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Obesity Pharmacotherapy

Adenylate cyclase 3: a new target for anti-obesity drug development

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Summary

Obesity has become epidemic worldwide, and abdominal obesity has a negative impact on health. Current treatment options on obesity, however, still remain limited. It is then of importance to find a new target for anti-obesity drug development based upon recent molecular studies in obesity. Adenylate cyclase 3 (ADCY3) is the third member of adenylyl cyclase family and catalyses the synthesis of cAMP from ATP. Genetic studies with candidate gene and genome-wide association study approaches have demonstrated that ADCY3 genetic polymorphisms are associated with obesity in European and Chinese populations. Epigenetic studies have indicated that increased DNA methylation levels in the ADCY3 gene are involved in the pathogenesis of obesity. Furthermore, biological analyses with animal models have implicated that ADCY3 dysfunction resulted in increased body weight and fat mass, while reduction of body weight is partially explained by ADCY3 activation. In this review, we describe genomic and biological features of ADCY3, summarize genetic and epigenetic association studies of the ADCY3 gene with obesity and discuss dysfunction and activation of ADCY3. Based upon all data, we suggest that ADCY3 is a new target for anti-obesity drug development. Further investigation on the effectiveness of ADCY3 activator and its delivery approach to treat abdominal obesity has been taken into our consideration.

Keywords: Adenylate cyclase 3, body mass index, obesity.

Abbreviations: ADCY3, adenylate cyclase 3; ATP, adenosine triphosphate; BMI, body mass index; cAMP, 3',5'-cyclic adenosine monophosphate; CNS, central nervous system; GK, Goto–Kakizaki; GWAS, genome-wide association study; T2D, type 2 diabetes; UTR, untranslated region.

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Introduction

Obesity has become a large and fast growing public health problem in the world. Since 1980, the prevalence of obesity has more than doubled. According to the reports in 2014, more than 1.9 billion adults (39% of populations) are overweight. Of them over 600 million (13%) are obese. Moreover, 42 million children under the age of 5 years old are obese or overweight (1–4). Obesity and particularly abdominal obesity (also known as central obesity) are widely

recognized as the major risk factors for non-communicable diseases, such as cardiovascular diseases (mainly heart disease and stroke), diabetes (mainly type 2), musculoskeletal disorders (especially osteoarthritis), cancers (endometrial, prostate, breast and colon), reproductive dysfunction, liver and kidney diseases (4–7). Although diet, exercise and behavioural modification have been used as obesity management approaches, drugs may amplify the adherence to behaviour change and improve physical function (8). In some patients, the treatment with dieting and physical exercise is not a viable option. For these patients, anti-obesity drugs can be a better alternative. Current anti-obesity drug candidates have aimed to reduce food intake by either curbing appetite or suppressing the craving for food or reducing the gastro-intestinal digestion of nutrients, e.g., lipids (9,10). However, several of these agents have been associated with severe psychiatric and/or cardiovascular side effects, highlighting the necessity to discover and develop the new anti-obesity drugs.

Adenylyl cyclases (ADCYs) are enzymes that catalyse the synthesis of 3',5'-cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). cAMP is a second messenger and used for intracellular signal transduction. This messenger is associated with function of kinases in several biochemical processes, including the regulation of carbohydrate, and lipid metabolism. In the family of ADCYs, there are 10 closely related members, including ADCY1-9 and ADCY-activating polypeptide 1. All these ADCY1-9 are located on the inner side of the plasma membrane, anchored at various locations in the interior of the cell and widely expressed in most organs and cell types in the body (11–13). Interestingly, an important paralog of ADCY3 is ADCY5, the fifth member in the same family. The ADCY5 genetic polymorphisms have been recently found to be associated with type 2 diabetes (T2D) but not obesity. Dupuis *et al.* have reported that the ADCY5 genetic polymorphism rs11708067 is associated with elevate fasting glucose levels and surrogate estimate of beta-cell function (HOMA-B) in the patients with T2D (14). Andersson *et al.* have demonstrated that this polymorphism is inversely associated with birth weight in T2D (15). In this review, we summarize genetic and epigenetic association studies of the ADCY3 gene in obesity, interpret the results from biological experiments with animal models and finally discuss the possibility of ADCY3-targeted therapies to treat abdominal obesity.

