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An Association of Prolactin Gene Polymorphisms with Some Milk Traits in Women

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Abstract

We investigated the effects of prolactin gene polymorphisms on milk contents in women. The main aim of this work is to determine the genotypes of prolactin and its relationship with some milk chemical contents in women. Genotyping was carried out at Molecular Genetic Laboratory at the College of Agriculture, whereas biochemical assays were performed at the Department of Diseases Analyses at the South Technical University. Blood samples were collected for the prolactin-related gene. DNA was extracted from fifty candidate women. The extra-pituitary prolactin gene promoter 1149 G/T was subjected to *XapI* restriction enzyme. In this analysis *PRL-Pxa, I* products result in three genotypes TT, TG and GG, as well as the population, is under Hardy-Weinberg equilibrium. Our results showed that the highest milk fat yield, milk protein, lactose and sold not fat (SNF) materials percentages were obtained by the genotype GG.

***Keywords*:** Prolactin gene; Polymorphisms; Women; Milk traits.

1. Introduction

Human prolactin is encoded by a single gene located on chromosome 6 and composed of 5 exons and 5 introns [1] .Transcription of prolactin gene is regulated by two independent promoter regions, in the pituitary gland transcription starts from the promoter of the 1b exon, whereas the second promoter is that of a non-coding exon is active in the extra-pituitary gland [2]. The length of human prolactin cDNA is 914 nucleotide with 618 open reading frame nucleotide, code for a prohormone consists of 227 amino acid [3]. Its peptide signal has 28 amino acids; for this reason, the mature human prolactin composed of 119 amino acids.

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In mammals, prolactin hormone is best known for regulation of lactation and reproduction; since it binds to mammary receptors and enhances DNA transcription as well as it suppresses sex hormones (LH and FSH) in lactations [4].

The main aim of this work is to determine the genotypes of prolactin and its relationship with some milk chemical contents in women.

2. Materials and Methods

Biochemical assays were measured at the Dairy laboratory at the College of Agriculture, genotyping was analyzed at the laboratory of Genetic Engineering at the College of Agriculture and Department of Diseases Analyses at the South Technical University.

2.1. Milk Samples

Fifty women were studied. 20 ml milk samples were collected from each woman once only. Milk components (fat, protein, lactose, non-fat dried material), Funk Gerber Lacto Flash – Germany.

2.2. DNA Extraction and amplification

Blood DNA extraction was carried performed using the Geneaid apparatus with some modifications. For detection of the obtained sample, DNA was electrophoresis in 1% agarose with ethidium bromide [5].

The extra-pituitary *PRL-XapI* genotypes were analyzed using polymerase chain reaction- RFLP method. A 338 bp fragments of G/T 1149 promoter gene was amplified using the primer forward 5'- AGA ATT GGA GTT CCA GTG CC-3' and reverse 5'- ATC ACA CTC AAC CAG TTG GC-3' [6].

2.3. Statistical Analysis

Data were analyzed using pop gene program [7] to evaluate F- statistics, Fis, and the observed homozygosity and heterozygosity mean for all the three different genotypes, comparisons were calculated using [8]. Additive values, dominance deviation, dominance mean [9].

3. Results and Discussion

3.1. Amplification Product

Amplification product from prolactin gene promoter was performed successfully as observed by others [6] and [10]. As seen from PCR results (Figure1), amplification of *PRL* primer was 338 bp (single band) using 100 DNA marker was observed in all blood samples.

Two alleles and three genotypes were obtained as following: DNA restriction fragments were obtained for *PRL-Xap1* polymorphisms; 280 and 56 for GG; 338, 280 and 56 for GT and undigested 338 for TT (Figure 2).

The role of genetic polymorphism of G/T PRL-1149 promoter region has been demonstrated in many autoimmune diseases like systemic sclerosis, multiple sclerosis, polymyositis arthritis, psoriatic arthritis and lupus [11, 12, 13, 14].

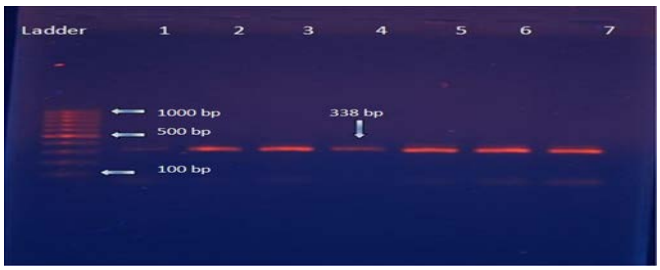

Figure 1: Amplification of PCR product of prolactin gene 338 bp.

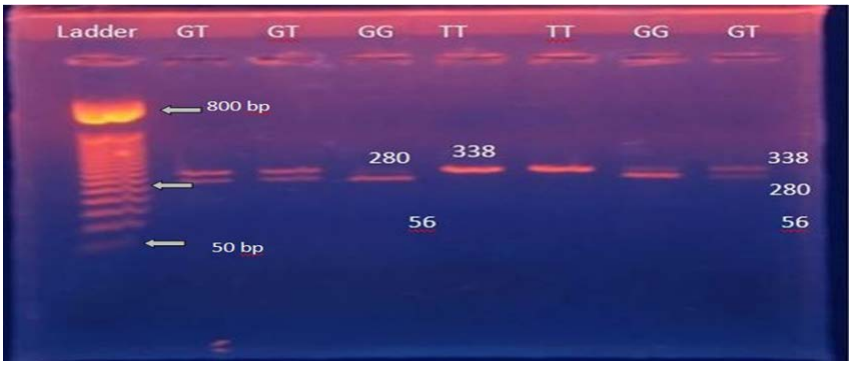

Figure 2: The polymerase chain reaction product of prolactin gene promoter bp 338 digested with *Xap-1* on 2% agarose gel electrophoresis and stained with ethidium bromide. TT genotype of undigested 338 PCR product; GG genotype = 280 and 56 bp.

