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# Genetic Effect of IGF1, PIT1 and Leptin Genes on Wool Weights in Makooei Sheep

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**Abstract**

The study of candidate genes, based on physiological results, is a critical tool to detect genes to be applied in marker-assisted choice processes. On the topic of sheep gene polymorphism knowledge the ovine IGF1, PIT-1, Leptin genes was analyzed as a probable genetic marker for growth characteristics in sheep. In the checked Makui sheep population, definitive calculational results demonstrated that the IGF1, PIT-1, Leptin genes great position in wool1 weight. Measurement of associations between genotypes as well as wool weights was performed with 100 tests. The measurement of a relation between these SSCP models with wool1 (A Fleece mass at lifetime), wool2 (Wool mass dual years of lifetime), wool3 (Fleece mass at three years of lifetime), wool4 (Fleece mass at four years of lifetime) showed a certain aftereffect of the entire models with wool1 mass in one year (A fleece mass at lifetime). Individuals with the L4, P4 additionally I2 of Leptin, PIT1 also IGF1, genes had a dominance wool1 mass (A fleece mass at lifetime) when contrasted to those of individuals with extraneousness genotypes. In addition the affects showed L3, P2 and I3 had mark down wool1(A fleece mass at lifetime) mass specialization when contrasted to those of individuals with difference genotypes. In the end, these events demonstrate that wool weights later one year of lifetime may be mainly influenced surrounding agents like Nutrition.

**Keywords:** PCR-SSCP, Leptin, PIT1, IGF1, wool weights, Makooei sheep breed.

## 1. Introduction

Makoei sheep is categorized as fat-tailed sheep, close to Turkish White Karaman, also outlined one of the influential multi-purpose sheep expedients in East as well as West Azerbaijan provinces of Iran. They are multi colored: black, white with black spots on face and feet. Indigenous genetic resources of the world are at the risk of extinction due to absorbent crossing with commercial breeds [1]. Genetic engineering is the tag of a group of skills used to detect, replicate, modify and transfer the genetic material of cells, tissues or complete organisms. Notable appeals of genetic engineering in animal breeding are:

1) Marker-assisted selection (MAS). The objective of this technology is to increase disease resistance, productivity and product quality in economically important animals by adding information of DNA markers to phenotypes and genealogies for selection decisions.

2) Transgenesis, the direct transfer of definite genes/alleles among individuals, species, or even Kingdoms, to alter their phenotypic expression in the recipients. Contrasted to the 'traditional' gain techniques based on phenotypic information only, these gene-b y-gene techniques allow theoretically a more complete management of animal genomes for animal breeding. In spite of high expectations and new technical developments, its actual efficiency is not always high, as they require a thorough knowledge of functional genomics, and pose additional technical, economical and ethical problems [2]. In animal industry, growth characteristics of animal are continually of basic attention during breeding for its source economical worth [3]. For growth characteristics, growth hormone (GH), growth hormone receptor (GHR), insulin like growth factor I (IGF-I), leptin (LEP), caprine pituitary specific transcription factor-1 (POU1F1), caprinemyostatin(MSTN) and bone morphogenetic protein (BMP) genes are necessary for bone formation, birth weight, weaning weight, body condition and muscle growth. Insulin-like growth factor I (IGF-I) plays a notable physiological role in the growth also development of mammals by functioning regionally in definite organs or entirely through circulating IGF-I [4]. Leptin is though t to have a min or role in GH regulation [5]. In mammals, leptin has b een associated with feed intake and energy metabolism, but the low closeness with the mamalian counterpart (nearly 25%) also its mRNA demonstration suggests that the applied position of this peptide might not have been kept among

vertebrates [6]. Insulin-l ike growth factors are a complex family which includes three hormones, three receptors and six, binding proteins, that interact in vertebrate s to regulate cell development and differentiation [7]. A product of the POU1F1 gene regulates the expression of GH, PRL, also TSH ˇ genes which simply play powerful roles in growth, development as well as metabolism in mammals [8].

