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Brief Report

Molecular Studies on Preproghrelin Gene: Signal Peptide, Ghrelin Coding Region and Variants – A Brief Report Focusing on rs34911341 Polymorphism

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Abstract

Background: Ghrelin is a hormone that exhibits effects in a lot of biologic processes such as food intake regulation, gastrointestinal motility and carbohydrate metabolism. Ghrelin is encoded by human preproghrelin gene (*GHRL*), containing 4 exons. However, experiments suggested a new molecular structure for this gene.

Objectives: This study aimed to clarify *GHRL* and its products by a simple representation, showing the variants described for this gene up to this moment.

Methods: The *GHRL* sequence for molecular comparisons was obtained from GeneBank. The dbSNP was used to search variants and the PubMed, SciELO and Science Direct databases to investigate related studies.

Results: The new molecular structure of *GHRL* includes a new exon 0 and an extended exon 1, located in the originally identified region of intron 1. Examples of *GHRL* products are signal, ghrelin and obestatin peptides and these molecules exhibit different effects on the organism. Thus, polymorphisms in these gene regions have been associated with a vast number of clinical effects including metabolic disorders. We identified 11 genetic variations in the region of *GHRL* which encodes the signal peptide and 23 polymorphisms in the ghrelin coding region. One of them, rs34911341, has been associated with some disorders such as diabetes, hypertension and obesity.

Conclusion: In this scientific article, we made *GHRL* a little bit more informative for readers. Furthermore, we highlighted the current reported genetic variations in *GHRL* associated with signal and ghrelin peptides, but only the rs34911341 variant has been associated with metabolic disorders.

Keywords: *GHRL*, Polymorphism, Diabetes, Obesity, Hypertension.

Background

Ghrelin has been massively described as a central and peripheral factor which induces effects on appetite, carbohydrate metabolism, gastrointestinal motility and pancreatic secretion (1). Furthermore, ghrelin has been found to significantly decrease thyroid hormone concentrations (2). Thus polymorphisms found in the ghrelin coding region of the human preproghrelin gene (*GHRL*) have been associated with a different number of clinical effects. It has been reported that *GHRL* is composed of 4 exons on the short arm of chromosome 3 (3). However, Seim et al (4) suggest a new molecular structure for this gene. Thus, this study aimed to clarify *GHRL* and its products by a simple representation, highlighting the main polymorphisms described for *GHRL* up to this moment, specifically for the coding region related to the signal and ghrelin peptides of the isoform 1 (major isoform).

Methods

The data for the comparison of the described molecular structure of *GHRL* (NCBI and Seim et al) (4) were obtained from GeneBank (NCBI Reference Sequence: NG_011560.1).

The dbSNP (http://www.ncbi.nlm.nih.gov/snp) was used to find polymorphisms described.

The Ferret software version 2.1, which uses 1000 Genomes Project information, was used to obtain genomic data of 2504 individuals. This information was subsequently analyzed for linkage disequilibrium using Haploview software version 4.2, Tagger option. A Minor Allele Frequency (MAF) of 0.05 and a coefficient of correlation (r^2) of 0.8 were the parameters used for this analysis.

Report Highlights

This scientific article was made in order to make the molecular structure of *GHRL* less difficult for readers, as well as to show the described variants in specific gene regions.

The PubMed (http://www.ncbi.nlm.nih.gov/pubmed), SciELO (http://www.scielo.org/php/index.php) and Science Direct (http://www.sciencedirect.com) databases were used to investigate published studies about genetic variations found in dbSNP. All the reference SNPs (rs) found in dbSNP were searched in all these databases.

Results and Discussion

Molecular Structure of *GHRL*

The *GHRL* gene (ghrelin/obestatin prepropeptide [Homo sapiens (human)] NCBI: Gene ID: 51738, OMIM: 605353, NCBI Reference Sequence: NG_011560.1) is located at 3p25.3 region and is composed of 7198 base pairs (Figure 1A). According to the sequence found in NCBI, *GHRL* presents 4 exons. However, Seim et al (4) proposed, after experiments, a new molecular structure for this gene. Compared with the sequence found in NCBI, Seim et al (4) proposed an exon called -1, which has the same sequence of the exon 1 (NCBI); a new exon 0, which has 736 bp and is found in intron 1 (NCBI); and an extended exon 1 (E1), with 188 bp, also located in intron 1 (4).

