

# Prader-Willi Syndrome and *PCSK1* mutation: a novel presentation of combined syndromic and monogenic obesity

E. KOSTOPOULOU, D.X. SPILIOTI, N.D. PANTZARIS, B.E. SPILIOTIS

Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, University of Patras School of Medicine, Patras, Greece

**Abstract.** – **OBJECTIVE:** Prader-Willi syndrome (PWS) is a genomic imprinting disorder predominantly caused by the absence of paternally expressed imprinted genes at chromosome 15q11.2-q13. The *PCSK1* gene is vital for the processing of hypothalamic POMC to ACTH and  $\alpha$ -MSH, leading to food intake suppression and increased energy expenditure. The aim of this study was to investigate whether our PWS patient had a defect in genes involved in the hypothalamic melanocortin-4 receptor (MC4R) pathway.

**PATIENTS AND METHODS:** A 27-year-old Greek man with PWS presented to the Adult Endocrine Clinic with morbid obesity and hyperphagia. He also had obstructive sleep apnea, growth hormone deficiency, gonadal failure and metabolic disturbances. At 6 years of age, chromosomal testing confirmed PWS with a deletion in the q11q13 region of the long arm of paternal chromosome 15.

**RESULTS:** At the age of 27 years, further genetic testing was conducted, and next generation sequencing revealed a *PCSK1\_pN221D* HET mutation which was confirmed by Sanger sequencing.

**CONCLUSIONS:** Our findings suggest that different genetic abnormalities may be present in an individual with PWS and that patients with PWS may need to be investigated for *PCSK1* mutations, as the finding may potentially offer a novel treatment perspective for them.

*Key Words:*

Prader-Willi syndrome, *PCSK1*, Obesity, Hyperphagia.

## Introduction

Prader-Willi syndrome (PWS) is a multisystem genomic imprinting disorder associated with a multitude of clinical problems, including hyperphagia, morbid obesity, cognitive dysfunction, neuropsychiatric abnormalities and hypogonad-

ism<sup>1</sup>. Early clinical features of PWS include hypotonia, feeding difficulties and failure to thrive, followed by obesity and prominent hyperphagia in later infancy or early childhood. Hypothalamic dysfunction may also be present resulting in growth hormone deficiency, hypothyroidism, hypogonadism and central adrenal insufficiency. Intellectual disability and pronounced behavioral problems together with sleep and neuropsychiatric abnormalities, are additional manifestations, whereas metabolic dysfunction and type 2 diabetes mellitus are common complications<sup>2</sup>.

In the majority of cases the underlying mechanism of PWS is a deletion of the paternal chromosome 15q11-q13 region while maternal uniparental disomy 15 is also responsible for 20-30% of the cases. Also, the chromosome 15q11-q13 region is genomically imprinted and an imprinting center defect or unbalanced translocation is found in 1-3% of the PWS cases<sup>3</sup>.

Several imprinted genes expressed exclusively from the paternal chromosome are believed to be involved in PWS, such as: SNURF-SNRPN, Necdin (NDN), MAGE Family Member, Melanoma antigen family L2 (MAGEL2), Makorin Ring Finger Protein 3 (MKRN3), Chromosome 15 open reading frame 2 (C15orf2) and HBII snoRNA cluster<sup>4</sup>.

Notably, in a small number of patients with PWS, a deletion encompassing the Small Nucleolar RNA, C/D Box 116 Cluster (SNORD116) was found in the critical region containing the functional PWS gene locus in the 15q11-13 region of the paternally expressed genes and these patients have a milder phenotype of PWS<sup>5</sup>. SNORD116 is expressed predominantly in the brain and lack of the expression of SNORD116 has been reported in mice which have the obese hyperphagic phenotype and cognitive deficits which mimic those found in PWS<sup>6</sup>.

It has been shown that PWS patients with a deletion of *Magel2* manifest hyperphagia and multiple clinical symptoms of PWS<sup>7</sup> and animal studies have shown evidence that *Magel2*-null mice display abnormal development of anorexigenic  $\alpha$ -melanocyte-stimulating hormone (MSH) axons in the arcuate nucleus (ARC) of the hypothalamus and aberrant activation of melanocortin-4 receptors (MC4R)<sup>8</sup>.

