# Sequence Verifications and Promoter Analysis of The Prolactin Gene

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**Abstract:**

**Prolactin or luteotropic hormone (PRL) is a protein hormone. Hyperprolactinemia is one of the most common endocrine disorders. We initiate to investigate if the genetic factor was behind some of the infertile cases suffering from hyperprolactinemia in slemani city. For this study, 36 samples from infertile women were taken and the genomic DNA was isolated. We desgined specific polynucleotide primers to amplify a portion of the PRL gene using PCR technique. The PCR products were subjected for DNA sequencing together with PCR products of 15 samples from healthy individuals. Silent mutations were found between individuals, but non of them correlated to the PRL gene variations between healthy and hyperprolactinemic cases. We perform promoter analysis of the PRL gene using Bioinformatics approcach to identify/determine specific transcription regulator(s) of the PRL gene.**

**Key words:** Prolactin, hyperprolactinemia, infertility, gene expression, promoter analysis.

## Introduction:

Prolactin (luteotropic hormone) is synthesized and secreted by lactotrope cells in the adenohypophysis (anterior pituitary gland). It is also produced in other tissues including the breast, the decidua, parts of the central nervous system and the immune system [1]. Prolactin is involved in many different biologic functions including behavior, immunology, endocrinology, metabolism, and reproduction [2]. More than 300 different biological functions have been attributed to PRL, the major ones being induction of differentiation and growth in mammary epithelia and stimulation of milk protein secretion [3].

A single gene encoding prolactin is found in the human genome, located on chromosome 6. The prolactin gene is 10.215 kb in size and is composed of 5 exons and 4 introns [4]. Transcription of the prolactin gene is regulated by two independent promoter regions. The proximal 5,000-bp region directs pituitary-specific expression, while a more upstream (distal) promoter region is responsible for extrapituitary expression [5]. The human prolactin mRNA is 914 nucleotides long and contains a 681-nucleotide open reading frame translated to prolactin prohormone of 227 amino

acids. The 28 amino acids signal peptide is cleaved and the mature human prolactin is formed (199 amino acids) [6].

Hyperprolactinemia is one of the most common endocrine disorders of the hypothalamicpituitary axis. It is more commonly diagnosed in women than in men, and, if it persists, it usually causes infertility, amenorrhea, and galactorrhea [7]. A history, physical examination, and pregnancy test are crucial. Women with hyperprolactinemia may present with classic symptoms of menstrual dysfunction (oligomenorrhea, amenorrhea) with or without galactorrhea and infertility; approximately 75% of patients presenting with galactorrhea and amenorrhea have hyperprolactinemia[8]. Hyperprolactinemia may also be associated with features of hyperandrogenism such as hirsutism and acne. On the other hand, the effects of hyperprolactinemia may be more subtle. Menses may be regular but with anovulatory cycles or luteal phase insufficiency and resultant infertility [9].

Aims of our study were to extend the understanding of the molecular marker of the female infertility and take a closer look on DNA sequence mutations and promoter analysis of the PRL gene.

## Materials & Methods:

***(1) Genome DNA and PCR:*** Two milliliters of venous blood was withdrawn from the cubital vein using disposable syringes, placed in EDTA containing tubes from 51 women, their ages were between (15-47) years, 15 of samples are from fertile women as control and 36 of samples are from hyperprolactenimic primary infertile women. These samples were either directly used or stored at -28 ºC [10]. Studied samples were collected from Sulaimania city - Iraqi Kurdistan region, after their diagnosis as primary infertile women by consultants. Genomic DNA Isolation from whole blood extraction from whole blood was done using Wizard®Genomic DNA purification Kit (Promega) according to manufacturer's description[11] and the oligonucleotides that have been selected for this study with their details were illustrated in (Table 1). The prearation of PCR reaction mixed on ice and was carried out in 25µl, by using 1X of Go Taq® Green master mix (Promega). The amplification condition were as follwoing, denaturation 45 seconds at 94 $^{o}$C, annealing 45 seconds at 55 $^{o}$C and extention 60 seconds at 72 $^{o}$C for 35 cycles. The quality of extracted DNA and PCR amplicons was checked with 1% agarose gels at 90V for 90 minutes.

