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# Genetic detection of prolactin intron 1 region in breast cancer patients of Iraqi women

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## Abstract

This study was made to inspect the genetic cause of breast cancer through the molecular base related with single nucleotide polymorphism (SNP) at prolactin gene in patients with the breast cancer .This study comprised thirty blood samples from patients suffering from breast cancer. Also thirty tissue samples of breast cancer patients were collected in which samples were formalin fixed paraffin embedded tissue. thirty blood samples from healthy persons were collected served as control group. The main ages of patients were 19 to 60 and same for control (healthy) group.The variation in gene that accountable for synthesis of hormone prolactin, was conducting using samples of breast cancer patients to demonstrate if this variation is important to breast cancer risk In addition to. Polymerase chain reaction (PCR), was done by using specific set of primers , in which 3 primers were selected to amplify the intron 1 region of the gene.

After optimization of the amplification condition, the product was sent for DNA sequencing for detection of variation of patient prolactin gene, so the association of this variation of prolactin gene to breast cancer patients was clear after studying the intron region. In intron 1 of the gene, 7 mutation was detected by using primer 1, in which two of them is deletion mutation and 5 was substitution, while in same intron of the gene but using primer 2, 6 mutation was detected all is substitution. The risk association between the prolactin intron gene association and breast cancer patients using information on national center for biotechnology information (NCBI), and Mega 6 program. The results of mutation detection in the PRL gene intron region showed that there is occurrence of mutations in of breast cancer patients samples

Keywords: Single nucleotide polymorphism; Breast cancer; Prolactin intron 1 gene

# 1. Introduction

Prolactin (PRL) is a polypeptide hormone of a pituitary origin, whose production is organized by dopamine and this hormone have many biological activity such as lactation and reproductive functions (Bernichtein *et al.*, 2010).

The human prolactin gene is present as a single copy on chromosome 6 it is about 12.215 kb) contains 5 exons and 4 introns and the transcription of it is regulated by two promoters is used in extra pituitary cells and tissues and downstream promoter that directs transcription in pituitary lactotrophs (Rui and Nevalainen, 2000). As the prolactin is an essential regulator of mammary development, the primary cells targeted by prolactin are the breast tissue cells in which it is involved in the development of mammary gland and in cellular growth and differentiation as well as in the initiation and maintenance of lactation (Mong *et al.*, 2011). studies demonstrated that prolactin could induce spontaneous mammary tumors and can stimulate proliferation (Liby *et al.*, 2003).

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A woman's genetic background contributes to her risk of having breast cancer because the risk of developing breast cancer increases in women if she has a family history of the disease (Lallo and Evans, 2012).

About 90 genes or genetic loci are involved in breast cancer susceptibility in general this was through rare, moderate to high penetrance mutations (lifetime risk >20%). The penetrance being the risk of a mutation carrier of developing a disease, or through common variants associated with risks that are only slightly increased compared to the wild-type allele (Couch *et al.*, 2014). In most cases the genetic variation plays a role and it is thought that genetics is the primary cause of 5-10% of cases (Gage et al., 2012).

Molecular oncology is now one of the greatest hopeful fields that may contribute significantly to diagnosis of breast cancer and its metastases address major problems with early detection, accurate staging, and monitoring of breast cancer patients.

The linkage between variation of both genes of PRL and PRLR with breast cancer was detected (Canbay et al., 2004; and Lee et al., 2007). Furthermore, this variation in these genes was shown to effect on breast cancer risk (Vaclavicek *et al.*, 2006) because it was expressed in both normal and malignant breast tissues (Ben-Jonathan *et al.*, 2002; and Clevenger et al., 2003).

## 2. Materials and methods

Patient selection and blood sample collection

Blood sample were collected from thirty (30), women suffering from breast cancer their ages ranged from (19-60) years, and 30 blood samples collected from healthy women. All samples were subjected for molecular analysis. The blood samples that collected in EDTA tube, was stored at -20 C until used for DNA extraction. The samples were obtained from hospital Kamal Al-Sammaraee. The collection period extended from July 2023 to December 2023. Also thirty tissue samples, with breast cancer were got from Al-Khadmyaa teaching hospital. The primers that have been selected for this study to amplify portion of prolactin intron region of the gene was designed, and the details were illustrated in (table 1).

## 2.1. DNA extraction

Total cellular DNA was extracted from blood samples by using the reliaprep blood genomic DNA MiniPrep System from Promega USA

Estimation the concentration and purity of the extracted DNA were measured by using nanodrop (UVIS Drop\ACTGene\USA Primers

No	Oligonucleotide	Oligosequnese	Prod.Size (bp)	GC%	Tm	Ref.
1	Forward primer	CGTAGGCTGGATTTGAAGGGT	312	52.38	54.36	NCBI
	Reverse primer	AGCGATAGATCAGGGTGCCT		55.00	53.83	
2	Forward primer	AGGGGGTAACATGCATAGCAG		52.38	54.36	NCBI
	Reverse primer	TCCCTGGATGGAGAGAGTCTG	416	57.14	56.31	
3	Forward primer	ATCCCGGGAAGTAAGCATGG		55.00	53.83	NCBI
	Reverse primer	TTGCTAGGGCTTTGGAGGTC	618	55.00	53.83	

Table 1 Sequences of primers used to amplify prolactin intron 1 region

## 2.2. PCR amplification

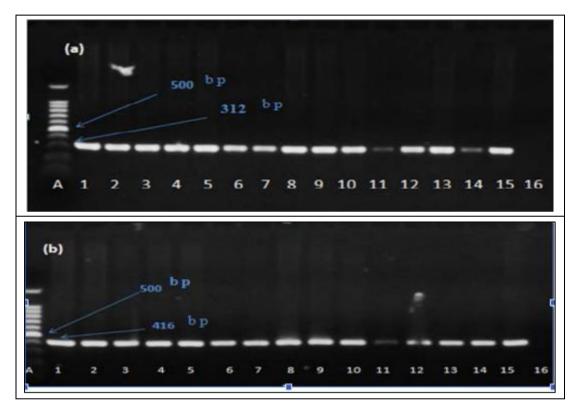
the preparation of PCR reaction mixed on ice and was carried out in 25  $\mu$ l of Go Taq Green masrer mix. The amplification condition were as following for primer 1, initial denaturation 94°C for 5 min, denaturation 94°C for 1 min, annealing 1 min at 55°C, extension 1 min at 72°C for 35 cycles, and final extension 72 °C for 10 min. Same program used for amplification of prolactin gene using primer 2 and 3, but with different annealing temperature, in which its 57°C for 1 min by sing primer 3.

- **DNA gel electrophoresis :** the quality of extracted DNA and PCR amplicons was checked with 1% agarose gels at 90V for 90 min.
- Statistical analysis: the statistical package for the social sciences (ANOVA version 15).

