Title

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Journal

International Journal of Obesity, 38(1)

ISSN

0307-0565

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Publication Date

2014

DOI

10.1038/ijo.2013.53

Peer reviewed

HHS Public Access

Author manuscript

Int J Obes (Lond). Author manuscript; available in PMC 2015 November 17.

Published in final edited form as:

Int J Obes (Lond). 2014 January ; 38(1): 148–151. doi:10.1038/ijo.2013.53.

The melanocortin system and insulin resistance in humans: insights from a patient with complete POMC deficiency and type 1 diabetes mellitus

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Abstract

The central melanocortin system is essential for the regulation of long-term energy homeostasis in humans. Rodent experiments suggest that this system also affects glucose metabolism, in particular by modulating peripheral insulin sensitivity independently of its effect on adiposity. Rare patients with complete genetic defects in the central melanocortin system can provide insight into the role of this system in glucose homeostasis in humans.

We here describe the *eighth* individual with complete POMC deficiency and the first with coincidental concomitant Type I Diabetes, which provides a unique opportunity to determine the role of melanocortins in glucose homeostasis in human. Direct sequencing of the *POMC* gene in this severely obese patient with isolated ACTH deficiency identified a homozygous 5′ UTR mutation −11C>A which we find to abolish normal POMC protein synthesis, as assessed *in vitro*.

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Disclosure Statement: The authors have nothing to disclose.

The patient's insulin requirements were as expected for his age and pubertal developmental. This unique patient suggests that in humans, the central melanocortin system does not seem to affect peripheral insulin sensitivity, independently of its effect on adiposity.

Keywords

POMC mutation; obesity; type 1 diabetes mellitus; melanocortin system and insulin resistance

Introduction

Proopiomelaniocortin (POMC) the protein precursor encoded by the *POMC* gene, is cleaved by prohormone convertases leading to ACTH production in corticotropes of the anterior pituitary and α-MSH in the arcuate nucleus of the hypothalamus. Homozygous or compound heterozygous loss-of-function mutations in *POMC* result in severe hyperphagia and obesity due to lack of MC4R activation by α-MSH in the hypothalamus and adrenal insufficiency due to defective ACTH synthesis in the pituitary (1) Several studies in mice have investigated the interaction between glucocorticoids, the melanocortin system and insulin sensitivity. *POMC*-null mice are hypersensitive to the adverse metabolic effects of glucocorticoids and develop insulin resistance upon glucocorticoid replacement (2, 3). This interaction has not been shown in humans with POMC mutations.

Complete POMC deficiency is rare, with only *seven* reported human cases (4–7). These patients present as newborns with adrenal insufficiency, requiring glucocorticoid replacement to prevent adrenal crises. Hyperphagia causing severe obesity begins in the first year of life.

Subject and Methods

Early Case History

The patient (patient OG215) was born at term by repeat Caesarian section to a 30 year-old G5 P2 Caucasian female with normal prenatal history. Birth weight 3.6 kg (50%). Parents are 1st cousins of Scottish and German descent. At 21 hours of life the patient developed severe hypoglycemia (blood glucose < 20 mg/dl), treated with IV glucose, then discharged from the nursery with no further evaluation.

At 6 weeks of age, the patient presented with hypoglycemic seizures that resolved with IV glucose. At blood glucose 21 mg/dl, CSF glucose was 5 mg/dl, serum insulin <5 uIU/ml, growth hormone 8.7 ng/ml, and serum cortisol <1 mcg/dl. CNS imaging did not reveal any pathology. The infant was discharged with a glucometer and was euglycemic on ad libitum feeds.

Genetic studies

With informed written consent, genomic DNA was extracted from white blood cells by standard methods. Coding regions of the POMC gene were amplified with the following primers (POMC-AF [5′GGCTCAAGGTCCTTCCTGGTGAGTGG] and POMC-AR, [5′GCCAAGATGGCAGTCATGGCCCAC], POMC-BF

[5′CCTCATGCCCTCGCGTCTTC] and POMC-BR[CTTGGCACCATCGCTGCGGGGCTC], POMC-CF[5′GAAGACTGCGGCCCGCTGCCT3′] and POMC-CR [5′CGTCATCGGCAGGGCCGTCG 3′]). Sequencing reactions were performed with a BigDye terminator kit (Applied Biosystems, Foster City, CA, USA) under standard manufacturer's conditions. Sequencing was performed on an ABIPRISM 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

