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Correlation analysis of *growth hormone* gene and growth traits and *in silico* protein investigation in European sea bass (*Dicentrarchus labrax*)

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Abstract

Genetic variations within a partial region of the *growth hormone (GH)* gene and their associations with growth traits were investigated in European sea bass (*Dicentrarchus labrax*) using DNA sequencing. Five haplotypes and ten novel single nucleotide polymorphisms were identified in the *GH* gene of European sea bass. *In silico* analysis revealed two amino acid substitutions between the reference and our partial protein sequence, specifically serine to leucine and serine to threonine. Both variations were located within the α1 helix, which also contains H34, a zinc-binding residue. The genotypes at the g.1611T>C locus of the *GH* gene were found to be significantly associated with total weight, fillet weight and head length. Additionally, the association between GH g.1557A>T genotypes and both preanal and abdominal lengths was statistically significant. Similarly, the genotypes of g. 1857 C>T loci having the synonymous mutation in phenylalanine amino acid were significantly associated with standard length. The haplotype 4 reported the highest weight and length traits than the other haplotypes. It was concluded that haplotype 4 could be used as a potential genetic marker for selective breeding programs of European sea bass under Mediterranean conditions.

Introduction

Growth hormone (GH) is a key regulator of fish biology, influencing growth, appetite, reproduction and immune function.[1-4] Its action through the hypothalamic-pituitary-somatotropic axis stimulates the release of insulin-like growth factors (IGFs), which mediate anabolic processes such as protein synthesis and cell division and regulate nutrient metabolism.[5-7] Growth is a multifaceted biological process influenced by factors such as nutrition and feed intake. It is primarily regulated by the hypothalamic-pituitary-somatotropic axis, which involves the secretion of pituitary GH and the activity of hepatic and extra-hepatic IGFs.[2,3] Besides this gene regulates metamorphosis in fish larvae and osmoregulation against rapid salinity and temparature changes.[8] The positive growth response to growth trials in aquaculture species such as coho salmon (*Oncorhynchus kisutch*), juvenile golden pompano (*Trachinotus ovatus*) and gilthead sea bream (*Sparus aurata*) has been reported to be strongly associated with molecular markers of GH, IGFs, IGF binding protein-5.[3,9,10]

Aquaculture production has emerged as a highly demanded category in the food sector, driven by rapid population growth, the depletion of natural fish stocks and evolving consumer preferences for sustainable and healthy food options. To support the long-term viability of aquaculture, it is crucial to improve both the growth perfromance and product quality of farmed species, particularly in response to evolving environmental and industry challenges.[3] The European sea bass (*Dicentrarchus labrax*, Linnaeus 1758), belonging to the Moronidae (Perciformes) family, is an economically significant aquaculture species. Global production of European sea bass reached around 286,968 tons in 2023, with Mediterranean countries like Türkiye, Greece, Egypt and Spain playing pivotal roles in this industry. European sea bass typically reaches a market size of 250 - 400 g in 18 - 24 months.[11,12] Key factors influencing productivity in sea bass aquaculture include growth rate, feed efficiency, mortality and disease resistance.[13] These aspects are critical to enhancing both production efficiency and sustainability in the industry. Mediterranean countries including Türkiye, Greece, Egypt and Spain, play a key role in the production of European sea bass and significantly contribute to global aquaculture production of the species.

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The *GH* gene has been reported as a candidate gene in studies of genetic variation associated with growth traits in farm animals and aquaculture species. The first gene transfer studies in goldfish (*Carrasius aurata*) utilized the *rGH* gene.[14] The transgenic *GH* genes could significantly increase the growth rate in more than 30 fish species due to the conserved regulatory functions of GH in somatic growth.[15] Various studies have been carried out on the relationship between *GH* gene polymorphisms and growth traits in fish species such as *Cynoglossus semilaevis*, red sea bream (*Pagrus major*), *Pelteobagrus fulvidraco*, *Oreochromis niloticus*, *Cyprinus carpio*, snapper and *Siniperca chuatsi*.[16-24]

The primary objective of this study was to investigate potential polymorphisms and their associations with various growth traits in European sea bass. In addition, the study aimed to evaluate whether the observed amino acid changes could affect the three-dimensional (3D) structure of the proteins involved, potentially affecting their function.

