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MOLECULAR METABOLISM

Cadm2 regulates body weight and energy homeostasis in mice

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ABSTRACT

Objective: Obesity is strongly linked to genes regulating neuronal signaling and function, implicating the central nervous system in the maintenance of body weight and energy metabolism. Genome-wide association studies identified significant associations between body mass index (BMI) and multiple loci near *Cell adhesion molecule2* (*CADM2*), which encodes a mediator of synaptic signaling enriched in the brain. Here we sought to further understand the role of Cadm2 in the pathogenesis of hyperglycemia and weight gain.

Methods: We first analyzed *Cadm2* expression in the brain of both human subjects and mouse models and subsequently characterized a loss-of-function mouse model of *Cadm2* for alterations in glucose and energy homeostasis.

Results: We show that the risk variant rs13078960 associates with increased *CADM2* expression in the hypothalamus of human subjects. Increased Cadm2 expression in several brain regions of $Lep^{ob/ob}$ mice was ameliorated after leptin treatment. Deletion of *Cadm2* in obese mice (Cadm2/ob) resulted in reduced adiposity, systemic glucose levels, and improved insulin sensitivity. *Cadm2*-deficient mice exhibited increased locomotor activity, energy expenditure rate, and core body temperature identifying Cadm2 as a potent regulator of systemic energy homeostasis.

Conclusions: Together these data illustrate that reducing Cadm2 expression can reverse several traits associated with the metabolic syndrome including obesity, insulin resistance, and impaired glucose homeostasis.

Keywords Cadm2/SynCAM2; Energy homeostasis; Insulin sensitivity; Genome-wide association studies; Leptin signaling

1. INTRODUCTION

The central nervous system is widely known to regulate energy expenditure and hormone sensitivity; however, the full extent to which our metabolism is managed by the brain and the key genes involved remain to a large degree unknown [1,2]. Recent studies have begun to identify neurons in regions of the brain beyond the hypothalamus which engage circuitry of the arcuate nucleus and mediate effects on energy balance [3]. While neuron-specific disruption of the insulin and leptin receptors both significantly influence body mass and glucose homeostasis, these key signaling mediators are widely expressed throughout the brain including the hippocampus, cerebral cortex, and cerebellum, indicating that the role of many independent sub-populations in energy homeostasis remains to be described [4–6]. Moreover, it has long been established that both insulin and leptin are released systemically in proportion to body fat mass; however it is of great interest to understand the extent to which these pathways coordinately regulate the cellular networks that influence metabolism by identifying molecular determinants which contribute to both signaling cascades [7].

Cadm2 (also known as SynCAM2, Igsf4d, and Nectin-like molecule 3) has been shown to be enriched throughout the central nervous system, and forms oligomers via its extracellular domain [8]. As immunoglobulin domain-containing adhesion proteins, Cadm2 and its related family members, including Cadm1, have been established to mediate the assembly of pre-synaptic specializations in neurons in the brain to direct homo- and heterophilic interactions across the nascent and mature synaptic cleft [9]. Genome-wide association studies meta-analysis for body mass index (BMI) recently identified several susceptibility loci which associate with body mass index and map near genes known to function in the central nervous system including *CADM1* and *CADM2* [10–12]. We recently showed neuronal Cadm1 regulates body weight and energy homeostasis via its expression within the hippocampus and hypothalamus [13]. Here

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we illustrate loss of Cadm2 protects mice from obesity and hyperglycemia by regulating locomotor activity and thermogenesis further underlining the functional role of this gene family in systemic energy homeostasis via the brain.

2. MATERIALS AND METHODS

2.1. Animals

Mice were housed in groups of 3–5 animals and maintained on a 12-hour light/dark cycle with ad libitum access to regular chow food or high fat diet (containing 60% kcal fat, cat. no. E15741-347, ssniff Spezialdiäten GmbH), in accordance with the Landesamt für Gesundheit und Soziales (LAGeSo). All experimental procedures were approved under protocols G 0216/16, G 0357/10, G 0204/14, O 0405/09, and T 0436/08. Cadm2KO mice were generated by the trans-NIH Knock-Out Mouse Project (KOMP) and obtained from the KOMP Repository (www.komp.org). Cadm2KO mice were characterized after backcrossing for four generations to C57BL/6 and then crossed to *Lep*$^{ob/ob}$ mice (Jackson Labs). Results were consistent in both genders; however, data from female mice is not shown.

2.2. Analytic procedures

2.2.1. Quantification of metabolic parameters

Blood glucose levels were obtained with the One Touch glucometer (Bayer) and plasma insulin measurements were measured by ELISA as described (Crystal Chem) [14].