HLA Genotyping

HLA genotyping was performed for eight classical HLA loci, including DRB1, DQA1, DQB1, DPA1, DPB1, A, B, and C, with a PCR-based sequence-specific oligonucleotide probe (SSOP) system that has been described elsewhere (8). Briefly, a series of oligonuleotide probes, corresponding to known polymorphic sequence motifs in the HLA genes, were immobilized onto a backed nylon membrane to create a "linear array." Relevant polymorphic exons (exon 2 for HLA class II genes; exons 2 and 3 for HLA class I genes) were amplified with biotinylated PCR primers. The PCR product was denatured, then hybridized to the appropriate linear array. After hybridization and wash, arrays were incubated with streptavidin-horseradish peroxidase, followed by the chromogenic substrate tetramethylbenzidine (TMB). Images were created by placing arrays on a flatbed scanner, and probe intensities were measured as pixel values with a proprietary genotyping software called StripScan™. Preliminary genotypes were determined with StripScan, and data from StripScan were imported into Sequence Compilation and Rearrangement Evaluation (SCORE™) software for final genotype calling.

Expression studies in cell culture

HEK 293 cells (American Tissue Culture Collection) were grown in modified Eagle's medium (MEM) with 10% calf serum, 2 mM L-glutamine, non-essential amino acids and antibiotics. Cells were transfected with plasmids containing wild-type (WT) or mutant POMC CDS using Lipofectamine 2000™ for 8 h. After 48h genomic DNA, mRNA and protein levels were analyzed by PCR, RT-PCR and Western blot, respectively. To estimate transfection levels, genomic DNA was isolated (lysis buffer: 10mM TrisHCl pH8.5, 5mM EDTA, 00.2% SDS, 0.2M NaCl, 0.1 mg/ml Proteinase K) and analyzed by PCR with primers POMC-CF and POMC-CR. RNA was isolated using TRIzol® Reagent (Invitrogen). After DNase treatment, 1 ug of total RNA was reverse transcribed by extension of random primers with M-MLV (Invitrogen). Relative POMC expression was determined by PCR using the primers POMC-CF and POMC-CR.

For protein expression analysis, transfected HEK 293 were treated with lysis buffer (20 mM Hepes pH 7,9, 125 mM NaCl, 1% NP-40, 1 mM EDTA, Protease Inhibitors (Mini, EDTA-free Protease Inhibitor Cocktail Tablet) (Roche), 5 mM β-glicerophosphate, 0,2 mM NaV, 10 mM Sodium Pyrophosphate) and whole-cell extracts were isolated. Protein was quantified with BCA™ Protein Assay Kit (Thermo Scientific) and 25ug were loaded into SDS-acrylamide gels for electrophoresis. Protein samples were analyzed by Western blot using rabbit anti-human ACTH (#AFP6328031 NHPP-NIDDK) antibody, donkey anti-

rabbit Horseradish Peroxidase linked Ig (Amersham Biosciences) and visualized with Pierce® ECL chemiluminescence detection system (Thermo Scientific).

Results

Pituitary function and obesity

At age 3 months, the patient's anterior pituitary evaluation showed isolated ACTH deficiency (baseline ACTH 3 pg/ml, cortisol 2 mcg/dl, and 60 minutes after 0.25 mg/m^2 IV cosyntropin cortisol 2.7 mcg/dl). These levels are diagnostic of ACTH deficiency in which the low, yet detectable, levels of cortisol are a reflection of ACTH-independent steroidogenesis in the human adrenal. The patient was started on hydrocortisone (~11mg/m2/day).

Thereafter, he developed an insatiable appetite such that his weight increased from the 50th %ile to >90th %ile by 6 months of age. Despite attempts to restrict his caloric intake and reduction in the hydrocortisone dose to 7 mg/m^2 the patient's hyperphagia continued. At age 13 years 6 months his weight was 83.5kg (Z-score of +2.36), height 153.9cm (Z-score of −0.76) BMI: 35.3 kg/m^2 (Z-score of + 2.48). As was found in a majority but not all patients with complete POMC deficiency the patient has red hair (Fig 1A). Evaluation for Prader-Willi syndrome and Fragile X syndrome were negative.

Genetic investigations

Sequencing of patient OG215′s DNA identified a homozygous C to A (−11C>A) transversion in the 5′untranslated region of the POMC gene that creates a novel ATG codon 11 base pairs upstream of the actual translational start site (Fig 1B). This mutation had previously been identified in two other POMC deficient patients (Fig 2) and had been proposed to completely abolish POMC expression. However, molecular studies formally demonstrating this had not been performed.