Materials and Methods

Sampling and DNA isolation. For the study, 200 European sea bass originating from two different hatcheries and reared together in the same cage environment at Tumay Seafood Corp. (İzmir, Türkiye) were randomly selected after reaching market size. After harvest, fish were graded into four market size groups and 25 individuals from each group were sampled. Four size groups were identified after harvest and 25 random fish from each size group were sampled from two hatcheries. Measurements including body depth, standard length, head length (HL), body length (BL), pre-anal length (PAL), abdominal length, post-anal length, head depth, total weight (TW), and fillet weight (FW) were recorded for each sample. The tissue samples used in this study were obtained from a previously published study using the same sampling protocols and methods.[25] A total number of 200 European sea bass samples were collected and sex determination was performed during the process, showing that all fish were male. This high percentage of males was consistent with previous studies reporting up to 95.00% male prevalence in farmed populations.[26] Thus, sex effects were excluded from further analyses. Each fish was weighed, photographed and morphometrically measured according to the methods of Massault *et al*.[27] Muscle tissue samples were stored in 96.00% ethanol at – 20.00 ˚C until DNA extraction. Genomic DNA was extracted using the GeneMATRIX Tissue and Bacterial DNA Purification Kit (EURx Ltd., Gdansk, Poland) following the manufacturer's protocol. The quality and quantity of DNA samples were assessed using 1.00% agarose gel electrophoresis and spectrophotometer (MN-913; Maestrogen, Hukou, Taiwan), respectively. The sampling carried out post-harvest, did not necessitate ethical approval at this phase, as it was not specifically mandated by the Ege University Animal Ethics Committee.

Primer design and PCR amplification of the *GH* gene. The European sea bass *GH* gene sequence was retrieved from GenBank® (Accession No. GQ918491) and primers were designed using the Primer-BLAST algorithm (https:// www.ncbi.nlm.nih.gov/tools/primer-blast/). The primer sequences of the *GH* gene were F: 5′-GTGATCAGTC GGGTTCAGGT-3′ and R: 5′-CGTTGTGTCTCGTGCTTGTC-3′. The PCR reactions (50.00 µL) containing 100 ng genomic DNA, 0.50 µM of each primer, and 2.00 X MyTaq™ Mix (Meridian Bioscience, Cincinnati, USA) were amplified using PCR temperature cycling conditions as initial denaturation at 95.00 ˚C for 3 min, 35 cycles of denaturation at 95.00 ˚C for 30 sec, annealing at 60.00 ˚C for 30 sec, and elongation at 72.00 ˚C for 60 sec followed by a final extension at 72.00 ˚C for 10 min. The PCR products were electrophoresed on 1.50% agarose gel using horizontal electrophoresis and visualized using RedSafe™ (iNtRON Biotechnology, Seongnam, Korea)

Sequencing. A region of 576 bp covering 1st partial intron, 2nd exon, 2nd intron, and 3rd partial exon regions of the *GH* gene region was sequenced in 200 individuals using the Genetic Analyzer System (3500XL; Applied Biosystems, Waltham, USA). The sequences were checked using ChromasPro (version 2.1.8; Technelysium Pty. Ltd. South Brisbane, Australia). The haplotype analysis was performed by the Haploview4.1 (https://www.broad institute.org/haploview/haploview). The nucleotide sequence of the *GH* gene region was translated using the Expasy resource portal (https://web.expasy.org/ translate/). The 3D tertiary structure of the proteins based on the *GH* gene region under study was predicted using the I-TASSER server (https://zhanggroup.org/I-TASSER/).

Statistical analysis. The single nucleotide polymorphism (SNP) genotypes were tested for the Hardy-Weinberg equilibrium using the Hardy-Weinberg in R Software (version 3.4.3; R Core Team, Vienna, Austria).[28] The associations between genotypes, haplotypes, and growth traits were analyzed using general linear model via SPSS Software (version 18.0; IBM Corp., Armonk, USA).

$$Linear\ Model\ I = Y_{jk} = \mu + G_j + e_{jk}$$

where, Y_{ijk} represents the traits, μ represents the intercept, G_j represents the fixed effect of *GH* genotype or haplotypes (j = 1, 2 or 3 for each SNP or haplotype) and e_{ijk} is the random error.