GHRL Products

The product of the *GHRL* is a ghrelin precursor called preproghrelin, a peptide which is composed of 117 amino acid residues (isoform 1). Post-translational processing might lead to different products such as a 23-amino acid signal peptide and a 94-amino acid segment, called proghrelin (1). Proghrelin is metabolized in ghrelin (des-acyl ghrelin, represented by 24-51 amino acids residues) and C-ghrelin, also called obestatin, (76-98 amino acid residues) (1) (Figure 1B). Furthermore, in order for ghrelin to come into its full activity, it is essential that this peptide should undergo modifications (5). This process consists of an acylation, a chemical process in which ghrelin receives an octanoyl group at the third serine amino acid residue (Ser3) (1). This acylation reaction is catalyzed by ghrelin O-acyl transferase (GOAT) and its product is the acyl ghrelin (Figure 1C).

GHRL Polymorphisms – Signal Peptide and Ghrelin Coding Regions

Until now, 11 single nucleotide polymorphisms were

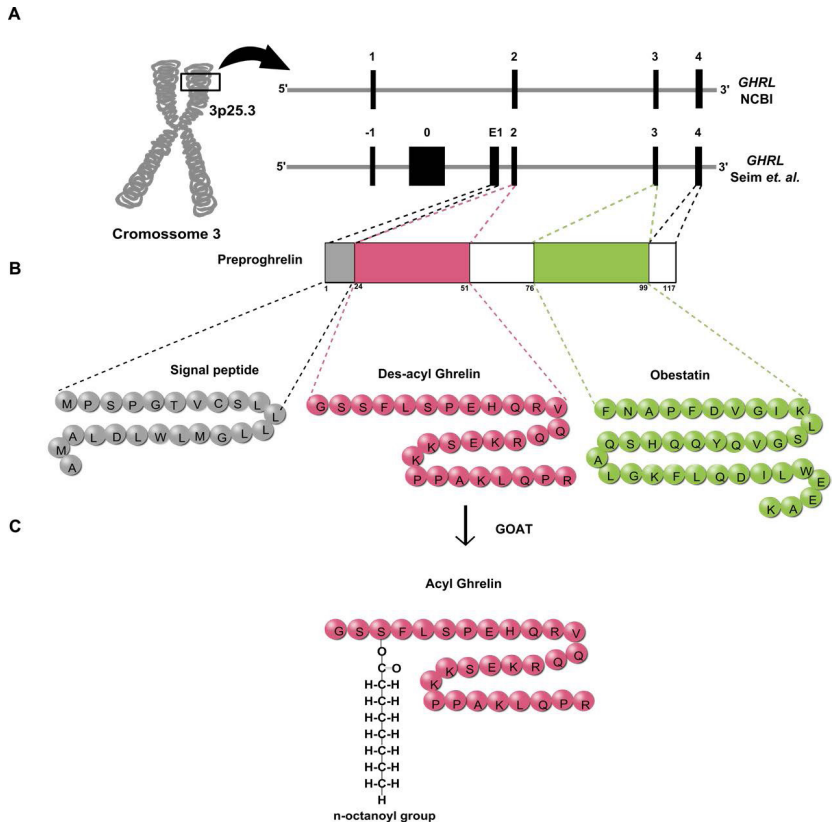

Figure 1. Representations of the Molecular Structures of *GHRL* and its Products.
A: Simplified comparison and representation of the *GHRL* from NCBI and Seim et al. The gray horizontal bars represent the whole gene and introns. The numbered boxes (black), the exons. E1: extended exon 1. **B:** *GHRL* products found after transcription and translation processes, as well as post-translational modifications. **C:** Products formed after GOAT catalysis at Ser3 residue.

Table 1. Variants Found in Signal Peptide and Ghrelin Coding Regions and Their Characteristics