Of particular interest though, is a study that showed that the protein and transcript levels of nescient helix loop helix 2 (NHLH2) and the pro-hormone convertase PC1 (encoded by the *PCSK1* gene in the hypothalamic leptin-melanocortin pathway involved in the regulation of appetite) were reduced in PWS patient induced pluripotent stem cell-derived neurons<sup>9</sup>. Furthermore, another study<sup>10</sup> showed that *Nhlh2*-null mice which are obese, hypogonadal, and display reduced linear growth have hypothalamic levels of *PCSK1* transcript and PC1 protein that are reduced by more than 50%.

### Case Presentation

We present a 27-year-old young man of Greek heritage with morbid obesity, hyperphagia and continuous food craving with a BMI of 88.3 (height: 158 cm, weight: 220.4 Kg) (Figure 1). His perinatal history revealed that he was the first child of non-consanguineous, non-obese Caucasian parents. He was born at 40 weeks of gestation by C-section and was small for gestational age. On the second day of life, he was admitted to the Neonatal Unit because of poor feeding and hypotonia. NG-tube feeding was initiated and he was discharged on a hypercaloric formula 3 weeks later.

The hypotonia and poor weight gain gradually improved within the first few months of life and his growth parameters and appetite increased significantly in early childhood. After the age of 3 years, he had hyperphagia with continuous food craving and his weight exhibited a rapid rise with a continuous increase (Figure 2A). He had a history of gross motor delay, as he walked at the age of 2 years, and later he had a mild learning disability and required supplementary home teaching during grade school. The patient was confirmed to have Prader-Willi syndrome at 6 years of age when chromosomal testing revealed a deletion in the q11q13 region of the long arm of the paternal chromosome 15. He was then referred

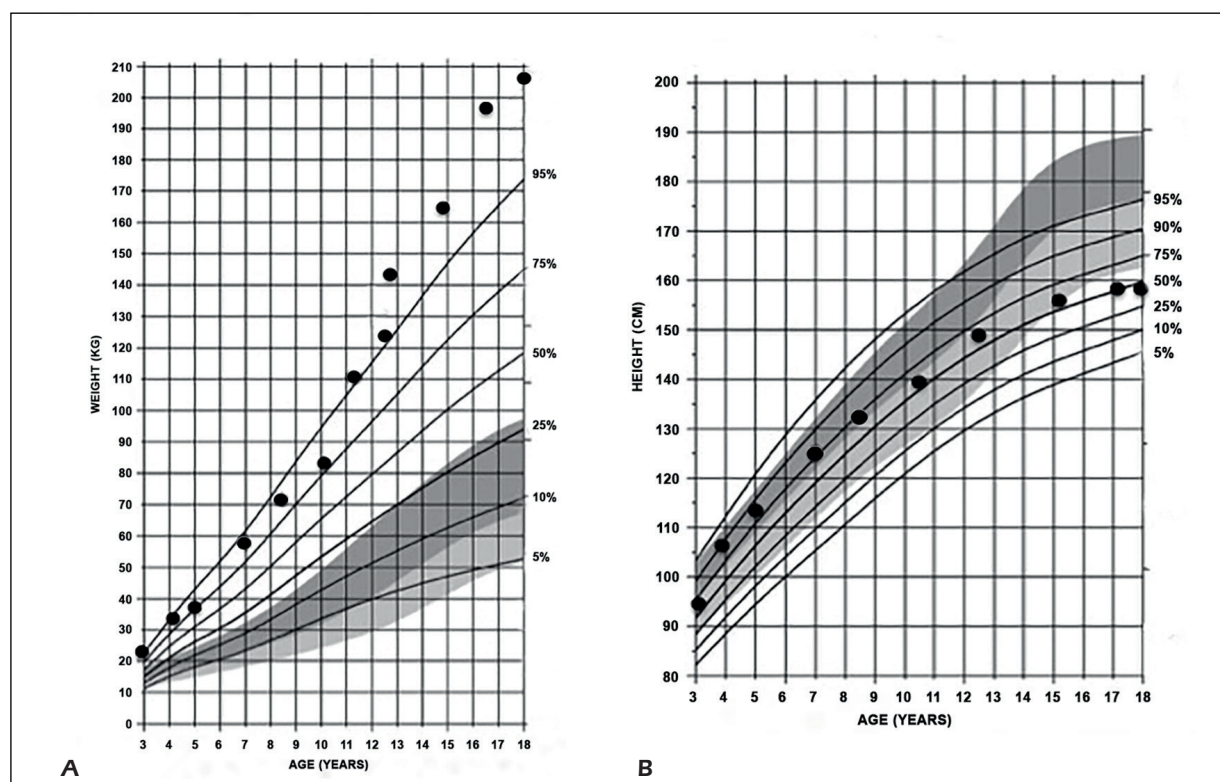
by his pediatrician to the Outpatient Clinic of the Division of Pediatric Endocrinology at the University Hospital of Patras, Greece.