The Bonferroni multiple range test was used to assess the significance of differences between genotypes at each locus. The thresholds for significant and highly significant differences were $p < 0.05$ and $p < 0.01$, respectively. Benjamin Hochberg's method was used to calculate the false discovery rate for correcting the associated SNP with growth traits.[29]

***In silico* protein analyses.** The ESPript program (http://espript.ibcp.fr) played a crucial role as a bio-informatics tool for unraveling the essential characteristics within protein structures.[30] This investigation focused on the amino acid sequences corresponding to the second and third exons, and this was 81 aa in length. In this study, the alignment of amino acid sequence of the partial GH protein (81 aa) was performed by Clustal Omega, displayed by ESPript Version 3.0 program using 3D protein structure of a GH protein (UniProt ID: Q05163) to determine the position and location of amino acid substitutions. Deep Multiple Sequence Alignment 2 functioned as a tool for creating multiple sequence alignments for both single- and multichain proteins. It enabled the development of multiple sequence alignments by incorporating homologous sequences obtained from genomic and metagenome sequence databases.[31,32] The GH protein sequence was modeled using the SWISS-MODEL homology modeling server (https://swissmodel.expasy.org/. The selection criteria for the optimal template were based on several factors, including amino acid sequence identity, coverage and the Global Model Quality Estimate value. After analysis, the somatotropin alphafold model from *Larimichthys crocea* (large yellow croaker) was identified as the most suitable template with a sequence identity of 86.27%.

Results

The European sea bass *GH* gene consisted of six exons and five introns with a total length of 4,015 bp and encodes 204 amino acids (Accession No. GQ918491). The region under study is located between 1,551 and 2,126 bp in the *GH* gene sequence. The genetic sequences of the partial region of the *GH* gene in the European sea bass were investigated in this study (Fig. 1). All SNPs and the partial DNA sequences of the *GH* gene in sea bass are reported for the first time in this study. The sequences were submitted to the NCBI GenBank® database (Accession No. MN329680-5 and ON035500-12).

The allele and genotype frequencies of the *GH* gene region under study in European sea bass samples are shown in Table 1. Three SNPs (1824 T>C, 1912 T>A, and 2052 G>C) have been reported only in three animals, so the allele and genotype frequencies of these SNPs are not included in Table 1. The *GH* gene g. 1557A>T, g. 1663C>G, g. 1684T>C and g. 1799T>C loci were in Hardy-Weinberg equilibrium, whereas, the g. 1611T>C, g. 1769T>C and g. 1857C>T loci were not in Hardy-Weinberg equilibrium. The genotype AA and GG were absent in *the GH* locus g. 1557A>T and g. 1663 C>G, respectively.

Associations between *GH* genotype and growth traits. The significant associations ($p < 0.05$, false discovery rate < 0.05) between the *GH* loci genotypes and morphological traits were estimated and no significant associations with body depth, BL, postanal length, abdominal length and head depth were observed (Table 2). However, we found significant correlations between TW, filet weight, HL, PAL, and standard length traits with the genotypes for g. 1557 A>T, g. 1611 T>C, g. 1857 C>T loci ($p < 0.05$). The genotypes for g. 1611 T>C were associated with TW, filet weight and HL ($p < 0.05$). Also, the genotypes for g. 1557 A>T were associated with the PAL and abdominal length ($p < 0.05$). The genotypes of g. 1857 C>T loci that caused the synonymous mutation in phenylalanine amino acid were found to be significantly associated with standard length ($p < 0.05$).

In haplotype analysis, one main haplogroup was identified among the 7 SNPs in the *GH* gene. The haplotypes included g. 1663 C>G, g. 1684 T>C, g. 1769 T>C, g. 1799 T>C, and g. 1857 C>T loci. The haplotype frequencies ranged from 0.015 to 0.825 among haplotypes 1-5 (Table 3).