Genomic and biological features of ADCY3

Adenylate cyclase 3 is the third family member of adenylyl cyclases, and its genomic and biological features are summarized in Table 1. The ADCY3 gene is located in chromosome 2p23.3 and consists of 21 exons with large 5'- and 3'-UTRs. In the ADCY3 gene sequence, one non-synonymous (rs1167272, Ser107Pro) and several intronic polymorphisms have been included into genetic association studies (16–21). In the ADCY3 gene promoter region, there are 10 binding sites for transcription factors, including FOXO4, Ik-2, Oct.B1, Oct-B2, Oct-B3, POU2F1a, POU2F2, POU2F1, POU2F2C and POU2F2B. Particularly, there are two CpG islands (cg16888658 and cg17644208) that have been selected for DNA methylation analysis (22,23). ADCY3 protein has 1,144 amino acids, and its molecular weight is 128,960 Da. This protein is membrane bound and Ca^{2+}–calmodulin sensitive (12). According to the previous reports, ADCY3 is expressed in olfactory sensory neurons, brain, spinal and retina, heart, lung, kidney, liver, pancreas, placenta and skeletal muscle. Recent studies with animal models have demonstrated that ADCY3 is presented in the certain regions of the brain, including striatum and hypothalamus (suprachiasmatic, paraventricular, ventromedial and arcuate nucleus) and adipocyte tissues (24,25). Moreover, there are two micro-RNAs Has-miR-128 and Has-miR-27a, which may be included in the regulation of ADCY3 expression in addition to transcription binding factors and DNA methylation alteration in the promoter.

Table 1 Bio-informatics of adenylate cyclase 3

Alliases	Adenylate cyclase, olfactive type; adenylate cyclase type III; ATP pyrophosphate-lyase 3; adenylyl cyclase 3; EC 4.6.1.1; AC-III; AC3; ADCY3
External IDs	HGNC: 234; Entrez gene: 109; Ensembl: ENSG00000138031; OMIM:600291; UniProtKB: O60266
Type	Olfactive and sensitive to Ca^{2+}–calmodulin
Chromosomal localization	2p23.3
Genomic DNA	NC_000002.12; NT_022184.16; NC_018913.2
mRNA	NM_004036
miRNA regulation	Has-miR-128; Has-miR-27a
Post-translational modifications	Ubiquitination at Lys1046, Lys1111 and Lys1120 NX_O06266 Glycosylation at Asn736 NX_O06266 Modification sites at PhosphoSitePlus O06266
Protein details	NP_004027.2; 1,144 AA/128,960 Da Belongs to the adenylyl cyclase class-4/guanylyl cyclase family Contains 2 guanylate cyclase domains
Biological function	Activated by calcium/calmodulin Mediates odorant detection (possibly) via modulation of intracellular cAMP concentration Expressed in olfactory sensory neurons, brain, spinal and retina, striatum and hypothalamus, heart, lung, kidney, liver, pancreas, placenta, skeletal muscle, adipocyte
Diseases associated	Obesity; thyroid adenoma; precocious puberty

Genetic and epigenetic studies of ADCY3 in obesity

In 2008, we reported the first genetic association study of ADCY3 in Swedish subjects with T2D and obesity (16).

The original hypothesis of our study was created based upon the results from experiments with Goto–Kakizaki (GK) rats in our laboratory (24). GK rat is an animal model for T2D that was developed initially by Goto and Kakizaki at Tohoku University, Sendai, Japan in 1975. GK rat develops T2D early in life, exhibits moderate hyperglycaemia and markedly reduces glucose-induced insulin release. This animal model, however, is non-obese, which is important to remember when interpreting data in our genetic association study. The Stockholm GK rat colony was generated by five pairs of F40 generation of the Japanese colony in 1989 (26). The ADCY3 gene is localized in rat chromosome 6. By detection of the ADCY3 mRNA gene expression, Abdel-Halim *et al.* found that this gene was up-regulated in the pancreatic islets of GK rats (24). The over-expression of ADCY3 in GK rats suggested that ADCY3 might be a susceptibility gene for T2D. In order to test this hypothesis, we first performed the variation screening of the ADCY3 gene sequences in Swedish subjects with T2D and non-diabetic controls and then conducted a population-based (cases and controls) genetic association study in a Swedish population (16). We found that three single nucleotide polymorphisms (SNPs) in the ADCY3 gene were associated with body mass index (BMI) but not with variation of fasting glucose and insulin levels in T2D. Keeping the issue that GK rat is a non-obese animal in our mind, we decided to test whether the ADCY3 genetic polymorphisms are associated with obesity or not. We thus extended sample collection to obese subjects and further genotyped the newly collected samples. Consequently, we provided evidence that ADCY3 genetic polymorphisms were associated with obesity in Swedish population. The minor alleles of these polymorphisms might confer the risk susceptibility to the development of obesity. However, the sample size of newly collected obese subjects ($n = 199$) in our first genetic study was small (16). We thus replicated the genetic association study with a relatively large cohort of obese and lean Chinese subjects ($n = 3{,}396$) and confirmed that ADCY3 genetic polymorphisms were associated with obesity and the major alleles might have the protective effects (17). Genetic polymorphisms in the ADCY3 gene and their association with obesity are presented in Table 2.