After his first Pediatric Endocrine Clinic appointment, he was referred to a dietician and a psychologist for monitoring of his dietary and developmental status. Throughout the following years during his follow-up visits, because of his hyperphagia and continuous food craving, despite substantial dietary intervention, his weight and BMI continued to increase excessively from a baseline body weight of 20 kg and BMI of 22.3 at 3 years of age to a body weight of 206.4 kg and BMI of 82.7 at his last Pediatric Endocrine Clinic visit at 18 years of age (Figure 2A). Also, at the age of 11 years, he started to show a reduced height velocity and he attained his final height of 158 cm at 17 years of age (Figure 2B). He was then lost to follow-up and he came to the Adult Endocrine Clinic at the age of 27 years, at which visit his weight was 220.4 kg with a height of 158 cm (BMI of 88.3).

As the years progressed, new clinical symptoms were added to the phenotype. He developed obstructive sleep apnea and has been on CPAP



**Figure 1.** Patient with Prader-Willi syndrome and heterozygous *PCSK 1* mutation.



**Figure 2.** A, Weight gain of patient with Prader-Willi syndrome and heterozygous *PCSK1* mutation as shown in a growth chart for Prader-Willi syndrome. B, Linear growth of the studied patient with Prader-Willi syndrome and heterozygous *PCSK1* mutation as shown in a growth chart for Prader-Willi syndrome.

during the night-time since the age of 10 years. Growth hormone (GH) provocative tests showed that he had GH deficiency, but because of his profound obstructive sleep apnea, he was never started on growth hormone treatment. Due to primary gonadal failure, intramuscular testosterone enanthate therapy was initiated. He also developed metabolic disturbances in the form of insulin insensitivity at the age of 15 years and Type 2 Diabetes mellitus at the age of 17 years, for which he is currently being treated with combined metformin 1000 mg and dapagliflozine 5 mg p.o. twice daily, as well as Liraglutide 1.5 mg./week i.m.

Of particular interest was the fact that he was able to finish high school (Greek Lykeion).

The blood testing and imaging that were performed throughout the years showed normal liver function tests, renal function, biochemical, thyroid and lipid profiles. From the age 17 years though, he has hepatic steatosis (NAFLD grade 1). His cardiologic exam was normal. Of note, the patient never experienced malabsorption symptoms.

Based on the multitude of new literature reports concerning abnormalities in genes encoding the hypothalamic appetite center in PWS and the patient's mild PWS phenotype with a lack of behavioral abnormalities, absence of profound cognitive dysfunction and a predominance of his appetite disorder and morbid obesity, it was decided to offer the patient further genetic testing regarding genes involved in the hypothalamic ARC regulating appetite and energy homeostasis. Therefore, the aim of this study was to investigate whether the patient had any defects in genes involved in the hypothalamic melanocortin-4 receptor (MC4R) pathway.

## Patients and Methods

At the age of 27 years, genetic testing for possible mutations in the hypothalamic MC4R pathway was performed in our PWS patient by Prevention Genetics (Marshfield, WI, USA).