The haplotype analysis revealed haplotypes of the *GH* gene had significant associations with growth traits (SL, HL, PAL, Post-Anal Length, BD, TW, and FW) ($p < 0.05$; Table 4). The HAP4 (GCTCC) has a significantly higher value of growth traits than the other haplotypes in the studied European sea bass samples.

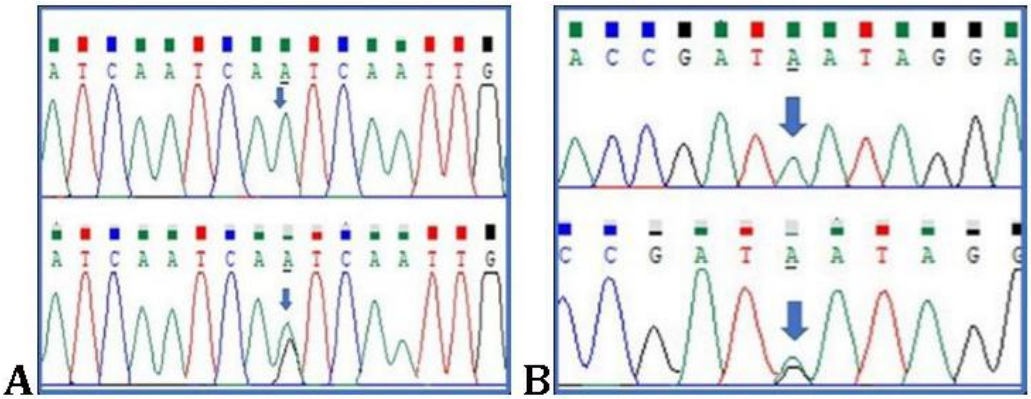

Fig. 1. Partial sequence chromatograms of the *growth hormone (GH)* gene showing the single nucleotide polymorphisms: **A)** g.1684T>C and **B)** g.1799T>C polymorphisms (reverse complement sequences shown by blue arrows).

Table 1. Allele and genotype frequencies of SNPs in *growth hormone* gene in European sea bass.

Loci		*GH* Genotypes			Allele Frequency		$\chi 2$
		AA	**AT**	**TT**	**A**	**T**	
g.1557 A>T	Obs.	-	190.00	10.00	0.98	0.02	0.13*
	Exp.	0.12	190.12	9.75			
		TT	**TC**	**CC**	**T**	**C**	
g.1611 T>C	Obs.	11	179.00	10.00	0.50	0.50	125
	Exp.	50.50	100.00	49.50			
		CC	**CG**	**GG**	**C**	**G**	
g.1663 C>G	Obs.	166.00	34.00	-	0.92	0.08	1.73*
	Exp.	167.45	31.11	1.44			
		TT	**TC**	**CC**	**T**	**C**	
g.1684 T>C	Obs.	160.00	38.00	2.00	0.90	0.10	0.02*
	Exp.	160.20	37.59	2.21			
g.1769 T>C	Obs.	152.00	36.00	12.00	0.85	0.15	17.3
	Exp.	144.50	51.00	45.00			
g.1799 T>C	Obs.	164.00	36.00	-	0.91	0.09	1.96*
	Exp.	165.62	32.76	1.62			
g.1857 C>T	Obs.	21.00	27.00	152.00	0.17	0.83	55.60
	Exp.	5.95	58.00	136.95			

* The locus is in Hardy-Weinberg equilibrium. Obs: Observed; Exp: Expected.

Table 2. The relationships between the genotypes of growth hormone loci and the growth traits in European sea bass (Mean ± SE).

