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Molecular studies on preproghrelin gene: Alternative splicing, obestatin effects, coding region and variants

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Introduction

Obestatin is a 23-amino acid peptide produced by post-translational modifications of the 117-residue preproprotein called Ghrelin and Obestatin Prepropeptide (GHRL) that undergoes cleavages, generating obestatin, ghrelin and a signal peptide. Despite being derived from the same prepropeptide, obestatin and ghrelin have opposite effects on weight gain, food intake, energy balance and gastric emptying [1,2]. While ghrelin has orexigenic properties (such as increased fat deposition and inhibition of insulin secretion), obestatin acts on anorexigenic effects slowing gastric emptying and suppressing food intake [3]. The physiological role of obestatin is considered by many authors multifunctional, acting in several tissues and promoting effects besides the antagonistic effects to ghrelin [3]. First, the G protein-coupled receptor-39 (GPR39) was indicated as an obestatin cognate ligand receptor, but this is not supported by several authors, indicating the need for new and more accurate research [4-6]. However, that peptide clearly shows an important role in metabolism involved with anti-inflammatory and cardioprotective effects [7-19]. Furthermore, polymorphisms found in *GHRL* gene are also being associated with different clinical conditions [20-23]. Thus, this article intends to describe examples of variants found in the *GHRL* coding gene obestatin-associated and to report the variants produced by alternative splicing processes in the whole gene.

Examples of obestatin-associated effects

Several physiological effects have been described for obestatin, however, the mechanisms are complex and need a better investigation. In the gastrointestinal system, obestatin acts increasing the secretion of enzymes released by the pancreas. Furthermore, that hormone reduces jejuni´s motility, gastric emptying, consequently reducing food intake and body weight [3]. Moreover, other mechanisms were proposed, such as those involved in the central nervous system and its association with positive effects on memory retention, increasing sleep promotion and inducing anxiolytic activity, opposing the effect of ghrelin on anxiety [1,8]. Obestatin also participates in cell proliferation. That peptide stimulates the primary cultures of human retinal epithelial cells (hRPE) [9], relevant effects on cell proliferation, apoptosis and progesterone releasing by ovarian cells [10]. Studies have shown that obestatin does not alter growth hormone (GH) and corticosterone secretion, but it was associated with vasopressin decreasing plasma levels, evidencing an important role in homeostatic regulation [11,12]. Obestatin has also been investigated in type 2 *Diabetes mellitus*, obesity, atherosclerosis, anorexia nervosa and cancer studies by different mechanisms [3,24].

GHRL polymorphisms – obestatin coding region

Up to now, 19 polymorphisms were found in the obestatin coding region, based on data obtained from dbSNP (Table 1). After researching in Pubmed, Scielo and Science Direct databases, only rs4684677, rs186599567 and rs149447194 were found described by authors.

The polymorphism rs4684677

The genetic variation Gln90Leu (Q90L, T/A, rs4684677) is a single nucleotide polymorphism (SNP) that leads to the exchange of the amino acid located at position 90 of preproghrelin in isoform 1. This variant was firstly identified in a German association study. The variant showed a significant difference (p=0.011) among extremely obese children and adolescents and students of University of Marburg with normal weight [13].

Furthermore, it was suggested that rs4684677 probably contributes to autoimmune thyroid disease predisposition in a case-control study that investigated polish children (p=0.002) [14], as well as increased women's waist circumference in a Spanish sample population [15]. No significant association was observed in a Brazilian population sample in a study conducted for gestational diabetes [16].

The polymorphisms rs149447196 and rs186599567

The polymorphism rs149447196 is characterized by G/A substitution, silent mutation (Asn → Asn) which corresponds to the 77th position in the preproghrelin, isoform 1 (2nd amino acid position in the obestatin peptide). The polymorphism rs186599567 is an A/G gene variant responsible for changing the 98th amino acid (Leu → Pro) of the isoform 1. This position is essential for the recognition of obestatin post-translational sites. Both rs149447196 and rs186599567 were investigated in association with congenital anorectal malformation and Hirschsprung disease, demonstrating the possibility of association [17].

Alternative splicing variants

A research in the NCBI nucleotide database showed variants generated from alternative splicing in the preproghrelin gene. Overall, there are 11 variants identified by biological evidence (numbered 1, 2, 3, 4, 5, 8, 9, 10, 11, 12 and 13) and another 2 (called X1 and X2)

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Table 1. Polymorphisms in obestatin coding regions and general characteristics