Our results were consistent with findings made by [10] and [6]. Treadwell [10] demonstrated the important role of SNP of G/T 1149 (rs1341239) of the extra-pituitary *PRL* in a sample of lupus women of African or European ethnicity; and suggested the genotype TT- 1149 may be a risk factor associated with a predisposition of lupus. This study is recommended to be planned with larger population sample and different ethnic background.

A recent study reported the presence of different regulation mechanisms and functional activity of pituitary *PRL*-1149 polymorphism [6]. *PRL*-1149 is organ-specific; its expressions in many immune-related organs like the spleen and lymphoid glands [16].

PRL was originally identified as a hormone of pituitary origin, but extra-pituitary tissues also express this protein [16]. Both pituitary and extra-pituitary prolactin coded by the same gene directed by two independent promoters. Extrapituitary *PRL* expression is a specialized independent single cell such as pituitary transcriptional factor-1 which activates pituitary *PRL* transcription [17]. Genetic polymorphisms of extra-pituitary promoter have been observed with which contain SNP G/T 1149 (rs 1341239) at the sequence GATA [14].

3.2. Genetic Description

Table 1 demonstrates the observed and expected number of genotypes for 50 women enrolled in this study. Genotype GT 27 and 24.03 respectively which is the most frequent among the studied group, followed by GG 17 and 18.48 whereas TT observed and expected numbers were 6 and 7.48 respectively. There were no statistical differences between the group's chi-square (χ^2) = 0.78 was not significant. The population was in equilibrium at this restriction site using the Hardy-Weinberg equilibrium. Probability value was (G) 0.79 as observed by [6] who recorded that the number of rheumatoid arthritis (RA) women of genotype GT was higher than both TT and GG genotypes. TT had the least observed arthritis women. Whereas for healthy women; GG and GT were more frequent. TT had the least number among the control group. The expected numbers of the control were higher; chi-square values (χ^2) = 0.09 and 1.11 for patients and control respectively. The population was in equilibrium using Hardy-Weinberg equilibrium.

Table 1: Distribution of *PRL*-1149 G/T Promoter genotypes values

Genotypes	Observed No.	Expected No.	χ^2	G^2
TT	6	7.48	0.78	0.79
GT	27	24.03		
GG	17	18.48		
Total	50	50		

3.3. Genetic Variation

Table 2 demonstrates the presence of two alleles; G and T and three genotypes (TT, GT, and GG). G and T allelic frequencies were 0.61 and 0.39 respectively. Genotypes frequencies were 0.12, 0.54 and 0.34 respectively. The prevalence of the heterozygous GT was higher than both TT and GG. Fixation index (Fis) for G and T alleles was -0.1349 and 0.1349 respectively, this indicates there no consanguineous marriage as approved by the negative sign. Our findings were in accordance with previously reported results by [6], who studied *PRL*-1149 polymorphisms in a sample of rheumatoid arthritis (RA) women. He found that G-allele frequency was higher in patients group compared with the control group, on the contrary, the T allele. The frequency of TT genotype was highest in control group in comparison with the patient's group, whereas the GG and GT genotypes were more abundant in patients than the control group. This difference between allele frequencies can be translated to develop RA among those carrying G allele, but the abundance of T allele seems to be protective. An association between T allele frequency and decreasing susceptibility to RA was observed [18]. The relationship between GG, GT genotypes and the predisposition to RA was noticed [6]. Further confirmation of this result was achieved. The same result was found by [14]; the dominance of GG genotype among the healthy group. GG genotype frequency was higher in RA patients so that could confer a risk for RA in the Iraqi society [6]. The *PRL*-114 G/T polymorphism among population can be due to ethnic- genetic

heterogeneity that might reflect migration history and the impact of natural selection force that shaped genetic variation in a population. [19, 20]. The further interesting finding has been recorded, an association of *PRL*-114 G/T polymorphism and RA patients; T allele frequency and TT genotypes were higher in RA patients than the control group and vice versa for G allele and GG genotype [6].

Table 2: Alleles Frequencies, Genotypes frequencies and Fixation Index (Fis)

Women total No.	**Alleles frequencies**		**Genotypes frequencies**			**Fis**	
50	G	T	TT	GT	GG	T	G
	0.61	0.39	0.12	0.54	0.34	-0.1349	-0.1349

3.4. Milk chemical Composition

Table 3 presents the results of milk chemical composition in women. There is a significant difference between *prolactin* genotypes for milk chemical composition percentages (fat, protein, ash, humidity, lactose, and non-fat dried materials). The genotype GG had higher milk chemical components were as follows: 3.78% fat, 4.26% protein, 0.26% ash, 5.58% lactose and 10.37% non-fat dried materials that both GT and TT genotypes. TT genotype had higher humidity (93.945) than both GT and GG genotypes. *Prolactin* is one of the candidate genes to be studied since it plays a crucial role in the initiation and maintenance of lactation and expression of milk protein genes. Lactation polymorphism is due to genetic background (ethnicity), as well as physiological and environmental factors have a crucial impact on milk traits.

Table 3: Milk chemical composition (%) in women ± Standard Deviation

Traits	Genotypes		
	TT	**GT**	**GG**
Fat	0.96±3.78	1.10±2.64	0.93±1.71
Protein	1.14±4.26	0.84±2.26	0.87±1.93
Ash	0.82±0.62	0.97±0.52	0.35±0.28
Moisture	1.28±85.57	0.79±90.90	0.82±93.94
Lactose	0.72±5.58	0.92±3.66	1.24±3.53
Solid not fat	0.89±10.37	0.99±6.16	0.60±6.31

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