Traits	SNP	CC/AA	TC/TA/CG	TT/GG	*p-value**
TW	g.1611 T>C	433.00 ± 25.45^{b}	438.50 ± 30.59^{b}	468.67 ± 12.22^{a}	0.040
FW	g.1611 T>C	228.40 ± 16.87^{b}	229.63 ± 16.44^{b}	248.15 ± 6.52^{a}	0.041
HL	g.1611 T>C	7.79 ± 0.32^{b}	7.89 ± 0.85^{b}	8.51 ± 0.44^{a}	0.016
PAL	g.1557 A>T	-	20.96 ± 0.21^{b}	21.60 ± 0.94^{a}	0.021
SL	g.1857 C>T	31.86 ± 0.95^{a}	30.57 ± 0.79^{ab}	29.34 ± 0.27^{b}	0.006

TW: Total weight (g); FW: Fillet weight (g); HL: Head length (cm); PAL: Pre-anal length (cm); SL: Standard length (cm).
^{ab} Values with different superscripts within the same row differ significantly at $p < 0.05$.

Table 3. The haplotype frequencies of the *growth hormone* gene of European sea bass.

Haplotypes	Allele combination	Frequency (%)
HAP1	CTTTT	0.825
HAP2	CTCTC	0.068
HAP3	GCCCC	0.060
HAP4	GCTCC	0.025
HAP5	CCCTC	0.015

HAP: Haplotype.

Table 4. The associations of haplotypes of growth hormone and growth traits in the European sea bass population (Mean ± SE).

Traits	HAP1	HAP2	HAP3	HAP4	HAP5	*p**
TW (g)	452.20 ± 12.42^{b}	466.32 ± 63.54^{b}	567.03 ± 25.39^{ab}	720.00 ± 29.81^{a}	473.33 ± 98.99^{b}	0.002
FW (g)	239.17 ± 6.61^{b}	246.32 ± 34.07^{b}	302.43 ± 11.21^{ab}	375.35 ± 11.29^{a}	251.86 ± 52.37^{ab}	0.002
HL (cm)	7.83 ± 0.08^{b}	7.93 ± 0.45^{ab}	8.39 ± 0.16^{ab}	9.52 ± 0.92^{a}	8.61 ± 0.97^{ab}	0.008
PAL (cm)	20.97 ± 0.01^{b}	21.05 ± 0.06^{ab}	21.12 ± 0.08^{a}	21.09 ± 0.13^{ab}	20.96 ± 0.00^{ab}	0.002
POSTAL (cm)	9.21 ± 0.09^{ab}	8.98 ± 0.33^{b}	9.61 ± 0.23^{ab}	10.63 ± 0.24^{a}	9.32 ± 0.54^{ab}	0.047
BD (cm)	7.41 ± 0.08^{b}	7.59 ± 0.38^{b}	8.44 ± 0.16^{ab}	9.23 ± 0.09^{a}	8.17 ± 0.58^{ab}	0.000
SL (cm)	29.26 ± 0.25^{b}	30.64 ± 1.23^{b}	32.68 ± 1.16^{ab}	36.12 ± 1.92^{a}	32.50 ± 2.29^{ab}	0.000

HAP: Haplotype; TW: Total weight; FW: Fillet weight; HL: Head length; PAL: Pre-anal length; POSTAL: Post-anal length; BD: Body depth and SL: Standard length.
^{ab} Values with different superscripts within the same row differ significantly at $p < 0.05$.

The variations of the European sea bass *GH* gene identified in this study and their comparison with the reference sequence from NCBI GenBank® are shown in Table 5. In the studied European sea bass samples, nonsynonymous mutations were detected from serine to leucine (S24L) and from serine to threonine (S42T) in the second exon region (Fig. 2). In addition, 2 synonymous SNPs were observed in serine and phenylalanine amino acids in the second exon and leucine amino acids in the third exon region of the *GH* gene (Fig. 3).

The analysis result showed that it was identified the differences existed at two locations between the reference (UniProt ID: Q05163) and our partial protein: serine to leucine and serine to threonine. S24L and S42T were found in α1 helix, including H34, a zinc-binding residue (Fig. 3).

In this investigation, the Deep Multiple Sequence Alignment 2 pipeline was employed to align the partial GH protein with homologous GHs from the Uniclust30, Uniref90, BFD, MGnify, and IMG/M databases (reference). In this examination, the alignment depth and for the amino acid sequences of GH was determined as 47.4809, respectively, while the total number of aligned sequences was 1093. The findings indicated substantial conservation of five residues (L22, E23, E44, C65 and P73) across all aligned sequences. Among these, three residues (L22, C65 and P73) were also conserved in GH; however, the corresponding residues E23 and E44 were found as F23 and F44, different from most of the aligned sequences. In addition, zinc-binding residue can be predominantly four amino acids (H, Y, S, N) in the position 34 along 1,093 homologous sequences (Fig. 4).