Functional studies of the −11C>A POMC gene variation

To confirm the effect of the (−11C>A) transversion on POMC expression we cloned the control (WT) and mutant (mut) POMC cDNA sequence upstream of a strong CMV promoter, transfected HEK 293 cells and determined POMC mRNA and protein expression. mRNA levels were similar between WT and mut indicating the lack of nonsense-mediated mRNA decay in the mut, however, no POMC protein was detected, confirming exclusive use of the upstream ATG for translation initiation in the mutant (Fig 1D).

Diagnosis of Diabetes mellitus and influence of POMC deficiency on insulin requirements

At 5.5 years the patient was diagnosed with type 1 diabetes mellitus presenting with diabetic ketoacidosis (blood glucose 700 mg/dl, bicarbonate 9 mEq/L, positive serum ketones, positive ICA-512 antibody in a carrier of the at risk HLA DR3/DR4 genotype (HLA class II DR-DQ genotype DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302)(8). The patient was managed with subcutaneous insulin (glargine and aspart).

As C-peptide levels were undetectable in this patient (<0.5 ng/ml), his exogenous insulin requirements are a direct gauge of his insulin sensitivity. At the onset of diabetes, the total daily dose of insulin varies from 0.5 to 1 unit/kg/day in pre-pubertal children. For patients recovering from DKA, up to 1 to 2 units/kg/day of insulin may be required. (9)

In this pre-pubertal patient with complete POMC deficiency insulin requirements have ranged from 0.5 to 1 unit/kg/day. His insulin requirements were not significantly affected by the glucocorticoid treatment. This indicates that this patient did not have insulin resistance above the level expected for his age.

Discussion

Studies in mice have suggested that POMC plays a role in the control of whole-body glucose homeostasis independently of its role on adiposity. Specifically, a number of genetic manipulations in the mouse have suggested that POMC neurons in the arcuate nucleus of the hypothalamus project to and act on distinct MC4R expressing neuronal populations to decrease insulin secretion or to increase insulin sensitivity independently of their effect on food intake and energy expenditure (10–12). As glucose sensing by POMC neurons becomes defective in obese mice on a high-fat diet it has even been suggested that loss of glucose sensing by those neurons has a role in the development of type 2 diabetes (13).

POMC null-mice are obese and glucocorticoid deficient but, unlike humans, can survive in the absence of glucocorticoid replacement. Corticosterone treatment of these mice as well as specific rescue of pituitary POMC in these mice increased their insulin resistance suggesting that hypothalamic POMC derived peptides play a critical role in glucose homeostasis that is masked by pituitary POMC deficiency in POMC null-mice (2).

In humans, severe obesity due to mutations in genes of the leptin-melanocortin pathway is associated with insulin-resistance, the level of which is, however, related to the level of obesity in leptin-receptor and MC4R deficient patients (14, 15) and we had previously observed a lack of insulin resistance in patients with a complete loss of MC4R (16).

Here we show that in a type I diabetic, POMC deficient patient, with adequate glucocorticoid replacement, hypothalamic POMC deficiency does not increase insulin requirements as glycemic control is adequately maintained with doses of insulin in the expected range for POMC sufficient individuals of his age and pubertal stage.

Therefore, based on this and previous observations, the chronic absence of a functional melanocortin system in humans does not seem to promote insulin resistance in a clinically significant way independently of its role in the maintenance of long-term energy homeostasis.

Acknowledgments

This work was performed with support from NIH RO1 DK068152IA and established investigator award from the American Heart Association (AHA#0740041N). IA was supported by a Genentech Clinical Fellowship, and an ADA Mentored fellowship award, SAR was supported by a Pediatric Endocrinology Training Grant T32-DK07161.