**Table 1: Sequences of the primer used to amplify portion of the PRL gene.**

| Oligonucleotides | Sequence (5'-> 3') | Start | stop | Tm | GC% | Reference | Product length |
|---|---|---|---|---|---|---|---|
| Forward primer | ATGATCCAAACACCCAGCTC | 339 | 358 | 59.93 | 50.00% | NC_000006 | 665 |
| Reverse primer | GATTTCATGGCACTGTCCCT | 1003 | 984 | 59.93 | 50.00% | | |

***(2) Nucleotides Sequencing:*** Ten of the PCR products (3 of normal as a control and 7 for abnormal) were used for DNA sequencing. 11 µl (containing approximately 200 ng) of DNA were combined with 1 µl of 1 nM of each of the forward and revers primers. Sequencing reactions were performed in

a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (Applied Biosystems), following conditions were applied for the touchdown step; denaturation 20 seconds at 94 $^{o}$C, annealing 20 seconds at 60 $^{o}$C and extention 20 seconds at 72 $^{o}$C for 10 cycles; and for the amplification step; denaturation 20 seconds at 94 $^{o}$C, annealing 20 seconds at 50 $^{o}$C and extention 20 seconds at 72 $^{o}$C for 25 cycles.

***(3) Bioinformatics for Identification of Transcription Factor (TF) of the PRL Gene:*** In silico analysis was made on the PRL promoter and the enhancer regions. The genomic sequence corresponding to 2000 bp upstream of the PRL transcription start site was analyzed. The computational scan for transcription factor–binding sites (TFBSs) was performed using TRANSFAC Database 7.0-public (http//:www.biobase-international.com) [12], and P-Match software [13]. A matrix (TFP60pm) was chosen and performed using alternative parameter settings to minimize the false negative or positive rates with regard to the PRL promoter sequence.

**Results & Discussion:**

In this study, PRL gene was schematically designed as shown in (Figure 1).


**Figure 1:** A schematic representation of human Prolactin gene indicating Exon/Intron predictions together with untranslated region of the mRNA transcript (5′-UTR) and 3′-UTR). The prolactin gene is 10.215 kb in size and is composed of 5 exons and 4 introns.

It was appeared that the extraction was accurate for all samples (hyperprolactinemic cases and normal controls) by showing clear bands represent the total DNA of the samples.Six hundred and sixty five bp were amplified by PCR using primer and indicated by agrose gel electrophoresis for 36 cases of hyperprolactinemic infertile women and 15 cases of healthy fertile women used as a controls(Figure 1).

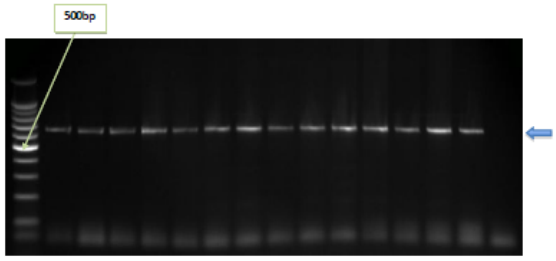


**Figure 2:** Agrose gel electrophoresis (1% agarose, 90 minutes, 90 V) of PCR products of the prolactin gene obtained by DNA amplification. Lanes: 2-15 for hyperprolactinemic infertile women 1-14, ladder lane 1: 100bp DNA ladder and lane 16: for negative control. The arrow indicates a 665 bp a specific product for the prolactin gene.

In this study, portion of the PRL gene was amplified by PCR for both of hyperprolactinemic cases and fertile healthy individuals. The position of PCR product was shown in (Figure 3). The PCR amplicon was 665 bp in size and indicated by agrose gel electrophoresis for all cases, including healthy control samples.