In population-based genetic association studies, candidate gene association study and genome-wide association study (GWAS) are common approaches. These two genetic association studies described briefly earlier have been carried out with the candidate gene approach, which is based on the selection of SNPs within the ADCY3 gene. With the development of high-throughput SNP genotyping methodologies, genetic studies in complex diseases have moved into GWAS era. GWAS is a hypothesis free design and focused on common variants with minor allele frequency >5%, and this approach tests the association between more than 300,000 genetic markers (usually SNPs) across the whole genome. In the recent years, four GWASs have replicated the genetic association between ADCY3 and obesity in European populations (18–21). Interestingly, a missense amino acid-changed polymorphism (rs11676272, Ser107Pro) in the ADCY3 gene was found to be associated with obesity. The minor allele A (107Ser) conferred to the increased BMI and fat mass (Table 2). Very recently, Felix *et al.* have conducted a GWAS in children and found that this polymorphism in the ADCY3 gene was the most significantly associated with BMI. The ADCY3 gene was topped up on the Manhattan plot of results of the discovery meta-analysis of 20 studies, while the transmembrane protein 18, fat mass and obesity-associated, fas apoptotic inhibitory molecule 2, SEC16 homolog B were followed after ADCY3 (21). Accumulating evidence from genetic studies has convinced us that ADCY3 has genetic effects in the development of obesity (27).

Obesity is a complex metabolic disorder influenced by genetic and environmental factors. In recent years, genetic studies have identified a number of confirmed genetic susceptibility variants for obesity. However, the genetic findings can only explain limited proportion of the overall heritable risk of this metabolic disorder, which challenges our expectations to translate genetic information into clinical practice (1–3). One of the reasons causing the missing information on heritability could be that epigenetic factors are involved in the complex interplay between genes and environment in the development of obesity. Epigenetic factors mainly including DNA methylation changes have been considered to be involved in the pathogenesis of obesity (28,29). DNA methylation levels are commonly analysed at clusters of CpG methylation sites in the gene and used for indication of epigenetic effects. DNA methylation analysis can be performed in the scales of global genome or specific gene region and in peripheral blood with mixed cell types. Dick *et al.* have recently used DNA samples extracted from whole blood and adipose tissues to study the epigenetic changes related to BMI and demonstrated that the analysis of blood DNA methylation can reflect changes in relevant tissues for obesity (30). Compared with accumulating data from genetic studies, epigenetic study of obesity is relatively limited. Liu *et al.* have investigated maternal obesity-induced methylation alterations with cord blood DNA samples and found that increased DNA methylation levels at the site of CpG island (cg17644208) within the ADCY3 gene were associated with BMI (23). Moreover, Voisin *et al.* have analysed two CpG islands in the ADCY3 gene; one (cg16888658) resides in the promoter region, and another one (cg01884057) is located together with SNP rs713586. Results indicated that increased DNA methylation levels in the promoter region may reduce the ADCY3 gene activity in obese subjects. The carriers with the risk allele C of SNP rs713586 had higher DNA methylation levels of CpG island (cg01884057) compared with the