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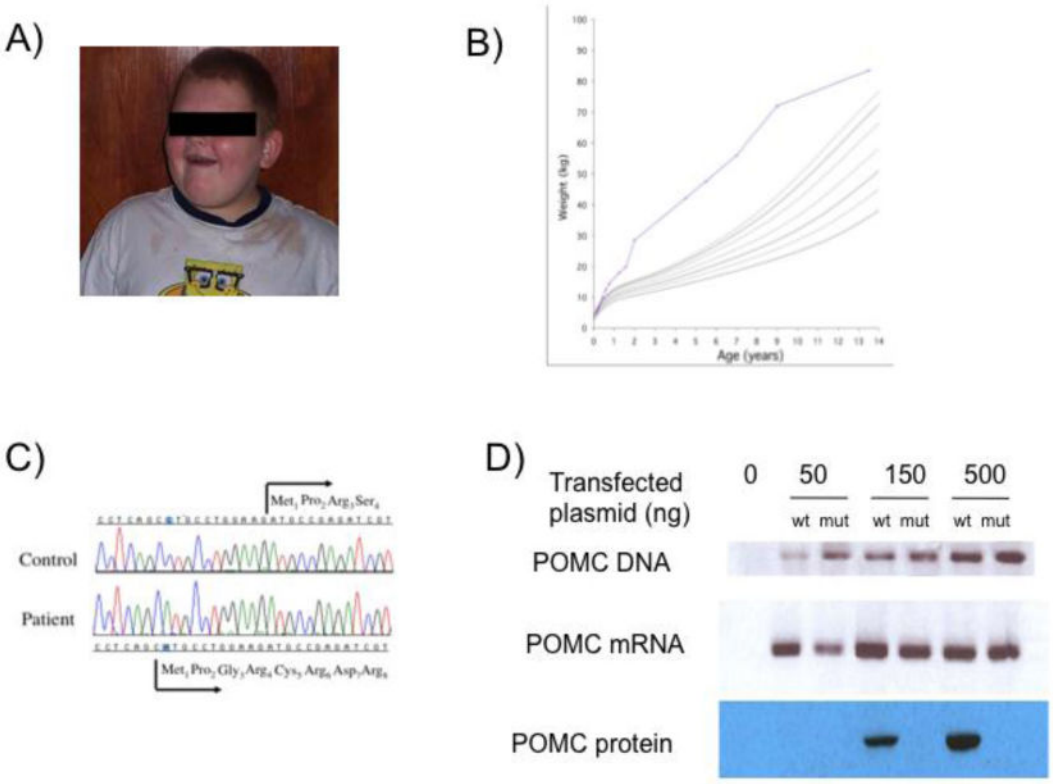


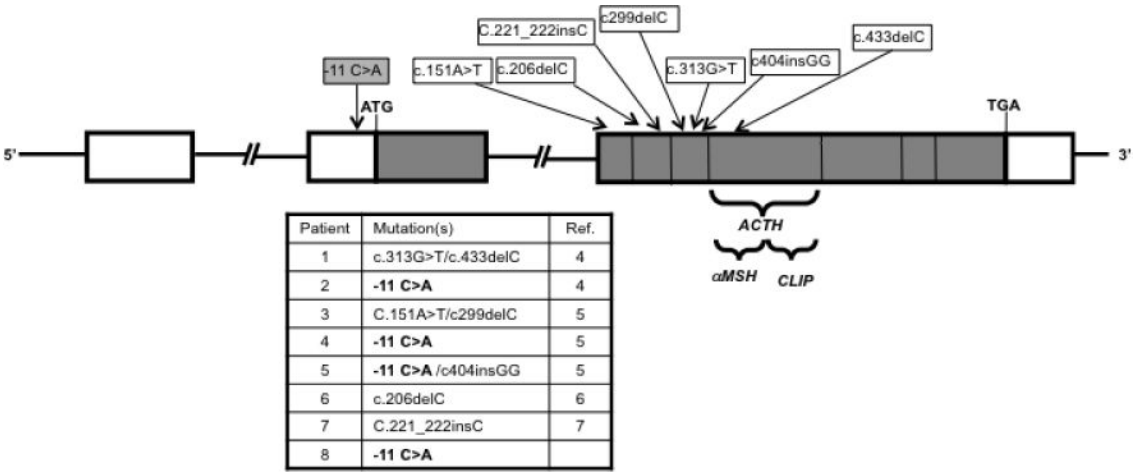
Figure 1.

A. Patient OG215 at age 10.5years.

B. Weight curve of patient OG215, US Boy percentile curves (P5, 50, 25, 75, 95, 97, 99) are shown for reference.

C. Direct POMC sequencing from a non-obese control and from patient OG215. A homozygous C->A change at -11 from the translational start site creates a novel premature ATG codon in patient OG215.

D. Functional study of the POMC −11C>A mutation: Hek293 cells were transfected with increasing concentrations of expression plasmids in which the expression of the Wild-type human POMC cDNA (wt) or the mutated −11C>A POMC cDNA (mut), with their entire 5′UTR, were driven by a CMV promoter. After 48h POMC DNA (to confirm transfection levels), mRNA and protein levels were quantified by PCR, RT-PCR and western blot. Despite the same amount of mRNA made from both constructs, no detectable POMC protein is translated from the -11C>A POMC cDNA.



Patient	Mutation(s)	Ref.
1	c.313G>T/c.433delC	4
2	**-11 C>A**	4
3	C.151A>T/c299delC	5
4	**-11 C>A**	5
5	**-11 C>A** /c404insGG	5
6	c.206delC	6
7	C.221_222insC	7
8	**-11 C>A**	

Figure 2.
POMC Mutations and genotype of the 8 described patients with complete POMC deficiency.