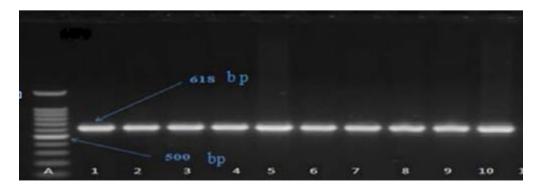
# 3. Result and discussion

## 3.1. Intron 1 amplification of Prolactin

samples of patients (blood, FFBE tissue) were subjected to molecular detection through PCR amplification of the PRL gene (intron 1 region) by using three specific primers predesigned for intron region of prolactin gene, the 3 primers set used in this PCR technique (PRL1, PRL2, PRL3), specific for intron 1 region of the prolactin from NCBI primer design with product length(312, 416 and 618bp respectively) which is shown in the figures (a,b and c).



**Figure 1 ( a, b)** Gel electrophoresis for amplification of intron 1 of PRL gene of breast cancer patients by using primer 1,(a) product size 312 bp and (b) 416. Electrophoresis was performed on 1.5% agarose gel and run with a 80v/mAMP current for 50min.Line A=100bp ladder, line (1-6) DNA isolated from blood samples of patients, line (7-12)DNA from patient tissue, line (13-15) DNA isolated from healthy, line (16) control negative



**Figure 1 (c)** Gel electrophoresis for amplification of PRL gene of breast cancer patients by using primer 3 which amplifies intron 1 of the gene, product size 618 bp. Electrophoresis was performed on 1.5% agarose gel and run with a

80v/mAMP current for 50min.Line A=100bp ladder, line(1-4) DNA isolated from blood samples of breast cancer patients, line(5-7)DNA from tissue, line (8-10) DNA isolated from healthy, line (11) control negative

## 3.2. Detection of intron 1 region of PRL gene mutations in breast cancer patients by sequencing

After amplification of genomic fragments corresponding to intron1 of the PRL gene the PCR products were (312, 618,416) shown in the above figures. By using the DNA of the above cases were selected to be sequenced in order to assess if any genetic variation in the PRL gene were known as predictors of breast cancer risk.

The sequencing was done to infected women, of breast cancer patients and for control. The results were directly matched with the Iraqi healthy, and compared to the information in the gene bank of the NCBI web site databases at www.ncbi.nlm.nih.gov using the BLAST search tool and also by using Mega 6 program. The current study utilized a forward and reverse primer for sequencing PRL gene (intron 1)of blood and tissue sample of breast cancer patients. It was found that the mutations were found around all PRL gene intron regions involved in this study, According to NCBI, this streach of intron 1 of gene, 9 mutations were detected.

The intron region of PRL region was detected and examined for the presence of any mutations or SNPs and the effect of this alteration in the function of the gene. This region was amplified using three primers, the first one amplifies the PRL at the gene region from 6169 to 6489 with product size 312bp. The second primer also amplifies the same region from 6556 to 7173, with the product size 618bp, the third primer amplified the region from 8737 to 9152, with the product size 416bp. The first and third primers were selected for gene sequencing. And it was shown that there were too many SNPs in the beginning of the gene region or in the end, so it was not considered. there were four SNPs in some samples, in breast cancer patient as shown in figure **2** 

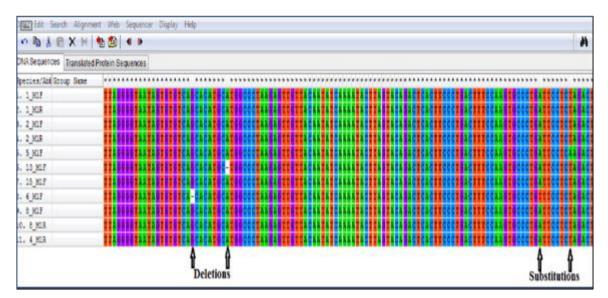
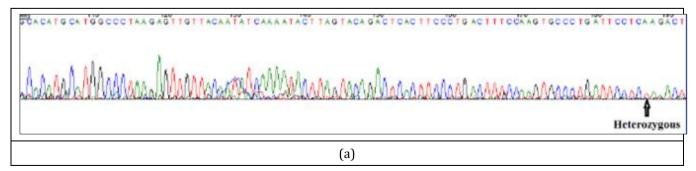
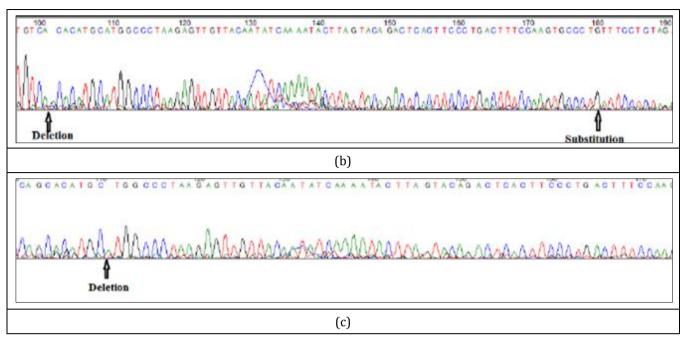


Figure 2 SNPs in intron one of hyperprolactemic and breast cancer patients by using primer 1, product size 312bp

## 3.3. The peaks of SNPs in these samples are obvious in figure (3) a, b and c





**Figure 3** Peaks of (a): breast cancer patient (Sample 5) shows the heterozygous SNP, (b): breast cancer patient (sample 6) shows the deletion and substitution mutations. (c): breast cancer (sample 13) shows the deletion mutation

The details about these mutations which appear in intron 1 in breast cancer patients when compared to NCBI are shown in figures (4) a, b and c.

Range 1	6199 to	6423 GenBank	<u>Braphics</u>	Vex Nex	t Match 🔺 Previous Match	
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Sbjct	6199	TCTGAAGAGCC	TGCTCTACTTTCAG	CTGAATCTTTTCAA	TACAGGCAAAAAAATTGGC	6258
Query	69	AGTGGGGGAAG	TTAGGGGTAATAGTG	GTCAGCACATGCAT	GGCCCTAAGAGTTGTTACA	128
Sbjct	6259	AGTGGGGGAAG	TTAGGGGTAATAGTG	GTCAGCACATGCAT	GCCCTAAGAGTTGTTACA	6318
Query	129	ATATCAAAATA	CTTAGTACAGACTCAC	TTCCCTGACTTTCC	AAGTGCCCTGATTCCTCAA	188
Sbjct	6319	ATATCAAAATA	CTTAGTACAGACTCAC	TTCCCTGACTTTCC	AAGTGCCCTGATTCCTCTA	6378
Query	189	GACTCCCCCAG	CCCCTCACATAGGTC	ACCCCTAAAGACAC	ACCA 233	
Sbjct	6379	GACTCCCCCAG	CCCCTCACATAGGTC	ACCCCTACAGTCTC	ACCA 6423	

