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G-protein-coupled receptor GPR21 knockout mice display improved glucose tolerance and increased insulin response

Jonitha Gardner [a], Sue Wu [a], Lei Ling [a,1], Jean Danao [b], Yang Li [a], Wen-Chen Yeh [a], Hui Tian [a,1], Hélène Baribault [a,*]

[a] Dept. of Metabolic Disorders, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA
[b] Dept. of Neurosciences, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA

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ABSTRACT

GPR21 is an orphan G-protein-coupled receptor. We found that mice deficient for the GPR21 gene were resistant to diet-induced obesity. Knockout mice were leaner than their wildtype counterpart, despite that no difference was observed in food intake. No differences were observed in the respiratory exchange rate and thermogenesis. However, knockout mice were more active than wildtype littermates, and this level of activity may be an underlying reason for the difference in energy balance. Mutant mice were more sensitive to insulin than their wildtype control and showed an improved glucose tolerance. Several inflammatory markers MCP-1, CRP and IP-10 were decreased in mutant animals, suggesting that GPR21 may also mediate its effect through anti-inflammatory mechanisms. We found that GPR21 is widely expressed in all tissues, with the highest levels found in the brain and in the spleen. Overall, these findings suggest that GPR21 may play an important role in regulating body weight and glucose metabolism.

1. Introduction

G-protein-coupled receptors (GPCRs or GPRs) contain seven transmembrane domains and transduce extracellular signals through heterotrimeric G proteins [1–3]. The family of GPCRs includes receptors for signals and small molecules such as light, peptidic hormones, neurotransmitters, amino acids, lipids, prostanoids and odorants. There are ~1000 genes encoding such receptors in the human genome and these receptors regulate almost all physiological processes, such as blood pressure regulation, inflammatory response and feeding behavior to name a few. Importantly, these molecules have been successful targets for the development of new therapeutic agents for a variety of therapeutic indications.

GPR21 was first identified along with GPR22 and GPR23 based on their homology to GPR20 [4]. GPR21 was isolated originally from the brain, yet, the transcript was not detected in the brain regions examined by Northern blot analysis of human RNA: thalamus, putamen, caudate, frontal cortex, pons, hypothalamus and hippocampus. GPR21 is located on chromosome 9, region q33 in humans and on chromosome 2 in the mouse. GPR21 has the highest identity to GPR52 (71%) [5]. GPR52 is also an orphan GPCR of currently unknown physiological function.

It has been suggested that the ligand for GPR21 may be a peptide, because GPR21 contains a lysine residue in an analogous position to that of the endothelin receptor which is important for its binding to a peptide agonist [4]. The ligand for GPR21 has not yet been identified. However, constitutive activity of the GPR21 receptor was observed when it was co-transfected with the promiscuous $G\alpha15_{/16}$ proteins in HEK293 cells [6]. GPR21 was also reported to activate the Gq pathway based on its effect on calcium-sensitive CHO cells [7].

The physiological role of GPR21 is still unknown. As part of an effort to identify GPCRs involved in obesity and glucose regulation, we tested the GPR21 knockout mice in a diet-induced obesity model. We found that GPR21 were resistant to gain weight in response to a high fat diet. GPR21 knockout mice exhibited an increase in glucose tolerance and insulin sensitivity when compared to their wildtype controls. These effects could be mediated through an increase in activity and a decrease in inflammatory response.

2. Material and methods

2.1. Animals

GPR21 knockout mice were produced by Deltagen (San Mateo, CA). The entire exon one from GPR21 was replaced by a 5.3 kb LacZ/Neo cassette in the GPR21 targeting vector, along with a total

Abbreviations: CRP, C reactive protein; IP-10, interferongamma-induced protein 10; MCP-1, monocyte chemotactic protein-1; SAP, serum amyloid protein.

* Corresponding author at: Amgen, Mailstop ASF1-1, 1120 Veterans Blvd., South San Francisco, CA 94080, USA. Fax: +1 650 589 3105.
E-mail address: helene@amgen.com (H. Baribault).
[1] Present address: NGM Biopharmaceuticals Inc., 630 Gateway Blvd., South San Francisco, CA 94080, USA.

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of approximately 3 kb of homology: ~2 kb 5′arm and ~1 kb 3′arm. The targeting vector was introduced into embryonic stem cells derived from the 129/OlaHsd mouse strain. Targeting events were first identified by PCR on the 5′ end with a GPR21 specific oligonucleotide ATG TGC TAG GGA CTG GGA GAG TAG G and three neo-specific oligonucleotides. The three PCR reactions gae the expected size of 3025, 3035 and 3600 bp respectively. PCR was used to confirm the targeting events on the 3′ end of the insertion. A GPR21 specific oligonucleotide CCG TGC TCA CAT TAC TCA TCC TAT GG and three neo-specific nucleotides were used in three separate PCR reactions and resulted in three bands of expected sizes: 2300, 2250 and 1550 bp respectively. Correct homologous recombination was further confirmed by Southern blot analysis of ES cells genomic DNA after digestion with HinDIII, using a probe located outside of the arms of homology. As expected from the insertion of the HinDIII site in the LacZ/Neo cassette, the targeted clone gave two bands while the untransfected clone showed one band only.