The study was approved by the Ethical Committee of the University of Patras School of

Medicine (IRB number: 64904, date of approval: 29/07/2019) and was in accordance with the Declaration of Helsinki of 1975, revised in 2013. Informed consent was obtained from the patient for participating in the study, blood sampling, and DNA testing.

### DNA Extraction

DNA was extracted from the blood sample using the JANUS<sup>®</sup> Integrated Workstation *via* magnetic bead technology. Isolated DNA was then normalized on the same instrument to a working concentration of 15 ng/L. DNA sample quality was assessed using a Nanodrop Spectrophotometer. Samples with a 260/280 ratio of ~1.8 and a 260/230 ratio of 1.8-2.2 were considered pure. Any samples with a 260/280 ratio less than 1.60 were purified prior to downstream use.

### Library Preparation

Patient-specific genomic libraries were prepared using 50-300 ng of purified DNA. Whole genomic DNA was enzymatically sheared, and the ends of each strand of DNA were modified so a universal adapter could be ligated to the ends. The DNA fragments were then hybridized to custom oligonucleotides (60-120 bp in length) that were complementary to the regions of interest. Hybridized fragments were then isolated and amplified through a limited number of PCR cycles.

### Next Generation Sequencing (NGS)

Patient DNA libraries were loaded onto an Illumina NovaSeq 6000, where single stranded DNA was duplicated *via* bridge amplification followed by visualization through sequencing by synthesis (SBS) technology. The NovaSeq utilized a flow cell with hollow channels filled with tethered oligonucleotides that were complimentary to the adapter sequences on either end of library-prepared DNA. The double-stranded ends of the DNA were denatured and hybridized to the flow cell. Amplification occurred on both strands simultaneously. Once double-stranded DNA was formed, the process repeated itself until no unused oligonucleotides were left on the flow cell surface. To prepare the strands for sequencing, linearization was performed. Reverse strands were cleaved, and the DNA strands were read by the sequencer using cyclic reversible terminator (CRT) dye technology. The resulting reads were then filtered by quality and aligned to a reference sequence by the Infinity v.1.0.0 pipeline for analysis.

### Gene Panel

DNA was sequenced for the presence of variants. The variants were determined using a GATK-based pipeline, followed by variant annotation to identify potentially clinically-relevant variants.

### Sanger Sequencing

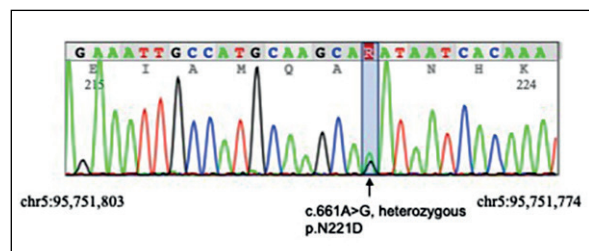
Genomic DNA was extracted from the blood sample for Sanger sequencing and Polymerase Chain Reaction (PCR) was used to amplify the targeted regions. After purification of the PCR products, cycle sequencing was carried out using the ABI Big Dye Terminator v.3.1 kit (Thermo Fisher Scientific, Waltham, MA, USA). PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Cycle sequencing was performed separately in both the forward and reverse directions. ABI trace files were visualized as a sequencing chromatogram by 4 peaks.

## Results

The NGS revealed a *PCSK1* *pN221D* HET mutation. Sanger sequencing of the *PCSK1* transcript confirmed a heterozygous A>G mutation at coding position 661, resulting in the missense variant p.N221D (Figure 3).