Table 2 Genetic and epigenetic association of ADCY3 with body mass index

Research approach	SNP and CpG ID	Type of SNP	Association	Population	Refernece
Population genetic association study	rs2033655 rs1968482	Y=C/T R=A/G	The minor alleles T and G were associated with obesity	Swedish	Nordman S *et al.* 2008
	rs753529 rs7604576 rs1127568	R=A/G	The minor allele G of rs753529 and common haplotypes constructed of these three SNPs were associated with obesity	Chinese	Wang H *et al.* 2010
Genome-wide association study	rs1172294	R=A/G	This SNP was a BMI-associated marker (ADCY3-POMC) and associated with increased pre-pubertal stature	European descent Finnish	Cousminer DL *et al.* 2013
	rs11676272	R=A/G missense Ser107Pro	The minor allele A was associated with BMI and fat mass	European	Stergiakouli E *et al.* 2014
				European descent	Warrington NM *et al.* 2015
				European children	Felix JF *et al.* 2016
Epigenome-wide association study[1]	cg16888658 rs713586 cg01884057	Y=C/T	In the promoter region of ADCY3, increased DNA methylation levels might reduce the gene activity This obesity-associated SNP was associated with DNA methylation changes at cg01884057. The carriers with C/C genotype had increased methylation levels compared with C/T and T/T genotypes. C is the minor allele.	Swedish	Voisin S *et al.* 2015
Epigenome-wide association study[2]	cg17644208		Pre-pregnancy maternal BMI might lead to alterations in offspring DNA methylation in ADCY3	African American and Haitian	Liu X *et al.* 2014

[1]With Illumina HumanMethylation450 BeadChip.
[2]With Illumina HumanMethylation27 BeadChip.
BMI, body mass index; FPG, fasting plasma glucose; SNP, single nucleotide polymorphism; ADCY3, adenylate cyclase 3; EFR3B, EFR3 homolog B; DNAJC27, DnaJ (Hsp40) homolog, subfamily C, member 27; DNAJC27-AS1, DNAJC27 antisense RNA1.

T allele carriers (22) (Table 2). Therefore, data from these two studies have suggested that epigenetic effects in the ADCY3 gene are involved in the pathogenesis of obesity.

Experimental analyses of ADCY3 with animal models

Among the members of ADCY family, ADCY1, 3 and 8 are Ca^{2+} dependent (11). Previous experimental studies with GK and Wistar rats in our laboratory have demonstrated that these three ADCYs are expressed in beta-cells of pancreatic islets (25). Particularly, ADCY3 was found to be over-expressed in pancreatic islets of GK rats compared with Wistar rat. Therefore, ADCY3 plays an important role in the regulation of insulin secretion. Figure 1 illustrates the cellular mechanism of ADCY3 in a pancreatic beta-cell. Briefly, ADCY3 catalyses the formation of cAMP from ATP. After then, cAMP-dependent phosphorylation of proteins by protein kinase A is involved in many cellular processes, including insulin release in pancreatic beta-cells as well as differentiation, proliferation and apoptosis. However, the interaction of ADCY3 with G protein-coupled receptors is still unknown.

We further analysed the ADCY3 activity in non-treated GK, insulin-treated GK and Wistar rats. We applied insulin treatment with micro-crystallized bovine insulin palmitic acid to normalize blood glucose levels in GK rats for 15 d and then comparatively analysed ADCY3 gene expression at mRNA levels. We found that ADCY3 expression patterns in pancreatic islets and brain (particularly in the striatum–hypothalamus region) were similar. In both pancreatic islets and striatum–hypothalamus of insulin-treated GK rats, the ADCY3 expression levels were intermediate between GK and Wistar rats. Compared with Wistar rats, the ADCY3 mRNA expression levels in GK rats were increased. Data suggested that normalization of plasma glucose levels in GK rats with insulin treatment tended to normalize the augmented ADCY3 mRNA expression. Based upon our observations of the similarity of ADCY3 gene expression patterns between pancreatic islets and brain, we had a hypothesis that ADCY3 might play its role in regulation of metabolism via CNS and insulin secretion (25).