Range 1	: 6200 to	6424 GenBank	Graphics	V Ner	et Match 🔺 Previous Match	
Score 379 bits	s(205)	Expect 1e-101	Identities 219/225(97%)	Gaps 3/225(1%)	Strand Plus/Plus	
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Sbjet	6200				ACAGGCAAAAAAATTGGCA	625
Query	73				GCCCTAAGAGTTGTTACAA	131
Sbjct	6260		TAGGGGTAATAGTGTG		GCCCTAAGAGTTGTTACAA	631
Query	132		TTAGTACAGACTCACT		AGTGCCCTGATTCCTCTAG	191
Sbjct	6320		TTAGTACAGACTCACT		AGTGCCCTGATTCCTCTAG	637
Query	192		CCCTCACATAGGTCAA			
Sbjct	6380		CCCTCACATAGGTCAA			
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Figure 4 a, b and c: NCBI of intron1 of prolacten gene by using primer 1 (a): breast cancer patient(sample 5), b: breast cancer patient (sample 13), c: breast cancer patient (sample 6)

The three SNPs of breast cancer patients are as follows: two in sample 6 where one is substitution TGA/TGT that converts stop codon to Cys at position 147, the other is deletion mutation in which GCA/ -CA in position 69. In the same region of the gene of breast cancer patients in sample 5, there is a substitution mutation in position 156 that converts TAG/AAG that convert Stop codon to Lys. The deletion mutation is in the same region of the gene, but in other patient it is in position 77 of sample 13 that converts GCA to GC-,also in other sample but in same region which is intron 1 of the gene, there is heterozygous SNP as is clear in the peaks Fig (5).

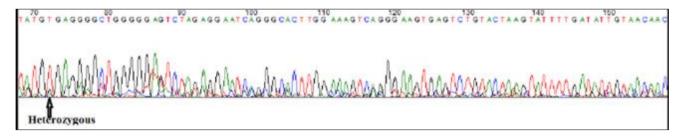


Figure 5 Peaks of intron 1 (sample 4) show the heterozygous SNP

Intron one was amplified also by using primer 3 product size 416bp and it was found that there are many substitution SNPs and also there are common SNPs between patients . In figure (6) a, the SNPs that are common between patients are clear in which AAA convert to AAT is in sample 6 and in sample 12, in position 104 in which Lys/Asn. The other substitution mutation is in sample 6 in which GCC is converted to TGC. In the same region, but in other samples, sample 5 TAA/TAT in position 272 convert stop codon to Tyr. But in 210 position of sample 7, the ACC/GCC converts Thr/Ala. The last one is sample 9 in 277 in which TAT/TTT that converts Tyr to Phe as its clear in figure (6) a, b

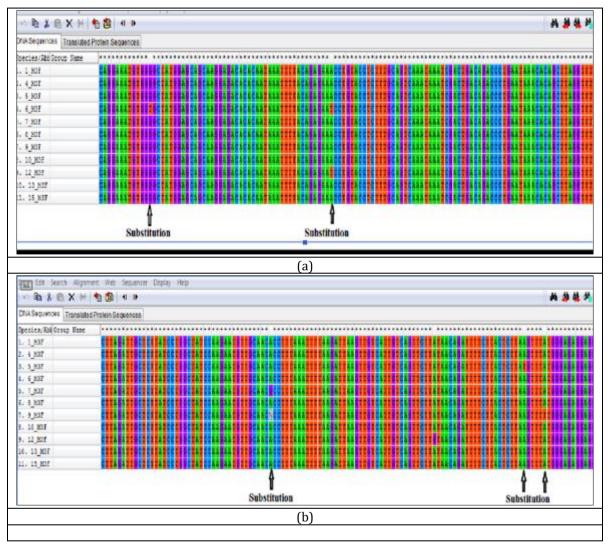
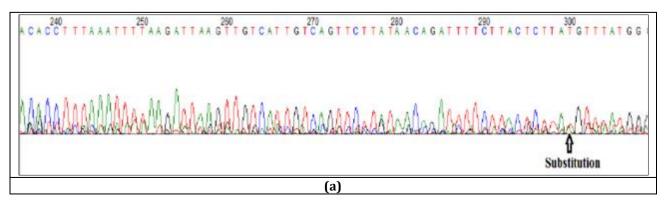
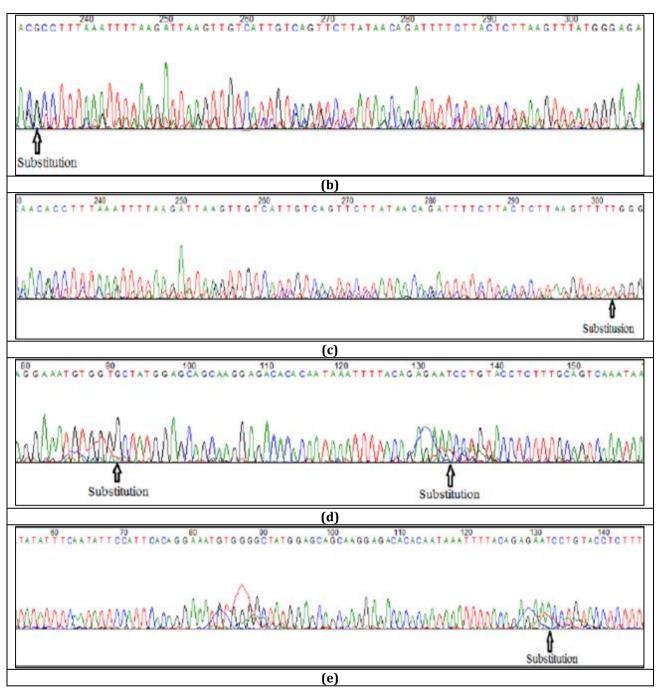


Figure 6 a and b: Substitution mutations of intron 1 by using primer 3 of breast cancer patients

The peaks for mutation samples that have mutation are clear in figure (7), a, b, c, d and e. The NCBI results of the mutations that are detected in intron one using primer (3) are obvious in figure(8), a, b, c, d, e and f.





**Figure 7** a, b, c, d and e: Peaks of intron 1 by using primer 3 for breast cancer patient. a, (Sample 5), b: Sample 7, c: Sample 9, d: Sample 6, e: Sample 12