Variant	NTc	AAp	AA and Characteristics	Taqman® Code
rs753640208	A/G	3	Ser-Ser; Silent; Polar	NA
rs559789290	C/T	6	Thr-Ala; Missense; Polar – Apolar	NA
rs147996515	C/T	7	Val – Ile; Missense; Apolar	C_169746153_10
rs774664563	-/CGGT	7	Val-Asp; InDel; Apolar - Polar	NA
rs760559023	G/T	9	Ser-Arg; Missense; Polar-Basic	NA
rs775447610	C/G	12	Leu-Val; Missense; Apolar	NA
rs540101934	C/T	14	Gly- Ser; Missense; Polar	NA
rs759055681	C/T	14	Gly-Asp; Missense; Polar	NA
rs370367548	C/G/T	15	Met – Ile; Missense; Apolar	NA
rs777169175	C/G/T	17	Trp-Ter	NA
rs769018720	A/G	18	Leu-Pro; Missense; Apolar	NA
rs747522812	A/C	20	Leu-Phe; Missense; Apolar	NA
rs780582809	C/T	21	Ala-Thr; Missense; Apolar - Polar	NA
rs758478747	A/G	21	Ala-Val; Missense; Apolar	NA
rs745943870	C/T	22	Met-Val; Missense; Apolar	NA
rs149396238	C/G	24	Gly-Ala; Missense; Polar – Apolar	C_169845502_10
rs375923081	A/G	24	Gly-Gly; Silent; Polar	NA
rs754016454	A/G	25	Ser-Phe; Missense; Polar – Apolar	NA
rs763897355	A/G	28	Leu-Pro; Missense; Apolar	NA
rs199668506	C/G	28	Leu-Leu; Silent; Apolar	C_191214822_10
rs200551646	C/T	29	Ser-Asn; Missense; Polar	C_191260430_10
rs771527525	-/CT	34	Arg-Ser; InDel; Basic - Polar	NA
rs368923730	C/T	36	Gln-Arg; Missense; Polar - Basic	NA
rs766038463	C/G	37	Gln-His; Missense; Polar - Basic	NA
rs762523533	C/T	40	Glu-Lys; Missense; Acid - Basic	NA
rs577851455	C/T	40	Glu- Gly; Missense; Acid - Polar	NA
rs761310643	A/C/G	41	Ser-Trp; Missense; Polar – Apolar	NA
rs146899970	C/T	41	Ser-Ser; Silence; Polar	C_169668597_10
rs772281507	G/T	47	Lys-Thr; Missense; Basic - Polar	NA
rs760055038	A/G	50	Pro-Ser; Missense; Apolar - Polar	NA
rs774815172	A/G	50	Pro-Leu; Missense; Apolar	NA
rs771038126	G/T	50	Pro-Pro; Silent; Apolar	NA
rs749364750	G/T	51	Arg-Arg; Silent; Basic	NA
rs34911341	C/T	51	Arg-Gln; Missense; Basic - Polar	C__25607739_20

Abbreviations: NTc, nucleotide change; AAp, amino acid position; NA, not available.
*Unlike the others polymorphisms described in table 1, only for this variant were found studies in the literature.
Obtained data from dbSNP (http://www.ncbi.nlm.nih.gov/snp).

found, described in the region of the *GHRL* which encodes the signal peptide and 23 variants in the ghrelin coding region (Table 1).

After linkage disequilibrium analysis, only 4 markers would be enough to map the entire *GHRL* gene. The rs73125655, rs26802, and rs42451 variants are found in introns and rs696217 in exon 2.

Only one (rs34911341) of the described variants in Table 1 was found in PubMed, SciELO and Science Direct databases. We considered that a brief report of this variant is relevant since it has been associated with severe diseases such as diabetes mellitus, obesity, and hypertension.

The Polymorphism rs34911341

The Arg amino acid residue at position 51 of the preproghrelin peptide was considered important for endoprotease recognition and action (6). The endoprotease catalyzes the proteolytic cleavage within the ghrelin formation process (3,7). However, it is not clear whether this genetic variation really changes the activity or biological properties of ghrelin (5).

A negative association was found between the rs34911341 polymorphism and type 2 diabetes mellitus (T2DM) and obesity in Danish and Malaysian populations (8,9); moreover, similar results were observed regarding this polymorphism in German (10) and Italian (11) studies investigating obesity.

Conflicting results were observed in other studies. The Arg51Gln variant was identified in 6 (all heterozygotes) Swedish obese subjects, with a prevalence of 6.3%, whereas it was not observed among the control subjects (7). Positive association signals were also observed among the rs34911341 variant and other metabolic disorders, such as T2DM in a Finland population ($P=0.009$) (6); gestational diabetes in a European-Brazilian population

($P=0.0001$) (12); hypertension in the Finnish population ($P=0.003$) (6) and Caucasians ($P=0.0084$) (13).

Conclusion

In this scientific article, we focused on the comparison of the described molecular structure of *GHRL* (NCBI and Seim et al), making it somewhat more informative for readers. Furthermore, in this first part, we also highlighted the main, or perhaps all reported *GHRL* genetic variations associated with signal and ghrelin peptides that might be useful to the ones interested in this gene and encourage other researchers in this field. Of the described variants, only rs34911341 has been associated with metabolic disorders, such as diabetes, hypertension and obesity.

Authors' Contributions

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

Conflict of Interest Disclosures

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

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