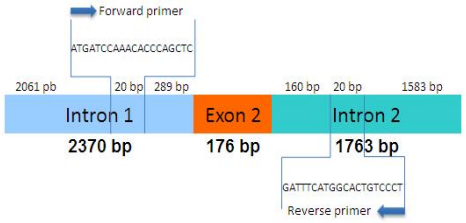


**Figure 3:** The position of PCR product in the prolactin gene.

The sequence of the PCR products for 10 samples {the reverses and forwards of three controls and the forwards and reverses of seven samples of hyperprolactinemic infertile women} were edited and deduced from the sequencing data. The sequences compared by BioEdit sequence alignment editor. Comparative analysis of the nucleotides sequence align was performed to assess the relation of patients isolate with control sequences and to find the different between DNA isolate from individuals in the Kurdistan region compared to the existing sequences in the NCBI databases. In the present study, it was the first time to find the relation between infertility and genes study in Iraq. There was some study done and find a novel mutation of estrogen receptor gene in girls with precocious Puberty [14], other were conducted on Growth hormone gene in fish and did not find any difference [15,16]. Therefore we tried to find some mutation in the PRL gene for ten of PCR products (3 of fertile controls and 7 of hyperprolactinemic infertile women). The two mutations that indicated from the DNA sequencing were in the intron 1 and 2, while there was not any mutation in the exon.

In this study, the transcriptional regulation of the PRL gene has been studied quite extensively. The silico bioinformatics used to analyze the promoter region of PRL gene (2000 bp upstream ATG start codon). Upon analysis of the PRL gene, HOX-1.3 and XFD-2 transcription binding-site are found which is the most specific transcription factor elements to master regulate the gene expression (Figure 4).

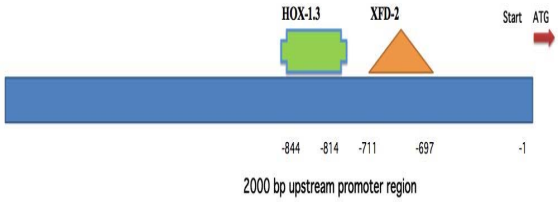


**Figure 4:** Prediction of the promoter region of the PRL gene at the 2000 bp upstream. The location of HOX-1.3 and XFD-2 transcription factors were indicated.

**(A) HOX 1.3:** The homeobox genes are a superfamily of genes encoding transcription factors that regulate developmental processes such as body patterning and organogenesis [17]. The mammalian HOX gene complex consists of 39 genes that are located on 4 linkage groups (clusters), dispersed over 4 chromosomes (HOXA, HOXB, HOXC and HOXD). HOX-1.3 is a transcriptional regulator of many target genes, including p53 and the progesterone receptor.HOX-1.3 is a Trans activator of p53 and may affect the response of breast cancer cells to DNA damage [18]. In primary breast carcinomas, loss of p53 expression is coupled with loss of HOX-1.3 expression, suggesting that the loss of HOX-1.3 expression is important in tumorgenesis [19]. The HOX-1.3 gene is located on mouse chromosome 6 and has been previously shown to be expressed in mouse embryos and adults [20].

**(B) XFD-2:** The recently described XFD (Xenopus fork head domain related) multigene family in the frog Xenopus

laevis that contain this DNA-binding domain [21]. Fork head proteins play an important role in embryonic pattern formation, regulation of tissue specific gene expression, and tumor formation [22]. These proteins contain a highly conserved 110 amino acid long DNA binding domain that was originally identified in Drosophila mutant fork head [23] and in the rat hepatocyte nuclear factor HNF-3 [24]. Because of their structure, these proteins are also referred to as winged helix proteins. They bind DNA as monomers and can act as transcriptional activators or repressors . They were renamed as Fox genes [25]. Fox genes represent a large gene family with more than 20 members in each of the higher vertebrate species [26].