Range 1:	8776 to	8989 GenBank G	iraphics	Vex Nex	t Match 🔺 Previous Match	
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Sbjct	8955		TCTTATCCTGGCTATC			
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Sequence Range 1 Score 667 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct	e ID: refl (361) 12 8775 72 8035 132 8095 192 8955	NG 029819.11 LA P138 GenBank S Expect 0.0 ACTATGATTTT ACTATGATTTT CAGGAAATGTO IIIIIIIIIIII CAGGAAATGTO GTACCTCTTTG TCTTAGATTGO IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	RefSeqGene on ch ength: 22610 Number <u>Sraphics</u> identities 363/364(99%) TGCATAATATATGTCT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	romosome 6 or Matches: 1 Caps 0/364(0%) TTGCATTATTTATA AGGAGACACACAAT IIIIIIIIIIIIIIIII AGGAGACACACAAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus TATTTCAATATTCCATTCA IIIIIIIIIIIIIIIIIII	8834 131 8894 191 8954 251 9014
Sequence Range 1 Score 667 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct Query	e ID: refl 12 8775 72 835 132 8895 192 8955 252	ACTATGATTTT ACTATGATTTT ACTATGATTTT ACTATGATTTT CAGGARATGTC GTACCTCTTTC GTACCTCTTTC TCTTAGATTGC TCTTAGATTGC TAAGTTGTCAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	RefSeqGene on ch ength: 22610 Number <u>sraphics</u> identities 363/364(99%) TGCATAATATATGTCT GGGCTATGGAGCAGCA CAGTCATATGGAGCAGCA CAGTCAAATAAAATCGA CAGTCAAATAAAATCGA TCTTATCCTGGCTATC TCTTATCCTGGCTATC	romosome 6 or Matches: 1 Caps 0/364(0%) TTGCATTATTTATA AGGAGACACACAAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus TATTTCAATATTCCATTCA IIIIIIIIIIIIIIIIIII	8834 131 8894 191 8954 251 9014 311
Sequence Range 1 Score 667 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct	e ID: refl (361) 12 8775 72 8035 132 8095 192 8955	ACTATGATTTT ACTATGATTTT ACTATGATTTT ACTATGATTTT ACTATGATTTT CAGGAAATGTC GIACCTCTTTC GIACCTCTTTC GIACCTCTTTC TCTTAGATTGC TAAGTTGTCAT	RefSeqGene on ch ength: 22610 Number Staphics Identities 363/364(99%) TGCATAATATATGTCT GGGGCTATGGAGCAGCA CAGTCAAATAATATGTCT CAGTCAAATAAAATCGA CAGTCAAATAAAATCGA CAGTCAAATAAAATCGA CAGTCAAATAAAATCGA CTCTTATCCTGGCTATC TCTTATCCTGGCTATCGA TGTCAGTTCTTATAAC	romosome 6 of Matches: 1 Caps 0/364(0%) TTGCATTATTTATA AGGAGACACACAAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus TATTTCAATATTCCATTCA TATTTCAATATTCCATTCA AAATTTTACAGAGAAACCT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATTTAAATTTTAAGAT	8834 131 8894 191 8954 251 9014
Sequence Range 1 Score 667 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct Query Sbjct	e ID: refi 3775 to (361) 12 8775 72 8035 132 8095 192 8955 252 9015	NG 029819.11 LA P138 GenBank S Expect 0.0 ACTATGATTTT ACTATGATTTT CAGGAAATGTO IIIIIIIIIII CAGGAAATGTO GTACCTCTTTG TCTTAGATTGO TAAGTTGTCAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	RefSeqGene on ch ength: 22610 Number Staphics Identities 363/364(99%) TGCATAATATATGTCT GGGCTATGGAGCAGCA IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	romosome 6 of Matches: 1 Caps 0/364(0%) TTGCATTATTATATATATATATATATATATATATATATAT	Strand Plus/Plus TATTTCAATATTCCATTCA TATTTCAATATTCCATTCA AAATTTTCAATATTCCATTCA AAATTTTACAGAGAAACCT ATAAACACAGGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGAAAGAA	8834 131 0894 191 0954 251 9014 311 9074
Sequence Range 1 Score 667 bits Query Sbjet Query Sbjet Query Sbjet Query Sbjet Query Sbjet Query	e ID: refl (361) 12 8775 72 8035 132 8095 132 8095 192 8955 252 9015 312	NG 029819.11 LA P138 GenBank S Expect 0.0 ACTATGATTTT ACTATGATTTT CAGGAAATGTO IIIIIIIIIII CAGGAAATGTO GTACCTCTTTG TCTTAGATTGO TAAGTTGTCAT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	RefSeqGene on ch ength: 22610 Number Staphics Identities 363/364(99%) TGCATAATATATGTCT GGGCTATGGAGCAGCA IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	romosome 6 of Matches: 1 Caps 0/364(0%) TTGCATTATTATATATATATATATATATATATATATATAT	Strand Plus/Plus TATTTCAATATTCCATTCA TATTTCAATATTCCATTCA AAATTTTACAGAGAAAACCT AAATTTTACAGAGAAAACCT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGTTT ATAAACACAGCTTAGGAAAGA TTAAGTTTATGGGAGAGGA TTAAGTTTATGGGAGAGGA TTAAGTTTATGGGAGAGGA TGCTTTCTTAATAATTCAG	883 131 889 191 895 251 901 311 907 371