The title wool is commonly approved as the generic description of the natural fibre of domesticated sheep (Ovisaries), although it is also used as the generic name of hair from animals such as goat, camel, vicuna, alpaca, angora rabbit and yak [9]. Wool characteristics like oily fleece weight, clean yield, fiber diameter also its coefficient of variation are very notable choice aims in sheep breeding methods, furthermore modern traits like as staple strength and staple length are of increasing importance in the wool industry. The greatest part of the wool produced by the indigenous sheep breeds in Iran is used in the hand woven carpets. It is calculated that 5.1 million m2 hand woven carpets are manufactured in Iran annually, due to the country desires 28 thousand tons of washed wool. About eight thousand tons of wool is imported as merino wool from Australia and New Zealand. Iranian wool is suitable for use in coarse-carpet industry, but it has some difficulties for use in the fine carpets. Best of the wool created (85%) is of course characteristic, applied mainly in the producing of hand-knotted carpets,5% is clothing category, 10% is coarse level, applied mainly for the manufacture of low grade carpets, blankets, etc. It is critical to exploit the central potential of domestic sheep, regularity to enhance the level of their wool also bring it on a par with its marvelous counterparts. Fiber diameter as well as staple strength is major wool quality characteristics that help to rate difference approaching to their act on fiber processing characteristics also the perfect level of the products. Staple strength is more costly to calculate, however due to the great association with the co-operative of difference of fiber diameter, it can be revealed at a part of the value. Wool characteristics have commonly high (fleece weight and fiber diameter) or medium (staple strength) degrees of heritability, yet experiment into definite wool quality genes or targeting particular wool quality characteristics (staple strength, staple length, position of break) is critical for the processing of the product [10]. The opportunity exists to utilize our knowledge of major genes that influence the economically important traits in wool sheep. Genes with Mendelian inheritance have been identified for many notable characteristics in wool sheep. Gene mapping assays have determined some chromosomal regions related with variation in wool quality also production traits. The question at present is to build on this knowledge base in a cost-effective way to deliver molecular tools that facilitate enhanced genetic improvement programs for wool sheep [10]. The plan of this assay was to determine the relations between IGF1, PIT1 also Leptin genotypes also wool weights applying single strand conformation polymorphism (SSCP) technique in Makooei sheep.

## 2. Methods

### 2.1 Sheep, blood sample collection and genomic DNA extraction

'Makoei' sheep breeds examined in this study were fat-tailed sheep with medium body size, white colour with black spots on face and feet. They are kept in east and west Azerbaijan provinces of Iran and their main products are meat and wool. Blood samples were collected into a 5 ml EDTA contained vacutainer tube and transferred to the laboratory for DNA extraction within 2 hours. Total DNA extractions were made with a modified salting out method from whole fresh blood. Quality and quantity of extracted DNA was measured on 0.8% agaroze gel prepared in 0.5× TBE buffer (45 mMTris base, 45 Mm boric acid, 1 mM EDTA pH 8.0) and visualized with ethidium bromide (1.0 µgml-1) and photographed under UV light.

### 2.2. PIT1 genotyping

The PIT1 gene was genotyped by PCR-SSCP (Polymerase chain reaction-single strand conformation polymorphism). A 295bp fragment was amplified from (exon 2).primers used were those designed by Bastos et al [11]. PIT-1-UP: 5'-GAGGGATAATTACAAATGGTCC-3 and PIT-1-down:5'-TGTTAACAGCTGTGGGACACAC-3'.PCR contained 25-50 ng genomic DNA, 10 pmoL of each primer, 2 µL 10X PCR buffer, 1.5 mM MgCl2, 200 µMdNTP and 1 unit Taq-polymerase, in a total volume of 20 µL. DNA amplifications were performed using Master cycler (Eppendorf, Germany) programmed for a preliminary step of 15 min at 95°C, followed by 35cycles of 45 s at 94°C, 1 min at 58°C and 45 s at 72°C, with a final extension of 5min at 72°C.