The ADCY3 gene is localized in mouse chromosome 12. After we reported the first genetic study of ADCY3 in obesity, Wang *et al.* developed a mouse model with knockout of ADCY3 to further explore the mechanism behind a role of ADCY3 in regulation of body weight. The $ADCY3^{-/-}$ mice exhibited pronounced obesity as they age primarily because of increased fat mass. The body weight of adult male $ADCY3^{-/-}$ mice was increased by 40% compared with

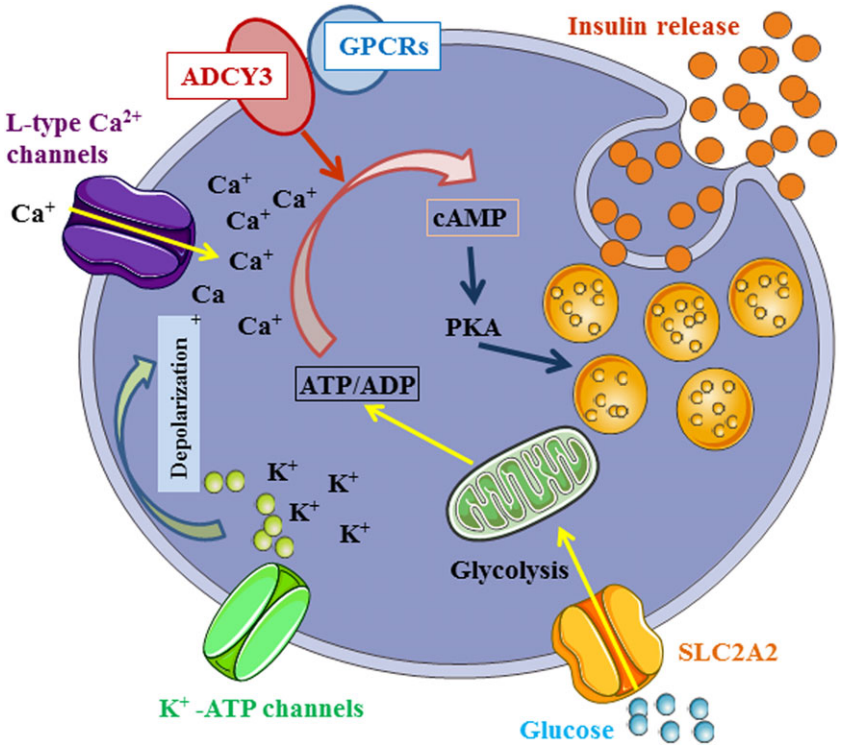

Figure 1 Cellular mechanism of adenylate cyclase 3 (ADCY3) in a pancreatic beta-cell. ADCY3 is membrane bound and Ca^{2+}–calmodulin sensitive. It catalyses the synthesis of 3',5'-cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). cAMP is a second messenger for intracellular signal transduction in beta-cells including activation of PKA and followed insulin release. SLC2A2, solute carrier family 2 (facilitated glucose transporter) member; GPCRs, G protein-coupled receptors; PKA, protein kinase A.

wild-type male mice, while female ADCY3$^{-/-}$ mice were 70% heavier than wild-type female mice (31). Both adult male and female ADCY3$^{-/-}$ mice exhibited obesity that were apparently caused by low locomotor activity, hyperphagia and leptin insensitivity. Furthermore, young ADCY3$^{-/-}$ mice exhibited decreased physical activity, increased food consumption and leptin insensitivity. Recently, Pitman *et al.* have recently analysed ADCY3 gene activity and cAMP production related to the metabolic response to high-fat diet in a line of N-ethyl-N-nitrosourea-mutagenized mice. They found that the gain-of-function mutation (M279I) of the NEU gene had increased NEU gene activity and cAMP production and consequently the mutant mice had dramatically reduced body weight and fat mass, low basal insulin and glucose levels compared with wild type of mice (32).

Based upon evidence from both genetic studies in obese subjects and biological analyses with animal models, we would conclude that ADCY3 has anti-obesity effects. The next question is how ADCY3 regulates body-weight changes, and whether this molecule can serve as a new target for drug development in treatment of obesity. Notably, ADCY3 is a membrane protein, which may interact with glucagon-like peptide-1 (GLP-1) receptor via G proteins. Liraglutide is a GLP-1 receptor analogue. This drug has a prolonged half-life and can be administered once daily to improve the control of blood glucose levels in the patients with T2D (33,34). Interestingly, recent clinical observations have demonstrated that GLP-1 analogues help the patients with body-weight reduction (35,36). Until now, this weight-reducing effect by GLP-1 has been accounted for by the peptide suppressing appetite through a direct effect on hypothalamic appetite centres (37). To understand the more complex mechanism behind GLP-1 effect on body weight, Liang *et al.* have very recently performed Liraglutide treatment experiments in the diabesity and obese mice, which were induced from db/db and C57BL/6J mice by HFD. Results indicated that ADCY3 activity at both mRNA and protein levels was up-regulated and negatively correlated with body weight in the mice after GLP-1 treatment (38) Therefore, the reduction of body weight with GLP-1 treatment can be explained partially by ADCY3 activation. This benefit of GLP-1 additional to control blood glucose levels for the patients with T2D is unexpected in the original objective for developing this drug but has created an approach against obesity.