**Conclusions:**

The conclusions can be summarized as follows:

1- The results of this research showed that hyperprolactinemia infertile women do not carry mutation in the genome sequence over a short spam between exon X and intron Y of the prolactin gene compared to fertilewomen.
2- Performing *in silico* bioinformatics to analyze the promoter region (2000 bp upsteam transcription) of the PRL gene, the results obtained reveal that HOX-1.3 and XFD-2 are most specific transcription factor elements to master regulate the gene expression, further experimental data needed to validate these findings.

## References

[1] Yen S. S. C, Jaffe R. B, and Barbieri R. L, "Reproductive endocrinology" 4th edition, W.B. Saunders Co, Philadelphia, pp. 257-283, (1999).

[2] Woodman D. D, "Laboratory animal endocrinology" Chichester, UK, (1997).

[3] Bole-Feysot, C., Goffin, V., Edery, M., Binart, N. and Kelly, P. A, "Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice" Endocr. Rev. 19, pp.225-268, (1998).

[4] Noh G. E, Lim H. K. and Kim J. M, "Characterization of genes encoding prolactin and prolactin receptors in starry flounder Platichthys stellatus and their expression upon acclimation to freshwater" Fish Physiol. Biochem. pp. 608-737, (2012).

[5] Berwaer M, Martial J. A, and Davis J. R, "Characterization of an upstream promoter directing extrapituitary expression of the human prolactin gene" Mol. Endocrinol. 8, pp. 635-642, (1994).

[6] Binart N, Bachelot A,and Bouilly J, "Impact of prolactin receptor isoforms on reproduction" Trends Endocrinol. Metab. 21(6), pp. 362-368, (2010).

[7] Mah P. M and Webster J, "Hyperprolactinemia: etiology, diagnosis, and management" Semin Reprod Med. 20, pp. 365-374, (2002).

[8] Luciano A. A, "Clinical presentation of hyperprolactinemia" J. Reprod. Med., 1999, 44, 1085-1090.

[9] Biller B. M, "Diagnostic evaluation of hyperprolactinemia" J. Reprod. Med. 44, pp. 1095-1099, (1999).

[10] Dyar A. H, "detection of Brucellosis causing bacteria (Brucella SPP.) by polymerase chain reaction (PCR)" High Diploma thesis, Sulaimani university, 2009.

[11] Miller S. A., Dykes D. D., and Polesky H. F, "a simple salting out procedure for extracting DNA from human nucleated cells" Nucl. Acids Rev. 16, pp.1215, (1988).

[12] Matys V, Fricke E, Geffers R, Gössling E, Haubrock M, Hehl R, et al, "TRANSFAC: transcriptional regulation, from patterns to profiles" Nucleic Acids Res. 31, pp. 374-378, (2003).

[13] Chekmenev D. S, Haid C, and Kel A. E, "P-Match :transcription factor binding site search by combining patterns and weight matrices" Nucleic Acids Res. 33, pp. W432-W437, (2005).

[14] Li B, Liu L, Fu X, Zhou W. Q, Zou D. T, Zhao X. Y et al, "A novel mutation of Estrogen receptor gene detected in girls with precocious puberty" Acta. Genetica. Sinica. 32, pp. 1011-1017, (2005).

[15] Vikas A, Thayanithy V, Ramanathan K, Thavamani J. P, and Sinnakaruppan M, "Cloning, sequencing and expression of cDNA encoding growth hormone from Indian catfish (Heteropneustes fossilis)" J. Biosci. 26, pp. 315-324, (2001).

[16] Janeth S. P, José L. Caldas W, Ronaldo de C. A. and Alexandre W. S. "Molecular cloning and sequence analysis of growth hormone cDNA of Neotropical freshwater fish Pacu (Piaractus mesopotamicus)" Genetics and Molecular Biology. 31, pp. 381-384, (2008).