Table 5. The variations of nucleotides identified in the *growth hormone* gene in European sea bass.

Positions	Reference sequence*	Studied samples	Region	Amino acid change	NCBI accession No.
1,557	A	A/T/W	Intron 1	-	MN329680, ON035502
1,611	T	T/C/Y	Intron 1	-	ON035500, ON035501
1,663	C	C/G/S	Intron 1	-	ON035503, ON035506
1,684	T	T/C/Y	Intron 1	-	ON035504, ON035505
1,769	C	C/T/Y	Intron 1	-	ON035507, ON035509
1,799	T	T/C/Y	Exon 2	Serine→Leucine	ON035508, ON035510
1,824	T	T/C	Exon 2	Serine**	MN329681, N329683
1,857	C	C/T/Y	Exon 2	Phenylalanine**	ON035511, ON035512
1,912	T	T/A	Exon 2	Serine→Threonine	MN329682, N329684
2,052	G	G/C	Exon 3	Leucine**	MN329683, MN329685

*GQ918491 reference sequence, **Synonymous amino acid change.

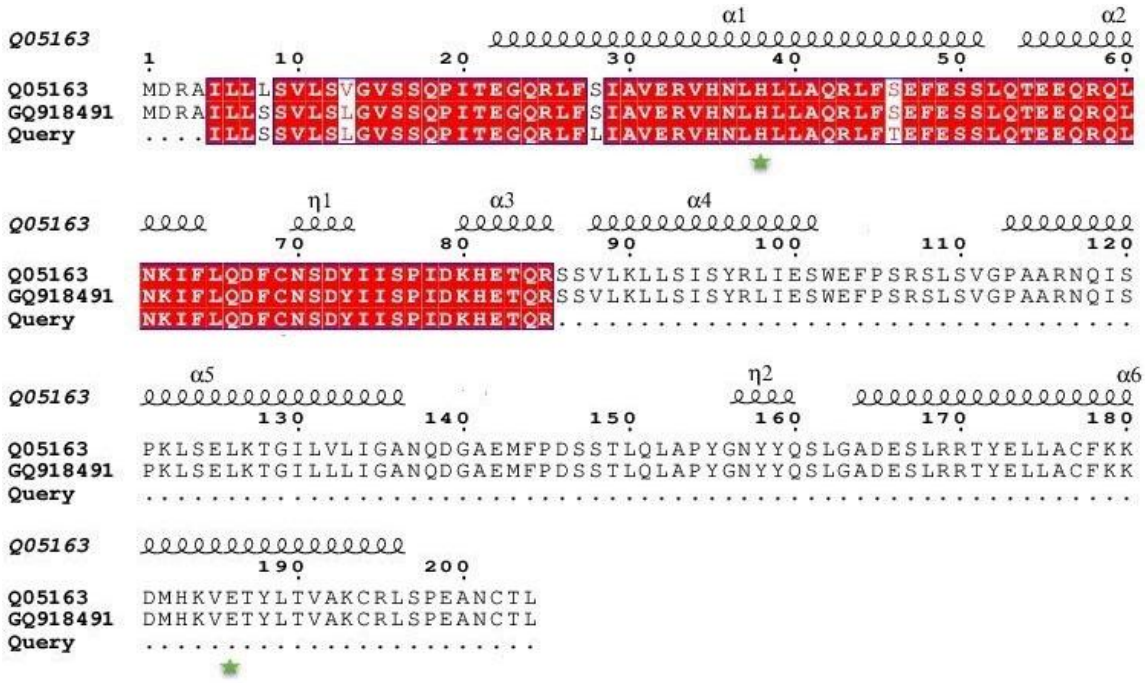

Fig. 2. The partial growth hormone protein (query), the reference protein sequence (GeneBank ID: GQ918491), and growth hormone from *Dicentrarchus labrax* (UniProt ID: Q05163) underwent multiple sequence alignment. In the figure, residues strictly conserved are highlighted with a red background and conservatively substituted residues are enclosed within boxes. The aligned sequences of Q05163 provided insights into the secondary structural elements, including α-helices (α) and short helices (η) indicated above them. The binding site was marked with green asterisks. ESPript was used to generate the figure.