ADCY3 and its interactive partners

Several studies have previously demonstrated that combined treatment with ephedrine and caffeine has beneficial effects on body weight, plasma triglycerides and lipid metabolism, which could be explained by chronic stimulation of cAMP via beta-adrenergic receptors and subsequently activation in thermogenic systems (39–41). However, the drug combination of ephedrine and caffeine was taken off the over the counter (OTC) market in 2004. We have herein reviewed ADCY3 as a new target for anti-obesity drug development. The question has been risen is whether ADCY3 has interaction in stimulation of cAMP via beta-adrenergic receptors. Further investigation of ADCY3 and its interactive partners, including the beta-adrenergic receptors, may need to be taken into our consideration.

STRING is a database of known and predicted protein interactions, which include direct (physical) and indirect (functional) associations. The data are derived from four sources, including genomic context, high-throughput experiments, co-expression and previous publications (42). According to the prediction from STRING, ADCY3 has interactions with other molecules, mainly, including heme-binding protein 1, sphingosine-1-phosphate receptor 1, chemokine (C-C motif) receptor 9, neuropeptide W and inosine triphosphatase (Fig. 2). Regulator of G-protein signalling 9 (RGS9) protein is one of G protein-coupled receptors and highly enriched in the striatum region of the brain. The dopaminergic circuits in the striatum are activated by stimuli associated with reward and also densely interconnected with the hypothalamus. Waugh *et al.* demonstrated that RGS9$^{-/-}$ mice, similar to ADCY3$^{-/-}$ animal model, became obese because of increased adiposity (43). There is an insertion/deletion polymorphism in the human RGS9 gene. We conducted a genetic association study in Chinese and Malaysian populations, including the same Chinese cohort in the ADCY3 genetic study. The D allele of this RGS9 insertion/deletion polymorphism was found to be associated with protective effects against obesity in adults and children (44). We further analysed ADCY3 and RGS9 gene expressions in brain of RGS9$^{-/-}$ mice but no interaction between these two genes was found (25). Therefore, it is necessary

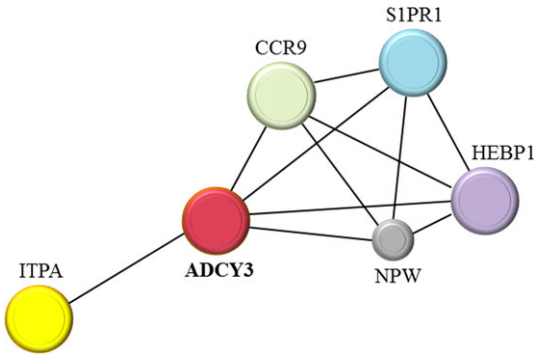

Figure 2 Possible interaction between adenylate cyclase 3 (ADCY3) and functional partners. This is a prediction of interaction between ADCY3 and other functional partners, including HEBP1 (heme-binding protein 1), S1PR1 (sphingosine-1-phosphate receptor 1), CCR9 (chemokine C-C motif receptor 9), NPW (neuropeptide W) and ITPA (inosine triphosphatase). The prediction is conducted by using a STRING interaction network.

to further investigate the relationship between ADCY3 and other proteins with comprehensive experiment designs because of their complexity.

Conclusions

Taking together all information from genetic, epigenetic and biological studies, we conclude that the minor alleles of ADCY3 genetic polymorphisms are associated with obesity. Reduction of ADCY3 gene activity due to its biological dysfunction at mRNA and protein expression levels or down-regulation by mutant variants and/or increased methylation levels in genomic DNA sequences is involved in the pathogenesis of obesity. Stimulation of the ADCY3 gene activity to be increased may be a useful approach for treatment of obesity. Therefore, ADCY3 is a new target for anti-obesity drug development.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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