the lateral hypothalamus sets physical activity in mice. Proc Natl Acad Sci. 2017 Apr;114(17):4525-30. DOI: 10.1073/pnas.1619700114
24. Duicu C, Mărginean CO, Voidăzan S, Tripon F, Bănes-

cu C. FTO rs 9939609 SNP Is Associated With Adiponectin and Leptin Levels and the Risk of Obesity in a Cohort of Romanian Children Population. Medicine (Baltimore). 2016 May;95(20):e3709. DOI: 10.1097/MD.0000000000003709

25. Tilinca MC, Barabas-Hajdu EC, Tusa Ferencz G, Nemes-Nagy E. Involvement of inflammatory cytokines in obesity and its complications. Rev Romana Med Lab. 2018;26(3):359-71. DOI: 10.2478/rrlm-2018-0019