Variant	NTc	AAp	AA and characteristics	Taqman code
rs1013246671	C/T	77	Asn-Ser; Missense; Polar	NA
rs149447194	G/A	77	Asn-Asn; Silent; Polar	C_169849178_10
rs752298108	C/T	78	Ala-Thr; Missense; Non-polar-Polar	NA
rs755045075	GGG/-	79	Pro/-; Indel;	NA
rs781599822	-/G	80	-/X; Indel;	NA
rs201358681	A/G	80	Phe-Ser; Missense; Non-polar-Polar	C_191183461_10
rs1044457750	-/C	85	Lys-Asn; Indel; Basic-Polar	NA
rs376322935	A/G	86	Leu-Pro; Missense; Non-polar	NA
rs751003045	C/A	88	Gly-Trp; Missense; Non-polar	NA
rs765646482	C/G/T	88	Gly-Gly; Silent; Non-polar	NA
rs376856625	C/T	89	Val-Ile; Missense; Non-polar	NA
rs753863091	G/A	90	Gln-*; Stop Gained;	NA
rs4684677	T/A	90	Gln-Leu; Missense; Polar-Non-polar	C__25607748_10
rs372665020	C/T	90	Gln-Gln; Silent; Polar	NA
rs781508813	G/T	93	Gln-Trp; Missense; Polar-Non-polar	NA
rs377110254	G/C	94	His-Asp; Missense; Basic-Acid	NA
rs544937594	C/G/T	95	Ser-Asn; Missense; Polar-Acid	NA
rs774405407	G/A	96	Gln-*; Stop Gained	NA
rs186599567	A/G	98	Leu-Pro; Missense; Non-polar	C_181918670_10

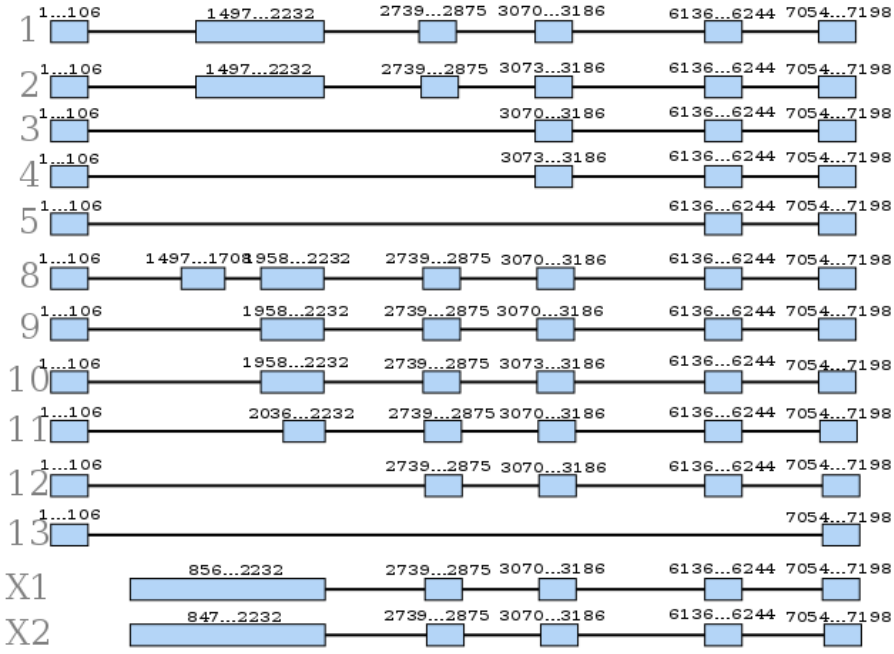

Figure 1. Regions transcribed from each of the variants of the preproghrelin gene. The numbers above the boxes mean the position of the nucleotides in the gene

identified by the computational prediction method Gnomon [18]. The variants have different sizes and differ from each other by including and/or excluding some exonic regions, as shown in Figure 1.

According to the NCBI nucleotide database, variants 8, 9, 11 and 12 contain the ligands ghrelin-28 and obestatin, differing from variant 1 only in the 5' UTR. Variants 2 and 10 are constituted by an alternate in-frame splicing site when compared with variant 1. These isoforms contain the ghrelin-27 and obestatin ligands and differ from each other in the 5' UTR.

Variants 3 and 4 lack two exons, while variant 5 lacks three exons in comparison to variant 1: all three are shorter variants but retain the reading frame. Variant 13 is classified as non-coding because it does not meet RefSeq criteria and lacks several exons present in variant 1.

Seim and colleagues [25] studied different amplicons of the ghrelin gene and were able to show multiple transcript variants originated by alternative splicing in different tissues, with the largest variety found in the stomach. However, the biological role of these variants is not fully elucidated, requiring further studies in the area.

Conclusion

In this brief report, we analyzed aspects of the portion of the *GHRL* gene coding for obestatin, its physiological role and some polymorphisms associated with clinical characteristics. In addition, once the role of transcript variants is still unknown, we searched

on databases to describe them in the whole gene, making it more accessible, updated and clearer to interested readers and researchers.

Authors and contributions

Study concept and design: LK, LTM (Ph.D.) and HRF (advisor, Ph.D.). Acquisition of data: LK, AdSK and ABL. Drafting the manuscript (and figure design and evaluation): LK, LTM, LMW and HRF. Critical revision of the manuscript for important intellectual content: LK, LTM, LMW, AdSK, ABL, KSK, HRF.

Conflict of interest disclosures

The authors declare no potential conflicts of interest relevant to this article.

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