### 2.3. IGF-1 genotyping

Two polymerase chain reaction (PCR) primers, IGF-1-up (5′-ATTACAG CTGCCTGCCCCTT-3′ ) and IGF-1-down( 5'-CACATCTGCTAATACACCTTACCCG-3') targeting a fragment of 265 bpwas employed in DNA amplifications as described before. PCR contained 25-50 ng genomic DNA, 10 pmoL of each primer, 2 µL 10X PCR buffer, 1.5 mM MgCl2, 200 µMdNTP and 1 unit Taq-polymerase, in a total volume of 20 µL. DNA amplifications were performed using Master cycler (Eppendorf, Germany) programmed for a preliminary step of 2 min at 95°C, followed by 31cycles of 45 s at 94°C, 30 s at 58°C and 45 s at 72°C, with a final extension of 3min at 72°C.

### 2.4. Leptin genotyping

The DNA amplification of the LEP gene was achieved by PCR. Two PCR primers, LEP-up (5-AGGAAGCACCTCTACGCTC-3) and LEP-dn (5´-CTTCAAGGCTTCAGCACC-3´), targeting a fragment of 471 bp was employed as described [12]. The PCRs were carried out in 50 µl volumes using PCR mastermix kit (Cinnagen, Iran) containing 2.5 units Taq DNA Polymerase in reaction buffer, 4 mM MgCl2, and 50 µM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer and about 100 ng of extracted DNA as template. The thermal profile consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 59°C and 30 s at 72°C, with a final extension of 5 min at 72°C. Amplification was carried out in Mastercycler (Eppendorf, Germany).

### 2.5. Single strand confirmation polymorphism (SSCP)

For single-strand conformation polymorphism (SSCP) analysis, several factors were tested to optimize the methodology: Amount of PCR product (4 – 15 µL), dilution in denaturing solution (20 - 85%), denaturing solution (A: 95% of formamide, 10mM NaOH, 0.05% xylene-cyanol and 0.05% bromophenol blue; B: same as A, plus 20mM of EDTA), acrylamide concentration (6 - 14%), 6 percentage for PIT1, 7 percentage for IGF-1 and 15 percentage for Leptin, percentage of cross linking (1.5 to 5%), presence (10%) or absence of glycerol, voltage (100 - 350 V), running time (2-12 h) and running temperature (4, 6, 10 and 15 °C). Each PCR reaction was diluted in denaturing solution, denatured at 95 °C for 5 min, chilled on ice and resolved on non-denaturing polyacrylamide gel.

### 2.6. Measuring the weights of wool

Greasy fleece weight (GFW) which was determined at shearing, and clean fleece weight (CFW), which was calculated as the product of GFW and yield, wool characteristics were measured by the Research Centre for Animal Breeding and Selection, Maku, Western Azerbaijan. The traits measured were wool weights such as wool1 (A fleece weight at age), wool2 (Wool weight two years of age), wool3 (Fleece weight at three years of age), wool4 (Fleece weight at four years of age).

### 2.7. Statistical analysis

Allele and genotype frequencies were calculated with Pop-Gene software v1.31. One hundred samples were used for statistical analysis of wool traits. SAS software was used to calculate least squares means, and for multiple comparisons among the different genotypes in Makooei sheep. Least square analysis using the General Linear Model (GLM) procedure was done to identify the fixed effects on a model that were to be included in the model and this then the model was made using the fixed effects (sex: 2 classes; type of birth:2 classes;IGF-1: 3 classes; PIT-1: 4 classes, according to the following statistical model:

$$Y_{ijklm} = \mu + G_i + P_d + L_n + S_j + ls_k + e_{ijklm}$$

where, $Y_{ijklm}$= wool traits, µ = the overall mean, $G_i$= the fixed effect of the ith genotype for IGF-1, Pd= the fixed effect of the dth genotype for PIT-1, $L_n$ = the fixed effect of the nth genotype for Leptin,$S_j$= the fixed effect of sex (j = 1, 2), $l_{sk}$= the fixed effect of litter size (k=1, 2), $e_{ijklm}$=the random residual error.