[17] Krumlauf R, "Hox genes in vertebrate development" Cell. 78, pp. 191-201, (1994).

[18] Aubin J, Lemieux M, Tremblay M, Be´rard J, and Jeannotte L, "Early postnatal lethality in Hoxa-5 mutant mice is attributable to respiratory tract defects" Dev. Biol. 192, pp. 432-445, (1997).

[19] Christian L, Michel T, Daniel B, Josee A, and Lucie J, "Multiple cis-acting regulatory regions are required for restricted spatio-temporal Hoxa5 gene expression" Dev. Dyn. 214, pp. 127-140, (1999).

[20] Shawn P. M, James G, Ward F. O, Robert A. L, and Elwood L, "Differential expression of the homeobox gene Hox-1.3 in F9 embryonal carcinoma cells" Proc. Natl. Acad. Sci. 85, pp. 5587-5591, (1988).

[21] El-Hodiri H, Bhatia-Dey N, Kenyon K, Ault K, Dirksen M, and Jamrich M, "Fox (forkhead) genes are involved in the dorso-ventral patterning of the Xenopus mesoderm" Int. J. Dev. Biol. 45(1), pp. 265-271, (2001).

[22] Gómez-Ferrería M. A. and Rey-Campos J, "Functional domains of FOXJ2" J. Mol. Biol. 329(4), pp. 631-644, (2003).

[23] Bülow M. H, Aebersold R, Pankratz M. J, Jünger M. A, "The Drosophila FoxA ortholog Fork head regulates growth and gene expression downstream of Target of rapamycin, PLoS One. 5(12):e15171, (2010).

[24] Norquay L. D, Yang X, Jin Y, Detillieux K. A, Cattini P. A, "Hepatocyte nuclear factor-3alpha binding at P sequences of the human growth hormone locus is

associated with pituitary repressor function" Mol. Endocrinol. 20(3), pp. 598-607, (2006).

[25] Kaestner K. H., Knochel W., and Martinez D. E, "unified nomenclature for the winged helix/forkhead transcription factors" Genes Dev. 15, pp. 142-146, (2000).

[26] Jackson B C, Carpenter C, Nebert D. W, Vasiliou V, "Update of human and mouse forkhead box (FOX) gene families" Hum. Genomics. 4(5), pp. 345-352, (2010).

# چە سبانـدنـی ریـزبەنـد و شـیکردنـەوەی پـرومـوتـوری جـینی پـرولاکـتین

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## پوختە

**هۆرمۆنی شیر هۆمۆنیکی بروتینیا. وە بەرزبونی ریژی هۆرمۆنی شیر لە خۆیندا یەکیەك لە نەخوشیە بلاوکاندا. بەرزبونەوەی بەرچاوی ئاستی هۆرمۆنی شیر لە ژنانی نەزوکدا وە بوونی ئەگەری هۆکاری جینی وەك هۆکاریك بۆ نەزوکی بەشی دوایی ئەم تۆیژینەوی تەرخانکرا بۆ جیاکردنەوەی ترشی** DNA **وبەکارهینانی پرایمری تاییەت بۆ زورینە کردنی بەشیك لە جینی هۆرمۆنی شیر بە ریگای** PCR **بۆ لیکولینەوە لە سەر زنجیرە کردنی نیوکلوتیدەکان لە سەر** (36) **ژنی نەزوك وە بەراوردکردنیان لە گەڵ** (15) **ژنی ئاسایی. هیچ گۆرانکاریەك یەدی نەکرا لە جینی هۆرمۆنی شیردا لە نیوان ژنانی نەزوك زئاسایدا. وشیکردنەوەی ناوچەی پرۆمۆتر ئە نجامدرا بۆ جینی هۆرمۆنی شیر بۆ دیاریکردنی ریخەرەکانی کۆپیکردنی جینی هۆرمۆنی ناوبراو بە بەکارهینانی** Bioinformatics Tools.