Targeted ES cell clones were injected into host blastocysts and resulting chimeric mice were bred to C57BL/6 J mice to generate F1 heterozygotes. F1 heterozygotes are backcrossed to C57BL/6 J mice for one generation. The resulting N1F1 heterozygous males and females were backcrossed to congenicity to C57BL/6 J mice as confirmed by microsatellite markers (Charles River Laboratories). GPR21 homozygous mutant mice were obtained from heterozygous mating according to a standard Mendelean transmission ratio. They appeared normal and reproduced normally.

Genotyping was performed by PCR using the following three oligonucleotides: GPR21–5′: ATA CAG AGG CGT AGT CTC CAG GGA G, neo-specific: GGG CCA GCT CAT TCC TCC CAC TCA T, GPR21–3′: AGT GAC AGT AGC TGC TCC TGA GAA C. The WT band had an expected size of 526 bp. The mutant band had an expected size of 319 bp.

Mice were housed in a specific-pathogen free animal facility with standard 12:12 dark-light cycle, with access to standard chow (Harlan Teklad Global Diets #2020) and water ad libitum. For

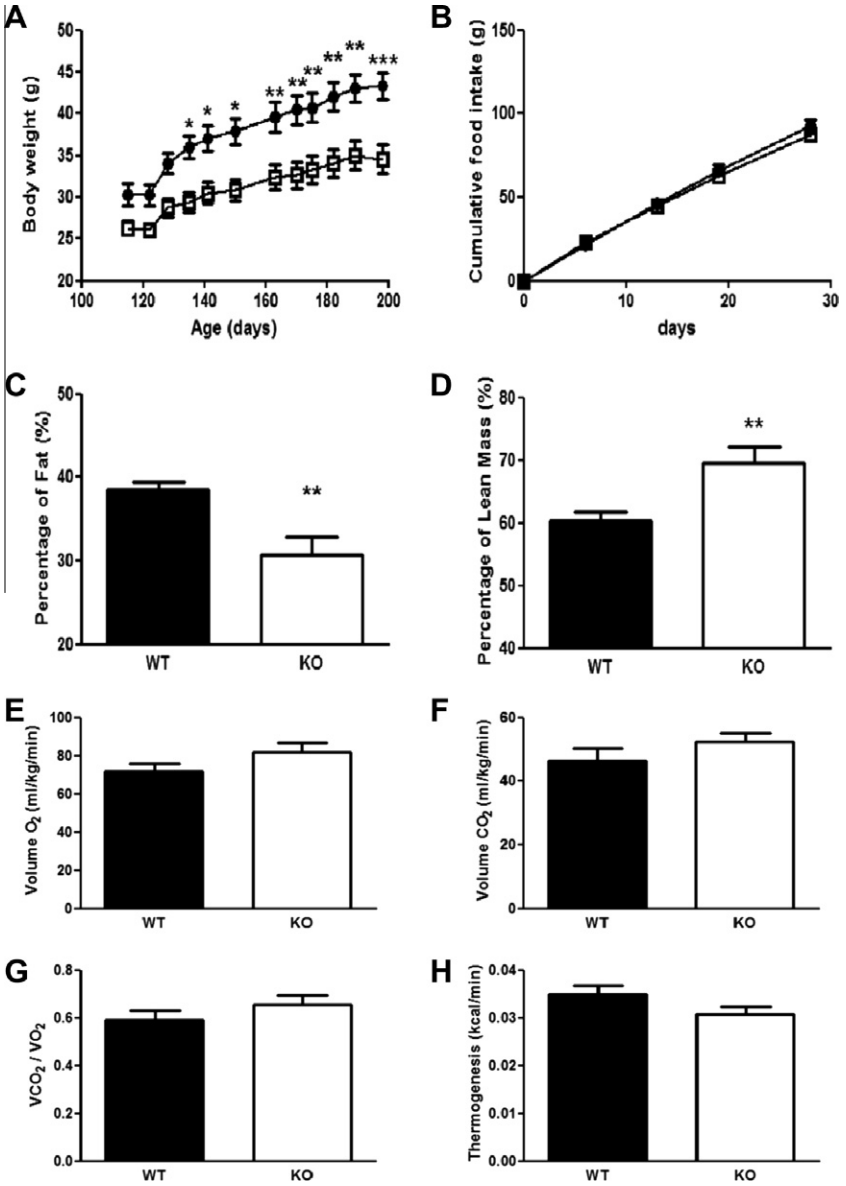

Fig. 1. Metabolic profile of GPR21 wildtype (closed symbols) and knockout (open symbols) mice were fed a 45% high fat diet for 12 weeks, starting at age 120 days. Body weight (A) and food intake (B) was measured weekly. The percentage of fat (C) and lean mass (D) were measured using a DEXAscanner. (E) Oxygen consumption (volume O_2 (ml/kg/min)) (F), carbon dioxide production (volume CO_2 (ml/kg/min)) (G), respiratory exchange ratio (VO_2/VCO_2) (C) and heat (kcal/min) (H) values were measured in GPR21 wildtype (closed bars) and knockout (open bars) mice. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.