Range 1	: 8774 to	9156 GenBank	Iraphica	V Neo	ct Match 🔺 Previous Match	
Score 649 bits	(351)	Expect 0.0	Identities 375/386(97%)	Caps 3/386(0%)	Strand Plus/Plus	
Query	10	111111 1111	1111111111111111111	111111111111111111	TATATTTCAATATTCCATT	69
Sbjet	8774				TATATTTCAATATTCCATT	8832
Query Sbjct	70 8833	111111111111	111111111111111111111	11111111111111111	ATAAATTTTACAGAGAAAC	129 8892
Query	130	CTGTACCTCTT	TGCAGTCAAATAAATC	GACTGACAGACCCT	GAATAAACACAGCTTAGGT	189
Sbjet	8893				GATAAACACAGCTTAGGT	895
Query	190	TITCTIAGATI	GCTCTTATCCIGGCIA	TCCAAGAATGTTGC.	AACACCITTAAATTTTAAG	249
Sbjet	8953	111111111111	11111111111111111111	11111111111111111	AACACCTTTAAATTTTAAG	901:
Query	250		ATTGTCAGTTCTTATA		~	309
Sbjot	9013	111111111111	attorcastcettata	11111111111111111		9072
Query	310				TCTGCTTTCGTCATCATTC	369
Sbjct	9073				TCTGCTTTCTTAATAATTC	9132
Query						
	370	IGACICICICC	ATCCCCGGTGAAAAA	395		
Homo s	9133 apiens	prolactin (PRL)		9156 ) romosome 6		
Sbjet Homo S Sequenc Range 1	9133 apiens   e ID: <u>refl</u>	prolactin (PRL). NG_029819.11 L	RefSeqGene on chi ength: 22610 Number of Graphica	9156 Tomosome 6 of Matches: 1	et Match 🛦 Previous Match	
Sbjct Homo S Sequenc Range 1 Score	9133 apiens e ID: <u>refi</u>	prolactin (PRL) NG_029819.11 L 9106 <u>GenBank</u> ( Expect	RefSeqGene on chi ength: 22610 Number Graphica Identities	9156 romosome 6 of Matches: 1 V Next	Strand	
Homo S Sequenc Range 1 Score 584 bits	9133 apiens   e ID: refl/ i 8767 to s(316)	prolactin (PRL) NG_029819.11 L 9106 GenBank ( Expect 3e-163	RefSeqGene on chi ength: 22610 Number Graphica Identities 334/342(98%)	9156 romosome 6 of Matches: 1	Strand Plus/Plus	
Homo S Sequenc Range 1 Score 584 bits Query	9133 apiens e ID: <u>refi</u>	prolactin (PRL) NG_029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT	ATCCA-GG-GAAAAA (c , RefSeqGene on ch ength: 22610 Number Graphics Identities 334/342(98%) CATGGATTTTGGCATA	9156 promosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC 111111111111111111111111111111111111	Strand	65 882-
Homo s Sequenc Range 1 Score 584 bits Query Sbjct	9133 apiens   i D: refl1 i D767 to s(316) 7 8767	prolactin (PRL) NG_029819.11 Lu 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT 1111111111	Caraphica Identities 334/342(98%) Caragantities Caragantities Caraphica	9156 promosome 6 of Matches: 1 V Next Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATTTCAAT 	882
Homo S Sequenc Range 1 Score 584 bits Query Sbjct Query	9133 aplens i D: refl i 8767 to 5(316) 7 8767 66	Prolactin (PRL) NG_029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT ITTAGGACAACT ATTCCATTCAC	ATCCA-GG-GAAAAA (c , RefSeqGene on ch ength: 22610 Number Graphics Identities 334/342(98%) ATGGATTTTTGGCATA AT-GATTTTT-GCATA CAGGAAAIGIGGGGCIA	9156 romosome 6 of Matches: 1 V Nex Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATTTCAAT IIIIIIIIIIIIIIIIIII	8824
Homo S Sequenc Range 1 Scora 584 bits Query Sbjct Query Sbjct	9133 apiens   i 8767 to i 8767 66 8825	prolactin (PRL) NG_029819.11 LA 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT 1111111111 TTAGGACAACT ATTCCATTCAC	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphics Identities 334/342(98%) CATGGATTTTTGGCATA II IIIIII AT-GATTTTT-GCATA CAGGAAATGTGGGGGCTA	9156 promosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATTTCAAT IIIIIIIIIIIIIIIIIII	8824 125 8884
Homo S Sequenc Range 1 Scora 584 bits Query Sbjct Query Sbjct	9133 aplens i D: refl i 8767 to 5(316) 7 8767 66	prolactin (PRL), NG_029819.11 L4 9106 GenBank () Expect 3e-163 TTAGGAC-ACT 1111111111 TTAGGACAACT ATTCCATTCAC AGAGAAACCTG	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphics Identities 334/342(98%) CATEGATTTTTGGCATA II IIIIIII CAT-GATTTTT-GCATA CAGGAAATGTGGGGGCTA IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	9156 promosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATTTCAAT IIIIIIIIIIIIIIIIIII	8824
Homo S Sequenc Range 1 Score 584 bits Query Sbjct Query Sbjct Query	9133 apiens   i 8767 to i 8767 66 8825	AGACTCTCTCC Prolactin (PRL) NG_029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphics Identities 334/342(98%) CATCGATTTTTCGCATA AT-GATTTTT-GCATA CAGGAAAIGIGGGGGCIA CAGGAAAIGIGGGGGCIA	9156 romosome 6 of Matches: 1 V Nex Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATTTCAAT IIIIIIIIIIIIIIIIIII	882 125 888 185
Homo S Sequenc Range 1 Score 584 bits Query Sbjct Query Sbjct Query Sbjct	9133 aplens D: refl 18767 to 5(316) 7 8767 66 8825 126	prolactin (PRL), NG_029819.11 L4 9106 GenBank () Expect 3e-163 TTAGGAC-ACT 1111111111 TTAGGACAACT ATTCCATTCAC ATTCCATTCAC AGAGAAACCTG 1111111111 AGGGAAACCTG CCTTAGGTTTT	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphics Identities 334/342(98%) CATGGATTTTTGGCATA II IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	9156 Promosome 6 of Matches: 1 V Nec Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATATTCAAT ATTATTTATATATATTTCAAT GACACACAATAAATTTTCAAT GACACACAATAAATTTTAC CAGACCCTGAATAAACACA IIIIIIIIIIIIIIIIIIIIIIIIIIIII	882 125 888 185
Homo s Sequenc Range 1 Score 584 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct Query	9133 apiens   be ID: refil (316) 7 8767 66 8825 126 8885	AGACTCTCTCC prolactin (PRL) MG_029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT TTAGGACAACT ATTCCATTCAC AGAGAAACCTG IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphica Identities 334/342(98%) ATGGATTTTGGCATA AT-GATTTT-GCATA CAGGAAATGTGGGGCTA CAGGAAATGTGGGGCTA CAGGAAATGTGGGGCTA CACTCTTTGCAGTCA CAGGAAATGTGGGGCTA CACTCTTTGCAGTCA	9156 romosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATATTCAAT ATTATTTATATATATTTCAAT GACACCACAATAAATTTTAC IIIIIIIIIIIIIIIIIIII	8824 125 8884 185 8944 245
Homo S Sequenc Range 1 Score 584 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct	9133 apiens   e ID: refl/ 9767 to 5(316) 7 8767 66 8825 126 8885 186	AGACTCTCTCC Prolactin (PRL), NG_029819.11 L 9106 GanBank (PRL), 9106 GanBank (PRL), 9106 GanBank (PRL), 9106 GanBank (PRL), Provide Comparison (PRL),	ATCCA-GG-GAAAAA (C) RefSeqGene on chi ength: 22610 Number Graphics Identities 334/342(98%) CATEGATTTTTGGCATA CAGGAAATGTGGGGGCTA STACCTCTTTGCAGTCA STACCTCTTTGCAGTCA CAGGAAATGTGGGGGCTA STACCTCTTTGCAGTCA CTTAGATTGCTCTTAT	9156 romosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATATTTCAAT IIIIIIIIIIIIIIIII	882 125 888 185 894 245
Homo s Sequenc Range 1 Score 584 bits Query Sbjct Query Sbjct Query Sbjct Query Sbjct Query	9133 apiens   be ID: refil 8767 to (316) 7 8767 66 8825 126 8885 126 8885 186 8945	AGACTCTCTCC prolactin (PRL) G 029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGAC-ACT 11111111111 TTAGGACAACT ATTCCATTCAC AGAGAAACCTC GCTTAGGTTTT ATTTCAGGTTTT ATTTTAGGATT	Crtagattgccttat	9156 Promosome 6 of Matches: 1 Caps 3/342(0%) ATATATGTCTTTGC ATATATGTCTTTGC ATATATGTCTTTGC ATATATGTCTTTGC ATATAATGGAGCAAGGA IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATATTTCAAT IIIIIIIIIIIIIIIII	882 125 888 185 894 245 900 305
Sbjet Homo S Sequenc Range 1 Score	9133 apiens   e ID: refil 9767 to 66 8767 66 8825 126 8885 126 8885 126 8945 246	AGACTCTCTCC Prolactin (PRL) G 029819.11 L 9106 GenBank ( Expect 3e-163 TTAGGACAACT ATTCCATTCAC AGAGAAACCTC IIIIIII AGAGAAACCTC CCTTACCTTT IIIIIIII GCTTAGGTTTI ATTTTAAGATT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Crtagattgccttat	9156 POMOSOME 6 of Matches: 1 VNO Caps 3/342(0%) ATATATGTCTTTGC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Strand Plus/Plus ATTATTTATATATATTTCAAT IIIIIIIIIIIIIIIII	8824 125 8884 185 8944 245 9004