The effect of SSCP patterns on the weights of wool for the interested traits was analyzed using the least square method of GLM procedure of SAS software and the least square means were compared by the Tukey test.

## 3. Results and Discussion

### 3.1 Genotype and allele frequencies

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of the 5'flanking region (Exon1) of the ovine IGF-I gene and (a part of intron2, exon3 and a part of intron3) PIT-1 and exon3 Leptin gene revealed three AA (G1), AG(G2), GG(G3) and four AA (p1), AB (p2), CC (p3), CD (p4)and five AB, BB, AC, BC and CC banding patterns respectively. In the experimented Makooei sheep populace, definitive calculation affects were determined in wool1 weight characteristic as to wool weights for IGF1, PIT1 also Leptin Genes. In addition, the measurement of a relation between these SSCP models with wool1, wool2, wool3, wool4 determined a positive function of the entire models with wool weight in one year. Individuals with the L4, P4 also I2 of Leptin, PIT1 as well as IGF1, genes had a dominance wool weight in one year when contrasted to those of individuals with difference genotypes. Additionally, the outcomes displayed L3, P2 and I3 had cheapened wool weight in one year characteristic when contrasted to those of individuals with difference genotypes. And there is very minor evidence about polymorphism of the Leptin, PIT1 as well as IGF1 genes also theirs relation with wool weight characteristics. Calculation of associations between genotypes as well as wool weights was performed with 100 tests. Classes of significance, least squares means, as well as standard errors are described in Table 1.

The native breeds, because of their natural choice opposite severe surroundings also adaptation to regional events are critical to resource-poor farmers as well as pastoralists. The molecular characterization of genetic variation is a basic level to control also protects native breeds. Genetic study of sheep populations is one of the most critical studies that have been begun throughout the last two decades in Iran. Part of these studies contains research on breed characteristics, family relation, genetic distances among breeds also determining genes influencing growth rate, reproduction as well

as manufacturing in native breeds applying microsatellite markers [1]. Genes within the somatotropic axis also converting growth factor super family have been demonstrated to induce growth variation in terrestrial livestock, as well as difference vertebrates [13]. Whilst the genetic improvement of sheep bred primarily for wool production has been slow relative to other livestock species, this cannot be blamed on the tools available to the breeders and advisor geneticists.

**Table 1**. Least square means and standard errors of the wool weights of Makooei sheep according to the various PIT-1, IGF-1, Leptin models.

| ***PIT-1*** | ***wool1*** | ***wool 2*** | ***wool 3*** | ***wool 4*** |
|---|---|---|---|---|
| **P1** | 0.444±0.019b | 1.49±0.057 | 1.41±0.056 | 1.73±0.062 |
| **P2** | 0.400±0.065b | 1.03±0.215 | 1.26±0.214 | 1.60±0.234 |
| **P3** | 0.525±0.021ab | 1.46±0.065 | 1.52±0.064 | 1.92±0.070 |
| **P4** | 0.675±0.092a | 1.40±0.373 | 1.30±0.37 | 1.75±0.406 |
| **P value 4.56*** ** | 1.44 | 0.89 | 1.72 | P value 4.56*** |
| **Leptin** | wool1 | wool 2 | wool 3 | wool 4 |
| **L1** | 0.9646±0.3907ab | 1.46± 0.101 | 1.52±0.104 | 1.99±0.113 |
| **L2** | 1.2709±0.3758ab | 1.36±0.121 | 1.43±0.118 | 1.77±0.129 |
| **L3** | 0.4843±0.5138b | 1.50±0.192 | 1.30±0.187 | 1.67±0.204 |
| **L4** | 1.6683±0.3794a | 1.47±0.069 | 1.48±0.067 | 1.84±0.073 |
| **L5** | 0.7208±0.4225ab | 1.49±0.081 | 1.40±0.080 | 1.69±0.087 |
| **P value** | 3.32* | 0.23 | 0.42 | 1.22 |
| **IGF-1** | wool1 | wool 2 | wool 3 | wool 4 |
| **AA(I1)** | 0.486±0.0425ab | 1.46±0.058 | 1.42±0.057 | 1.76±0.064 |
| **AG(I2)** | 0.517±0.0445a | 1.44± 0.067 | 1.49±0.065 | 1.85±0.073 |
| **GG(I3)** | 0.3456±0.062b | 1.61±0.154 | 1.38±0.153 | 1.83±0.169 |
| **P value** | 3.43* | 0.55 | 0.43 | 0.45 |