## Discussion

The *PCSK1* gene consists of 14 exons located on chromosome 5 in humans<sup>11</sup>. It encodes the Pro-protein convertase 1/3 (Prohormone convertase/PC1), which performs the proteolytic cleavage of prohormones to their intermediate forms or functional peptides in neural and endocrine tissues, such as the pituitary gland, the hypothalamus, the



**Figure 3.** Sanger sequencing of heterozygous *PCSK1* mutation. Green, red, black, and blue correspond to adenine, thymine, guanine, and cytosine nucleotides, respectively.

intestine and the pancreas. These prohormones are implicated in the regulation of food intake, glucose and energy homeostasis. PC1 is vital for the processing of proopiomelanocortin (POMC) to ACTH and then to  $\alpha$ -MSH in the pituitary gland, of proinsulin to insulin in the Langerhans islets and of proglucagon to GLP-1 and GLP-2 in the small intestine<sup>12,13</sup>. It is well known that the activation of the POMC neurons by leptin triggers  $\alpha$ -MSH production and release from the POMC neurons, which subsequently activates melanocortin receptor 3 (MC3R) and melanocortin receptor 4 (MC4R). As a result, food intake is suppressed and energy expenditure is increased.

Specific polymorphisms in the gene have been associated with increased risk of obesity<sup>14,15</sup>. It has been reported that there is an increased risk of obesity by 8.7-fold in heterozygous carriers of mutations in the *PCSK1* gene, including the mutation that was detected in our patient (N221D), as well as those heterozygous mutations may result in extreme obesity<sup>16</sup>. The *PCSK1* gene was one of the first genes linked to monogenic obesity<sup>17</sup>. As opposed to other forms of monogenic obesity, such as mutations of *leptin* or *leptin receptor*, *POMC* and *MC4R*, *PCSK1* gene mutations can cause central and enteric endocrinopathy that result in both poor growth in early stages of development and excessive weight gain later on<sup>18</sup>.

The clinical phenotype of *PCSK1* gene deletion includes hyperphagia and early onset obesity, polyuria, polydipsia, abnormal glucose homeostasis mainly in the form of impaired glucose tolerance, malabsorptive diarrhea, which is the most common clinical presentation, decreased linear growth and growth hormone deficiency and can include hypogonadotropic hypogonadism, central hypothyroidism and central hypocortisolism<sup>19</sup>. Many clinical features are very similar to the ones seen in Prader-Willi syndrome, with the exception of malabsorptive diarrhea, which is only seen in *PCSK1* gene deletion, and hypotonia and mild developmental delay, which are only observed in PWS.

Confirmation of a link between PWS and diminished *PCSK1* expression would be of particular interest in terms of treatment. Setmelanotide, an MC4R agonist, has been used successfully to significantly reduce the hyperphagia and weight of two patients with POMC deficiency<sup>20</sup>. It is interesting to speculate whether this could potentially have the same effect in our patient with Prader-Willi syndrome who has a concomitant heterozygous mutation of the *PCSK1* gene.

To our knowledge, this is the first patient with Prader-Willi syndrome reported to have a heterozygous mutation in the *PCSK1* gene and also the first reported case of combined syndromic and monogenic obesity. This is an important finding not only because of the rarity of the co-existence of two rare conditions, but also because it suggests that patients with Prader-Willi syndrome may also need to be investigated for *PCSK1* gene mutations, particularly in the presence of clinical characteristics suggestive of *PCSK1* gene deletion. This not only broadens the understanding of Prader-Willi syndrome, but also of obesity as a whole. Furthermore, it potentially offers an entirely new perspective in the management of this condition.

## Conclusions

The advances in genetic research have broadened the differential diagnosis in pediatric obesity and hyperphagia. More significantly, it has become apparent that different genetic abnormalities may be present in the same patient and may have a cumulative effect on the phenotype. It seems that one genetic diagnosis does not exclude the existence of a second genetic diagnosis as is shown in our patient who has PWS and a heterozygous *PCSK1* gene mutation. This is important not only for the diagnosis, but also for the treatment and the prognosis of Prader-Willi patients in the future.

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## Conflict of Interest

Eirini Kostopoulou is involved in a research protocol with Rhythm Pharmaceuticals, Inc. Boston, Mass. USA, Diamantina X. Spilioti has nothing to declare. Nicholas D. Pantzaris has nothing to declare. Bessie E. Spiliotis is involved in a research protocol with Rhythm Pharmaceuticals, Inc. Boston, Massachusetts, MA, USA.

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## ORCID ID

Bessie E. Spiliotis: 0000-0001-7922-4012.

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