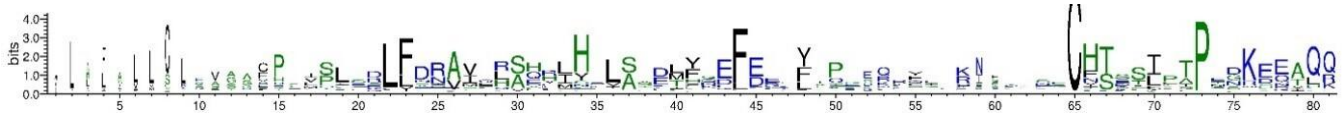

Fig. 3. Deep multiple sequence alignment of the partial growth hormone protein.

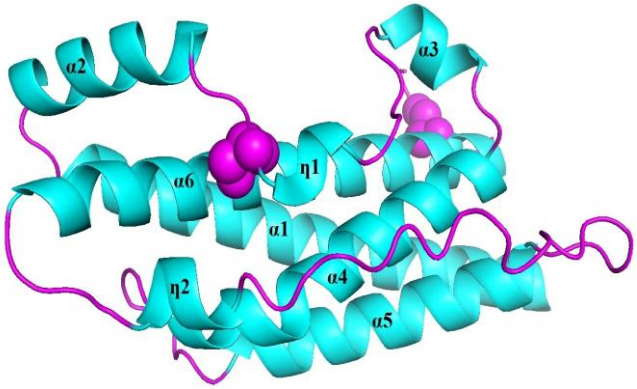

Fig. 4. Three-dimensional model of the full-length protein combining partial growth hormone protein with the reference growth hormone protein. Two spheres represents the disulfide bonds.

Discussion

In this study, a 576 bp long region of the European seabass *GH* gene covering 1st partial intron, 2nd exon, 2nd intron, and 3rd partial exon was investigated. The *GH* gene of European sea bass populations contained five SNPs in the first intron, four SNPs in the second exon and an SNP in the third exon region. Two SNPs, g.1557 A>T and g.1611 T>, located in the first intron region were found associated with TW, FW, HL, and PAL traits. Introns, particularly the first ones, are essential for accurately localizing specific mRNAs within the cytoplasm, encompassing functions related to mRNA export.[33] Intergenic regions can accumulate more gene mutations in comparison with exons due to their longer length.[34] Although intronic regions do not code for proteins, they play a regulatory role in mRNA splicing, gene transcription, translation and expression.[24,35] The SNPs in noncoding regions can modulate gene expression and hence regulate various properties such as growth, disease resistance etc.[36-38] In studies conducted on the genome wide association, it has been reported that disease or a trait-associated SNPs are more frequently found in intronic regions.[33,39-41] Early-stage growth in *Salvelinus alpicus* was significantly affected by the SNP in the intragenic region of the GH Releasing Hormone locus, which regulates GH secretion.[42]

Five SNPs were detected: g.1799 T>C, 1824 T>C, g.1857 C>T, 1912 T>A, and 2052 G>C, in the second and third exons of the *GH* gene. The SNP g.1857 C>T (present in the second exon of the *GH* gene) caused a synonymous mutation in phenylalanine amino acid and was revealed to be associated with SL. Li *et al*., have reported two SNPs ofg.5045T>C and g.5234T>G in the 5th exon and 5th intron regions of the *GH* gene to be associated with growth performance in the *S. chuatsi* population.[23] Three SNPs detected in the *GH* gene in yellow catfish (*P. fulvidraco*) were significantly associated with yield characteristics such as body thickness, caudal pedicle length and BL.[18]