Same characters in column demonstrate no definitive compliance (P> 0.05); Dissimilar letters in column show definitive compliance (P< 0.05); wool1 (A fleece weight at lifetime), wool2 (Wool weight two years of lifetime), wool3 (Fleece weight at three years of lifetime), wool4 (Fleece weight at four years of lifetime).

In most countries where wool sheep are grown, there is a quite sophisticated wool market and market intelligence, and the important price determinants are well quantified and communicated. The amount of wool grown is an act of the surface region of the animal, the wool follicle density, also the volume of fiber per follicle manufactured in a given time. Ideally, the inducement is to breed animals exhibiting great follicle densities, low fiber diameter, as well as a great length growth rate of fiber. Each of these characteristics are recognized to be genetically also environmentally related. In special, a great negative genetic relation between follicle density as well as fiber diameter is clear in adult sheep from a variety of breeds also strains [14]. This negative relation is imagined in associated replies in desire lines for which the desire criteria have been based on one or difference of the elements of fleece weight. In as much as pleiotropic acts are not predictable without a mechanistic accepting of complete of the genes included, pleiotropy composes a substantial barrier to the breeding of advantageous high-quality wool-producing animals.

The biology of skin and wool growth in sheep has been extensively studied since the 1950's and the developmental processes at the cellular level are reasonably well understood. The basic units used in the study of biology of wool growth are the wool follicle and the fiber growing from it. Although there is evidence for higher-level organizational mechanisms, such as the trio group of primary follicles and associated secondaries, it is at the level of the individual follicle that most studies have focused. The wool subprogram used a combination of gene association and functional biology studies to identify genes and gene networks amenable to manipulation or selection to improve wool production and quality. Significant progress was made in identifying genes involved in wool follicle initiation, hair cycle regulation, recessive black pigmentation and fleece rot. Little information about the candidate genes affecting wool weights is available in makooei sheep. Therefore, it is suitable candidate genes markers that correlate with wool weights in the makooei sheep and must be identified before this tool can be applied to breeding populations. Lan et al. demonstrate that distributions of genotypic and allelic frequencies at AluI polymorphism of the goat POU1F1 gene are significantly different among meat breeds and cashmere breeds, implying that there is definitive relationship between cashmere traits and allelic frequencies of POU1F1 gene. Recently, the 3UTR 110T > C mutation (DQ827397:g.365T > C, PstI polymorphism) has been found to be associated with goat cashmere yield [15-16]. POU1F1 regulates PRL, the concentration of which is associated with wool growth and wool traits in sheep and may be responsible for the same effects in goats [17]. Therefore, the associations of four polymorphic