Range 1	: 8835 to	9083 GenBank G	raphics	V Nex	a Match 🔺 Previous Match	
Score 449 bits		Expect 8e-123	Identities 247/249(99%)	Gaps 0/249(0%)	Strand Plus/Plus	
Query	1	CAGGAAATGTG	GGGCTATGGAGCAGCA	AGGAGACACACAT	AAATTTTACAGAGAATCCT	60
Sbjet	8835	111111111111	GGGCTATGGAGCAGCA			8894
Query	61	GTACCTCTTTG	CAGTCAAATAAATCGA	CTGACAGACCCTGA	ATAAACACAGCTTAGGTTT	120
Sbjct	8895				ATAAACACAGCTTAGGTTT	8954
Query	121	TCTTAGATTGC	TCTTATCCTGGCTATC	CAAGAATGTTGCAA	CACCTITAAATTTTAAGAT	180
Sbjct	8955				CACCITTAAATTTTAAGAT	9014
Query	181				TTAAGTTTATGGGAGAGAGA	240
bjct	9015				TTAAGTTTATGGGAGAGAGA	9074
Query	241		249			
tipmo		prolactin (PRL)	9083 (e ), RefSeqGene on ch .ength: 22610 Number	romosome 6		
Sequen	sapiens	GGAGAATAT prolactin (PRL)	), RefSeqGene on ch .ength: 22610 Number	of Matches: 1	oct Match 🔺 Previous Match	
Range Score	sapiens	GGAGAATAT prolactin (PRL) NG_029819.11 L o 9083 GenBank	(c), RefSeqGene on ch ength: 22610 Number Graphics	or Matches: 1		
Range Score 556 bil Query	sapiens ice ID: ref 1: 8774 b ts(301) 14	GGAGAATAT prolactin (PRL) NG_029819.1] L o 9083 GenBank Expect 6e-155 AACTATGGAT	(Graphics Identities 300/311(99%)	Gaps 1/311(0%)	Strand Plus/Plus ATATATTTCAATATTCCATT	0.000
Range Score SSG bi Query Sbjot	sapiens nee ID: ref 1: 8774 to ts(301) 14 8774	GGAGAATAT prolactin (PRL) NG_029819.1  L o 9083 GenBank Expect 6e-155 AACTATGGAT 	(G ), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) TTTGCATAATATATG IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%)	Strand Plus/Plus ATATATTTCAATATTCCATT	8832
Range Score 556 bil Query	sapiens ice ID: ref 1: 8774 b ts(301) 14 8774 74	GGAGAATAT prolactin (PRL) NG_029819.1] L o 9083 GenBank Expect 6e-155 AACTATGGAT IIIIII III AACTAT-GAT CACAGGAAAT	(G ), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) TTTGCATAATATATG IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%)	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT AATAAATTTTACAGAGAAT	0.000
Range Score SSG bi Query Sbjot Query	sapiens nee ID: ref 1: 8774 to ts(301) 14 8774 74 8833	GGAGAATAT prolactin (PRL) NG_029819.11 L 0 9083 GenBank Expect 6e-155 AACTATGGAT 11111 111 AACTAT-GAT CACAGGAAAT 11111 111 CACAGGAAAT	(C), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG GTGGGGCTATGGAGGAG IIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) TCTTTGCATTATTTF TCTTTGCATTATTTF SCAAGGAGACACACF CGACTGACAGACCCT	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT ATAAATTTTACAGAGAAT AATAAATTTTACAGAGAAA GGAATAAACACAGCTTAGGT	8832 133 8892
Range Score SSG bi Query Sbjot Query Sbjot	sapiens nee ID: ref 1: 8774 to ts(301) 14 8774 74 8833	GGAGAATAT prolactin (PRL) NG_029819.1] L o 9083 GenBank Expect 6e-155 AACTATGGAT IIIIII III AACTAT-GAT CACAGGAAAT CACAGGAAAT CTGTACCTCT IIIIIIIIII	(G), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG IIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT ATAAATTTTACAGACAAT AATAAATTTTACAGACAAA	8832 133 8892 193
Range Score S56 bil Query Sbjot Query Sbjot Query	sapiens ice ID: ref 1: 8774 to ts(301) 14 8774 74 8833 134 8893	GGAGAATAT prolactin (PRL) NG_029819.11 L 0 9083 GenBank Expect 6e-155 AACTATGGAT 11111 111 AACTAT-GAT CACAGGAAAT CACAGGAAAT CACAGGAAAT CTGTACCTCT 11111111 CTGTACCTCT TTTCTTAGAT	(C), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG GTGGGGCTATGGAGGAGA IIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) TCTTTGCATTATTTA TCTTTGCATTATTTA TCTTTGCATTATTTA TCTTTGCATTATTTA TCTTTGCATTATTTA TCCAAGAGACACACA CGACTGACAGACCCT ATCCAAGAATGTTGC	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT AATAAATTTTACAGACAAA TGAATAAAATTTTACAGACAAA TGAATAAAACACAGCTTAGGT	8832 133 8892 193 8952
Range Score 556 bil Query Sbjot Query Sbjot Query Sbjot	sapiens ice ID: ref 1: 8774 to 1: 8774 to 1: 8001) 14 8774 74 8833 134 8893 134 8893 194	GGAGAATAT prolactin (PRL) NG_029819.1] L o 9083 GenBank Expect 6e-155 AACTATGGAT IIIIII AACTAT-GAT CACAGGAAAT CACAGGAAAT CTGTACCTCT TTTCTTAGAT	(G ), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTTGCATTATTTF TCTTGCATTATTTF	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT AATAAATTTTACAGACAAA MATAAATTTTACAGACAAA TGAATAAACACAGCTTAGGT	8892 133 8892 193 8952 253
Range Score S56 bil Query Sbjot Query Sbjot Query Sbjot Query	sapiens ice ID: ref 1: 8774 to ts(301) 14 8774 74 8333 134 8893 194 8953	GGAGAATAT prolactin (PRL) NG_029819.11 L 0 9083 GenBank Expect 6e-155 AACTATGGAT 11111 111 AACTAT-GAT CACAGGAAAT CACAGGAAAT CTGTACCTCT TTTCTTAGAT 11111 111 TTTCTTAGAT ATTAAGTTGT	(C), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG GTGGGGCTATGGAGGAG IIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) ICTTTGCATTATTTA ICTTTGCATTATTTA ICTTTGCATTATTTA ICTTTGCATTATTTA ICTTTGCATTATTTA ICTTTGCATTATTTA ICTTGCATTATTTA ICTTGCATTATTATTA ICTTGCATGACAGACCCT ICTTGCACAGACACACA ICTCCAAGAATGTTGC ACCAGATTTTCTTAC	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT AATAAATTTTCAATATTCCAT AATAAATTTTACAGAGAAA GAATAAACACAGCTTAGGT CAACACCTTTAAATTTTAAG CTCTTAAGTTTATGGGAGAG	8892 133 8892 193 8952 253 9012
Sequen Range Score S56 bil Query Sbjot Query Sbjot Query Sbjot Query Sbjot	sapiens ice ID: ref 1: 8774 to 1: 8774 to 1: 8774 to 1: 8774 0774 74 8833 134 8833 134 8893 194 8953 254	GGAGAATAT prolactin (PRL) NG_029819.11 L 0 9083 GenBank Expect 6e-155 AACTATGGAT 111111 111 AACTAT-GAT CACAGGAAAT 111111 111 CACAGGAAAT CACAGGAAAT 111111111 CTGTACCTCT TTTCTTAGAT ATTAAGTTGT 11111111	(G ), RefSeqGene on ch ength: 22610 Number Graphics Identities 308/311(99%) TTTGCATAATATATG STGGGGCTATGGAGGAG TTGCAGTCAAATAAAT TGCAGTCAAATAAAT TGCAGTCAAATAAAT TGCTCTTATCCTGGCT IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICAAGGAGACACACF ICAAGGAGACACACF ICTCAAGAATGTIGC ACCAGATTTICTTAC	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT AATAAATTTTCAATATTCCATT AATAAATTTTACAGACAAA IGAATAAAACACAGCTTAGGT CAACACCTTTAAATTTTAAG	8832 133 8892 193 8952 253 9012 313
Range Score SSG bil Query Sbjot Query Sbjot Query Sbjot Query Sbjot Query	sapiens ice ID: ref 1: 8774 b 1: 8774 b 14 8774 74 8833 134 8893 134 8953 194 8953 254 9013	GGAGAATAT prolactin (PRL) NG_029819.11 L 0 9083 GenBank Expect 6e-155 AACTATGGAT 111111 111 AACTAT-GAT CACAGGAAAT 111111 111 CACAGGAAAT CACAGGAAAT 111111111 CTGTACCTCT TTTCTTAGAT ATTAAGTTGT 11111111	(G ), RefSeqGene on ch ength: 22610 Number Graphics Identities 300/311(99%) ITTTGCATAATATATG STGGGGCTATGGAGCA STGGGGCTATGGAGCA IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 1/311(0%) ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICTTIGCATTATTIF ICAAGGAGACACACF ICAAGGAGACACACF ICTCAAGAATGTIGC ACCAGATTTICTTAC	Strand Plus/Plus ATATATTTCAATATTCCATT ATATATTTCAATATTCCATT ATAAATTTTACAGAGAAA IGAATAAACACAGCTTAGGT GAATAAACACAGCTTAGGT CAACACCTTTAAATTTTAAG CTCTTAAGTTTATGGGAGAG	8832 133 8892 193 8952 253 9012 313