Similarly, Tian *et al*., reported that the 4 SNPs in the *GH* gene, with two of these SNPs located in the 4th exon and the others in the 5th exon and 5th intron, have been observed.[43] They have suggested that these polymorphisms are significantly associated with growth traits and could be used for marker assisted selection) in *S. chuatsi* populations. In a study, a total number of four polymorphic SNPs out of 32 SNPs (one in the fifth exon and 31 in intronic regions) in the *S. chuatsi GH* gene were found to be significantly associated with economically important growth characteristics.[24] Liu *et al*., found SNPs in the third exon and intron of the *GH* gene to be associated with the growth traits in common carp in Southern China.[20] The SNPs in introns, promotors, and 5′UTR regions of the *GH* gene have an important effect on growth traits. Jaser *et al*., identified ten SNPs in *GH* gene of Nile tilapia (*O. niloticus*), including nine in the proximal promoter region and one in the 5′UTR.[44] They reported that five genotypes of these SNPs were associated with higher market weight. Furthermore, their findings suggested that SNPs significantly related to growth rate may be interdependent and linked to GH expression levels during the growth phase. Taken together, these findings highlighted the potential of *GH* gene polymorphisms as valuable molecular markers for improving growth traits through marker-assisted selection in aquaculture species.

The haplotypes showed significant associations with some measured growth traits of European sea bass in this study. The HAP4 showed greater TW and FW traits than the other haplotypes. The HAP1 was the most common haplotype (82.00%) which could be due to its presence in the ancestral gene and other haplotypes emerged because of mutations in the evolutionary process. On the other hand, it was found that HAP1 was significantly lower than the other haplotypes for the TW, FW, HL, PAL, BD, and SL traits of European sea bass. Similar to our results, Hu *et al*., identified four haplotypes and ten diplotypes in Heilongjiang carp (*C. carpio haematopterus)*, German mirror carp (*C. carpio* L. *mirror*) and Purse red carp (*C. carpio var. wuyuanensis)* breeds in China.[21] They showed that the H2H2 diplotype fish have significantly higher body weight and net weight than the other diplotypes in the Heilongjiang carp breed. Sun *et al*. found one of the four diplotypes detected in the *S. chuatsi GH* gene was significantly associated with higher body weight, total length, and BL.[24] These results suggest that specific *GH* gene haplotypes, particularly HAP4, could be useful genetic markers for improving growth performance in European sea bass breeding programmes.

The 3D structure of a protein depends on the sequence of amino acids and a mutation can change the structure of the native proteins.[45] In this study, two nonsynonymous (S24L and S42T) amino acid substitutions were observed in the native GH protein, based on reference gene (GenBank® ID: GQ918491). The S24L mutation could

affect the 3D structure of GH because leucine is a relatively large and nonpolar amino acid, whereas, serine is a smaller and more polar amino acid.[46] This may affect the folding structure, thereby, efficiency and stability of the protein. Amino acids, especially Leucine, play a dual role - not only as substrates for the synthesis of new proteins but also as signaling molecules that initiate the process of protein synthesis.[47-49] Mutations in exons are generally harmful and eliminated by selection.[50] The GH is a major hormone controlling growth and metamorphosis in fish larvae.[6] Therefore, the S24L mutation may be eliminated from the population in the next generations. Because in S24L, we found two genotypes, predominantly homozygote and low-frequency heterozygote. Based on *in silico* predictions suggesting a potential adverse effect of the CC genotype on protein structure, we recommend cautious interpretation of its role in breeding programs. Further functional and phenotypic studies are required to confirm this observation.

Genetic variations in the *GH* gene in European sea bass populations reared in Mediterranean conditions were investigated in this study. We detected ten SNPs in the intron and exon regions of the *GH* gene. Based on the outcomes of this study, we suggested HAP4 should be used as a potential marker to improve the accuracy of selection in European sea bass in Mediterranean conditions due to its positive association with body weight and length traits. The association of these SNPs with growth traits needs to be investigated further. In addition, the association, the reported SNPs with other traits of economic importance (meat quality and reproduction traits) and their interactions with other genes should be studied. These SNPs have the potential to be used for marker-assisted selection in European sea bass breeding.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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