fragments of POU1F1 locus with greasy fleece weight and fiber length are analyzed in IMWC goats. The result showed that exon 3 (P4 fragment) and exon 5 (P6 fragment) have significant effects on fiber length and greasy fleece weight, respectively. In addition, the Glu109His, Glu110ter, Glu206Glu, Arg213Lys and Arg218Lys may also contribute to greasy fleece weight and fiber length [18]. Shen M et al, commented that the IGFBP-3 gene diversified genotypes minimally influenced many wool characteristics of Chinese Merino sheep [19]. The individuals of genotype AA, AB, also BB had no definitive contrast in post-shearing weight as well as neat wool measure. Staple length (SL) was diminished with the genotype of AA, AB, also BB, additionally the contradiction between AA and AB was important (P<0.01). oily fleece weight (GFW) also follicle density in individuals of genotype AA was definitively cheapen than that in individuals of genotype AB (P<0.01) additionally BB (P<0.05); mean fiber diameter (AFD) in individuals of genotype AA was definitively higher than that in individuals of genotype AB (P<0.01) as well as BB (P<0.05). Z. KarimiKurdistani described an cause of the intron 4/HaeIII polymorphism was definitively related with yearling weight (YW), post-weaning moderate workaday approach from 3-months to 12-months (ADG4), 6-months to 12-months (ADG6), 9-months to 12-months (ADG7), and first cut fleece weight (FSFW) ($P \leq 0.05$)[20]. Animals that acquired the GG genotype for the intron 4/HaeIII mutation demonstrated possibly additional adequate for entire mentioned characteristics. In the experimented Makooei sheep populace, definitive calculation events were determined in wool1 weight characteristic as to wool weights for IGF1, PIT1 also Leptin Genes. In addition, the measurement of a relation between these SSCP models with wool1, wool2, wool3, wool4 manifested a certain act of the entire models with wool weight in one year. Individuals with the L4, P4 also I2 of Leptin, PIT1 as well as IGF1, genes had the excellence wool weight in one year when contrasted to those of individuals with difference genotypes. In addition the affects showed L3,P2 and I3 had cheapen wool weight in one year characteristic when contrasted to those of individuals with difference genotypes. Additionally there is very little knowledge about polymorphism of the Leptin, PIT1 also IGF1 genes as well as theirs relation with wool weight characteristics. In the end, these events demonstrate that wool weights following one year of age may be mainly influenced surrounding agents like Nutrition. Furthermore the environs in addition act an important position. Environmental agents can be separated into pre-weaning also post-weaning affects. The nutritional input of the ewe during pregnancy and lactation is the governing agent of the pre-weaning environment. The ewes' nutrient input must be adequate to satisfy her own bodily requires for keeping and wool growth as well as supply sufficient nutrients to her foetus for growth also development until weaning. Post-weaning affects greatly reflect the impact of altering seasonal terms and therefore nourish availability on the working of the subsisting follicle population. In dates of adequacy like the spring flush of pasture growth a greater number of nutrients are possible to the follicles in the skin developing an increase in either wool growth also average fibre diameter. However as the nutrients attainable to the foetus pre-weaning, during pregnancy as well as lactation, can have a significant impact on the development the follicle population there is an adventure through advanced control of the breeding ewe flock, to increase the wool manufacture as well as wool quality level (Sue Hatcher and PR Johnson). Abundant assays have been described on class of ewe nutrition required at different stages of the reproductive cycle to optimize both wool and meat production per hectare [21-22]. Also abundant studies have been commented on the association of protein content in the ration to wool manufacture. Hajihosseinlo et al described Single nucleotide polymorphism in growth hormone gene exon-4 using PCR-SSCP in Makooei Sheep [23]. Fraser viewed that a "relative excess" of protein gave a 20 percent elevation in raw also clean fleece weights whereas the feeding of a protein-deficient ration approached in a 20 percent diminish in fleece weights[24]. Other researchers found that feeding increased amounts of casein to a basal ration which approved admissible gains during pregnancy had no cause on wool growth[25]. The affect of added protein feeding on wool growth. Such assessments recommend that variation in IGF-1, PIT-1, Leptin genes in domestic animals may be important in growth rate. The main objective of the present study was the assessment of relationships of putative polymorphisms of IGF-1, PIT-1, Leptin genes with wool weights in Iranian pure breed makoei sheep.

## 4. Conclusion

Our results indicate that these three polymorphisms contributed to the variation in the traits analyzed and reinforce the possibility of using these polymorphisms in molecular marker-assisted selection and breeding programs. Additional research with a bigger population of the same breed in order to have more animals of each genotype is required. Also research with signs affiliated with wool quality properties like staple strength, staple length; locate of Brea, Fiber diameter. Finding out genes of major outcome approaches the opportunity to revise production efficiency, product quality and product diversity, through utilizing them in breeding programs. Moreover, a study with other commercial breeds is also needed to determine the general effects of these polymorphisms, particularly since

commercial breeds are obtained from crosses of two or more breeds.

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