**Figure 8** a, b, c, d, e and f: NCBI appear mutation of intron of PRL gene forbreast cancer patient. a:( sample 6), that appear the substitution mutations , b: NCBI of patient (sample 7). c: (Sample 9), as it appears the substitution mutation. d: patients (sample 5). e sample 12 , f: sample 6 forward

Genetic factors are important for the disease in many samples of patients, but it's not clear which region of the gene exactly contributes exactly to disease. The intron region of the PRL gene show mutations in breast cancer patients and some region of the gene shows a common mutation in some bases in some patient samples. The mutations detected in intron 1 region of PRL gene of breast cancer patients give evidence that these mutations play a part in this disease.

This result was the same in the study to Vaclavicek et al., 2006 who detected a new SNP in PRL gene showing a significant association between promoter SNPs (G/T) and (A/G) of PRL gene and breast cancer in which this effect was carried by the TGTG haplotype. It was significantly associated with an increased breast cancer risk.(Vaclavicek et al., 2006). Besides, a rare homozygous genotypes of the (A/T) SNP near exon 2 and the (G/A) SNP near exon 5 were more frequent in the patients than in controls. But in same study the existence of a (Arg/Stop) SNP, in exon 4 of PRL, gene could not be confirmed after sequencing a 96 breast cancer sample

While in this study a two heterozygous SNPs were detected (Stop/Trp) in two breast cancer samples. From this study, it was shown that here is mutation in PRL gene and some mutations are common with breast cancer patients. So this association was detected between the PRL gene mutation and breast cancer risk. This has been most extensively in connection, of hyperprolactinemia, with breast cancer as , Mong et al., (2011) when he carried out an association study and first confirmed that the SNP in PRL gene is strongly associated with metastasis of breast cancer in Taiwanes subjects (Mong et al., 2011).

In this study, there are SNPs in breast cancer patients and the same in exon 2 and exon 4. This may be PRL is involved in mammary gland growth and differentiation, so overexpressing of prolactin or variation happens in prolactin gene, patients will increased mammary tumorigenesis (Ben-Jonathan et al., 2002). This agrees with a study by Faupel-Badger et al., (2010), who reported that higher prolactin levels were associated with an increased breast cancer risk.

It is also agrees with Lee et al., 2007, who discovered a low frequency synonymous SNP in exon 3 (A/G) also in exon 5, but not in exon 2, and also a missense SNP in exon 4 when he made a comprehensive analysis of common genetic variation in PRL and PRLR genes in relation to plasma prolactin levels and breast cancer risk. Hormone PRL physiologically influences the mammary gland in several ways during development, growth and stimulation of milk protein gene transcription (Wennbo et al., 1997).

Besides PRL is important in pathological conditions such as mammary tumor growth in which PRLR has been formed in 40-70% of human breast tumors and PRL stimulate growth of several human breast cancer cell lines invitro indicating a possible auto/paracrine function of PRL in many cases of tumor growth. The role of it on breast cell proliferation is that the tumor growth promots effects of PRL signaling in the mammary gland which are well documented in animal models (Nevill et al., 2002; Ormandy et al., 2003).

This result also agrees with Nitze et al., (2013), who found that as prolactin has been implicated in tumorgenesis, it is important for proliferation and differentiation of the breast epithelium. It is shown that PRL and receptor are coexpressed in breast cancer tissues and cell lines and thus PRL has been suggested to promote the growth of the carcinomas in autocrine/paracrine fashion (Ben-Jonthan ,2002).

The mechanisms that have been suggested to explain the possible action of prolactin include the increased synthesis and expression of prolactin receptors in malignant breast tissue and prolactin induced increase in DNA synthesis in breast cancer cell invivo(Vyas, 2012).

This agrees with the study of Plutnikov (2009) who shows that as the PRL signaling is mediated by its similar receptor, so prolactin receptor is commonly stabilized in human breast cancer due to decrease in phosphorylation of residue serum which when phosphorylated facilities PRLR degradation so the import of PRLR turnover results in augmented PRL signaling and PRL induced transcription.

In the present study, it was found that there are many mutations in intron region of the gene as its clear in the above figures conserving the mutations in intron 1. This agree with the result obtained by the Iraqi study which reported the mutations in intron 1 and 2 of prolactin gene of infertile hyperprolactinemic women.