2.2.2. Antibodies for western blot analysis

The following primary antibodies were used for western blotting at 1:1000 dilution: Cadm1 (MBL CM004-3), Cadm2 (Synaptic systems, 243203), UCP-1 (Cell Signaling, #14670), STAT3 (Cell Signaling, #9139), p-STAT3 (Cell Signaling, #4113), TH (Cell Signaling, #2792), β-Actin (Sigma, A1978), GAPDH (Abcam, ab8245), α-Tubulin (Sigma, T6557). Variance in the banding patterns of Cadm proteins in different panels may result from variation in acrylamide percentage or levels of protein glycosylation. Image densitometry of 16-bit TIF images for all western blots was performed using ImageJ.

2.2.3. Primary cell cultures

Primary neurons were derived from hippocampi of mice at age postnatal day 2 and dissected in cold HBSS (Invitrogen), followed by digestion with papain. After centrifugation, tissue pellet was resuspended in Neurobasal (Invitrogen) supplemented with B27 (Invitrogen) and Glutamax (Invitrogen) for plating. Hippocampal cells were cultured in 24-well dishes with 12 mm coverslips (5000 cells per coverslip) coated with poly-D-lysine and laminin and immunohistochemistry was performed as described previously [15]. After post-fixation in 4% PFA, sections were blocked with 10% BSA/PBS, then incubated with primary antibody overnight at 4 °C, followed by the fluorochrome-conjugated secondary antibodies for 1 h. Fluorescence was imaged under a confocal microscope (Zeiss LSM700). Digital images were analyzed with Fiji/ImageJ.

2.3. Mouse phenotyping

All phenotyping analysis was performed in a 'blinded' manner; genotypes were unknown to the investigator during the experimentation and age of animals is stated in figure legends. All genotypes were present during all experiments and randomization was implemented to the extent that all animals were identified by number prior to analysis.

2.3.1. Body temperature, composition, and energy expenditure analysis

Body temperature was measured by rectal probe thermometry at ambient room temperature. Body composition analysis was measured using Minispec Model LF90 II (6.5 mHz) (Bruker Instruments). VO_2, VCO_2, food intake, and locomotor activity were measured using the PhenoMaster System (TSE, Germany), and energy expenditure was analyzed as previously described using ANCOVA [16]. Animals were placed into individual cages with weight sensors quantifying ad libitum access to food. VO_2 and VCO_2 level were measured for 1 min in a 9 min interval for 4 consecutive days and locomotor activity was measured continuously by breaks of light beams. The first 24 h of measuring time was excluded from the analysis to allow for acclimation to the new cage environment. Measurement of energy expenditure was normalized to lean body mass as previously described [13].

2.3.2. Tolerance tests

Glucose and pyruvate tolerance tests were performed following an overnight fast (16 h) and injected intraperitoneally with either glucose (2 g/kg body weight) or pyruvate (2 g/kg body weight in saline) as described [14]. Insulin tolerance tests were performed after same day fast (6 h) by injecting insulin (Sigma) intraperitoneally (0.75 U/kg body weight). Murine leptin (Peprotech) (0.75 μg/g body weight) was injected intraperitoneally twice daily (09:00 and 19:00) for 3 days. Body weight and food intake were measured daily at 08:30.

2.4. eQTL analysis

We have downloaded currently unpublished eQTL data from the GTEx consortium analysis version 6, including results from ten distinct brain regions [17]. We specifically queried the data for association tests that were performed for the gene *CADM2* against SNP rs13078960. We adjusted for multiple hypothesis testing using the method of Benjamini and Hochberg required false discovery rate (FDR) below 15% to call associations significant [18]. Boxplots were obtained through the GTEx portal.

2.5. Statistical analysis

All results are expressed as mean ± standard error (SEM), and statistical analysis is summarized in Supplementary Table 1. Comparisons between data sets with two groups were evaluated using an unpaired Student's t-test. One-way and two-way repeated-measures ANOVA analysis has been performed using GraphPad Prism Software Version 7 for comparisons of three or more groups. Post hoc statistics were performed using Sidak's multiple comparison test. A *P*-value of less than or equal to 0.05 was considered statistically significant. The presented data met the assumptions of the statistical tests used. Normality and equal variances were tested using GraphPad Prism software. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications [13].

3. RESULTS

3.1. SNP associates with increased *CADM2* in human subjects

Recent genome-wide association studies (GWAS) identified a single nucleotide polymorphism (SNP) (rs13078960) in proximity to *CADM2*, which associates with increased body mass index (BMI) in human subjects [12]. We analyzed expression quantitative trait locus (eQTL) data from 10 distinct regions of the brain available from the GTEx consortium and observed that this risk allele (the allele associated with increased BMI) is associated with increased expression of its proximal