Nore et al. (2013) reported the mutations in hyperprolactinemic patients in intron region of the PRL gene, and thus they considered them as genetic markers for breast cancer Moreover, it was found that there are mutations in breast cancer patients in the same region of the gene which make the association of the mutation in this region of the gene and breast cancer clear. Thus, it agrees with the (Vaclavicek et al., 2006), who showed a significant association between the promoter SNPs of the prolactin gene and breast cancer. positive results for the association of PRL gene polymorphism in breast cancer patients.

Direct Actions on Mammary Epithelia Extensive studies of the direct actions of PRL on breast cancer cells *in vitro* have demonstrated increased proliferation and cell turnover (Marano et al 2014)

Various studies have demonstrated the proliferative, anti-apoptotic, and angiogenic effects of PRL in human breast epithelium, also pointing to a presumptive pro-carcinogenic action(Arendt et al 2011), (Clevenger et al 2003)

Also many studies showed that, genetic variation in human PRL gene has also been associated with breast cancer risk, specific clinicopathological features, and clinical outcome of the disease(Booms et al 2019),( Ellingjord-Dale et al).. (López-Ozuna et al 2016).(Mohr et al 2016)

## 4. Conclusion

In the present study it was found that, there were many mutations , SNPs were detected in intron 1 region of prolactin gene of blood and tissue samples collected from blood and tissue of breast cancer patients in compared with healthy samples .The positive results for the association of polymorphism in PRL intron 1 region of the gene with the breast cancer disease confirmed as the same SNP were found in the same position of many patients. As shown a significant association were detected between SNPs of the prolactin gene and breast cancer thus the mutation in intron region of the PRL gene, can be considered as genetic marker for the infection with breast cancer .

## **Compliance with ethical standards**

#### Disclosure of conflict of interest

No conflict of interest to be disclosed.

#### References

- [1] Mong, F. Y.; Kuo, Y. L.; Liu, C. W.; Liu, W. S. and Chang, L. C.(2011). Association of gene polymorphisms in prolactin and its receptor with breast cancer risk in Taiwanese women. Molecular biology reports, 38(7), 4629-4636.
- [2] Liby, K.; Neltner, B.; Mohamet, L.; Menchen, L. and Ben-Jonathan, N. (2003). Prolactin overexpression by MDA-MB-435 human breast cancer cells accelerates tumor growth. Breast cancer research and treatment, 79(2), 241-252.
- [3] Lalloo, F. and Evans, D. G.(2012). Familial breast cancer. Clin Genet. 2012; 82:105–14. Fox., S. I .Human physiology. 8th ed.
- [4] Couch, F. J.; Nathanson, K. L. and Offit, K. (2014). Two decades after BRCA: setting paradigms in personalized cancer care and prevention. Science. 20.343:1466–1470.
- [5] Gage, M.; Wattendorf, D. and Henry, L. R. (2012). "Translational advances regarding hereditary breast cancer syndromes". Journal of surgical oncology 105 (5): 444–451.
- [6] bernichtein, S., Touraine, P. and Goffin, V. (2010). New concepts in prolactin biology. Journal of Endocrinology, 206(1), 1-11.
- [7] Rui, H. and Nevalainen, M. T. (2000). Prolactin. Oppenheim JJ, Feldman M Cytokine reference on-line. London, UK: Academic Press, Harcourt, 267-283.
- [8] Vaclavicek, A.; Hemminki, K.; Bartram, C. R.; Wagner, K.; Wappenschmidt, B.; Meindl, A.; Schmutzler, R. K.; Klaes, R.; Untch, M.; Burwinkel, B. and Forsti, A. (2006). Association of prolactin and its receptor gene regions with familial breast cancer. Jol.Clinical. Endocrinology and metabolism:91(4):1513-1519.
- [9] Ben-Jonathan, N; Liby, K.; McFarland, M. and Zinger, M.(2002). Prolactin as an autocrine/paracrine growth factor in human cancer. Trends Endocrinol Metab. 13:245-250.
- [10] Delgrange, E.; Trouillas, J.; Maiter, D.; Donckier, J. and Tourniaire, J. (1997). Sex-Related Difference in the Growth of Prolactinomas: A Clinical and Proliferation Marker Study 1. The Journal of Clinical Endocrinology and Metabolism, 82(7), 2102-2107.
- [11] Bernichtein, S., Touraine, P. and Goffin, V. (2010). New concepts in prolactin biology. Journal of Endocrinology, 206(1), 1-11.
- [12] Rui, H. and Nevalainen, M. T. (2000). Prolactin. Oppenheim JJ, Feldman M Cytokine reference on-line. London, UK: Academic Press, Harcourt, 267-283.
- [13] Marano RJ, Ben-Jonathan N. Minireview: Extrapituitary Prolactin: An Update on the Distribution, Regulation, and Functions. Mol Endocrinol (2014) 28:622–33. doi: 10.1210/me.2013-1349 PMC free\
- [14] Arendt LM, Rugowski DE, Grafwallner-Huseth TA, et al. Prolactin-induced mouse mammary carcinomas model estrogen resistant luminal breast cancer. Breast Cancer Res. 2011;13(1):R11.
- [15] Clevenger CV, Furth PA, Hankinson SE, et al. The role of prolactin in mammary carcinoma. Endocr Rev. 2003;24(1):1–27.

- [16] Ellingjord-Dale M, Lee E, Couto E, et al. Polymorphisms in hormone metabolism and growth factor genes and mammographic density in Norwegian postmenopausal hormone therapy users and non-users. Breast Cancer Res. 2012;14(5):R135.
- [17] López-Ozuna VM, Hachim IY, Hachim MY, et al. Prolactin pro-differentiation pathway in triple negative breast cancer: impact on prognosis and potential therapy. Sci Rep. 2016; 6:30934.
- [18] Mohr A, Lüder Ripoli F, Hammer SC, et al. Hormone receptor expression analyses in neoplastic and non-neoplastic canine mammary tissue by a bead based multiplex branched DNA assay: a gene expression study in fresh frozen and formalin-fixed, paraffin-embedded samples. PLoS One. 2016;11(9):e0163311
- [19] Faupel-Badger, J. M.; Sherman, M. E.; Garcia-Closas, M.; Gaudet, M. M.; Falk, R. T.; Andaya, A. and Figueroa, J. D.(2010). Prolactin serum levels and breast cancer: relationships with risk factors and tumour characteristics among pre-and postmenopausal women in a population-based case-control study from Poland. British journal of cancer, 103(7), 1097-1102..
- [20] Lee, D. Y.; Oh, Y. K.; Yoon, B. K. and Choi, D. S. (2012). Prevalence of hyperprolactinemia in adolescents and young women with menstruation-related problems. Am J Obstet Gynecol . 206(3):213.
- [21] Wennbo, H.; Gebre-Medhin, M.; Gritli-Linde, A.; Ohlsson, C.; Isaksson, O. G. and Törnell, J. (1997). Activation of the prolactin receptor, but not the growth hormone receptor is important for induction of mammary tumors in transgenic mice. Journal of Clinical Investigation, 100(11), 2744-2751.