

Association between *rs10046* Polymorphism of *CYP19A1* gene and aromatase enzyme activity in Acromegaly Iraqi patients

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Abstract

Aromatase activity in the acromegaly is an important marker to determine sexual behavior. There is evidence showed that aromatase activity is associated with polymorphisms in the 3' untranslated region T>C *rs10046* (SNPs) single nucleotide polymorphisms in *CYP19A1* gene. From the whole blood genomic DNA was extracted to perform genotyping analysis and *rs10046* SNP in the *CYP19A1* gene by using PCR-restriction fragment length polymorphism technique, and serum aromatase levels were determined using the solid phase enzyme –linked immunosorbent assay based on sandwich method. The results show that in aromatase activity levels of acromegaly patients there was a highly significantly decrease when contrasted with healthy control groups in both sexes ($P \leq 0.01$) and showed that there was highly significant in TT and CC alleles while was the non-significant difference in TC allele when contrasted with healthy control groups ($p > 0.01$). Also the TT and CC alleles tended to be correlated with an increased acromegaly risk in this groups as specified via odds ratios > 1 , although these differences were significant, while the TC allele tended to be correlated with a decreased acromegaly risk in this group as specified via odds ratios > 1 , although these differences were significant.

Keywords: Aromatase activity; SNP; *rs10046*; TT allele; CC allele.

Introduction

Acromegaly is a chronic, rare, and debilitating disease that outcome from too much production of growth hormone. Clinically, the disease is correlated with soft tissue enlargement, skeletal growth is excessive, i.e. too much production of growth hormone (GH) because of pituitary adenoma⁽¹⁾. Growth hormone induces the secretion and expression of insulin-like growth factor 1(IGF-1), so phenotypes correlated with acromegaly may be due to either GH signaling and insulin-like growth factor 1 IGF-1 signaling or a combination of both⁽²⁾. Systemic complications usually connected with the chronic hypersecretion of GH and IGF1 include acromegaly, soft tissue changes and arthralgia, and comorbidities include type 2 diabetes, hypertension, carpal tunnel syndrome and sleep apnea⁽³⁾. The aromatase enzyme cytochrome P450 is vital for the biosynthesis of estrogens in few tissues, that plays a crucial role in the biosynthesis of (C18 steroids) estrogens from androgens (C19steroids) in all vertebrates⁽⁴⁾. In humans, aromatase enzyme expression is regulated by various tissue-specific promoters in brain, ovary, and adipose tissue⁽⁵⁾. *CYP19A1* is located at chromosome 15 and spans about 123 kb in length by standards of other *CYP* genes, that encoding the aromatase(*CYP19*) is extraordinarily significant since its more than 50 kbp, at chromosome 15q21.2. It contains 9 coding exons

and various untranslated first exons whose expression are regulated by tissue-specific promoters⁽⁶⁾. In a wide assortment of tissues aromatase is expressed, its has been found in brain, placenta, liver, ovary, skin, testis and adipose tissue⁽⁷⁾. The tissue-specific promoters regulate the aromatase gene at the transcriptional levels in the gonads, brain, and placenta⁽⁸⁾. The protein result of aromatase gene has so far been found the same despite of the tissue-specific expression patterns of aromatase. The aromatase gene expression control at all levels (mRNA, protein, functional and enzymatic activity)has been considered as important, because of the critical role of aromatase enzyme⁽⁹⁾, as well in males, for example in the cases of prostate cancers or gynecomastia⁽¹⁰⁾. In every pituitary tissue there was expression of aromatase enzyme inclusive autopsy samples and were most increased in patients with active acromegaly than in another adenoma groups. In functional adenoma patients aromatase expression was elevated in patients with than without postoperative complete remission. These result proposed that expression of aromatase perhaps be a useful prediction marker of GH- excreting pituitary adenomas⁽¹¹⁾. Prior studies examining the associations of selected *CYP19A1* variants, including the polymorphism in the 3' untranslated region T>C *rs10046* single-nucleotide polymorphisms in *CYP19A1* have been seen to be correlated with

circulating estrogen levels;

specifically *rs10046* (T) ⁽¹²⁾.

Patients

One hundred and twenty male with female (age 20-60) years were recorded in this study, sixty patients with active acromegaly who have attended the national diabetes center and center and specialist center for Endocrinology and diabetes, Baghdad, Iraq from December 2015 to June 2016 and sixty healthy individual with matches as a control group. Physicians diagnosed all patients. Blood was collected, each

blood sample was split into two fractions. The first fraction was conveyed into tube containing ethylene di amine tetra acetic acid disodium salt (EDTA) for whole blood for genotyping analysis. The other fraction was separated in plain polyethylene test tube, left at room temperature to clot then the tube was centrifuged at 704 ×g for ten minutes, and the sera were separated.

Determination of Serum Aromatase Activity:-

Serum aromatase levels were determined using human ARO (Aromatase) kit obtained from Mybiosource company, is enzyme linked immunosorbent assay (ELISA) depend on the sandwich principle, at a wavelength of 450±2 nm the optical density is measured by spectrophotometric using microplate reader RT-2100C obtained from

Hagto company. The optical density value is proportional to concentration of aromatase enzyme and then calculated the aromatase concentration in serums by comparing the optical density in the serums to the standard curve(that be created by plotting the mean of optical density value for each reference standard against the concentration) .

DNA extraction

Collected blood samples in (EDTA) tubes from healthy controls and patients groups, genomic DNA was extracted from the wholes blood by G-Spin™ Total DNA extraction kit

obtained from iNtRON's Biotechnology company. The quality and integrity of DNA was checked on 2% agarose gel electrophoresis before doing PCR

Detection of *rs10046* gene by using PCR-restriction fragment length polymorphism (RFLP-PCR):

Achievement of optimization PCR reactions were after many endeavors

to reveal the best annealing temperature and both primer and

DNA concentrations. By use of maximum PCR premix kit (i-Taq), and PCR a number of the amplification cycles, since *CYP19 rs10046* polymorphisms were designed for amplification a PCR method via using two primers⁽¹³⁾. Briefly, genomic DNA was amplified by primers, forward (5'-TAGAGAAGGCTGGTCAGTGCC-3' and reverse 5'-CTCTGGTGTGAACAGGAGCA-3'), then followed by digestion with restriction enzyme (Nco I). Primers set supplied by Integrated DNA Technologies company in Canada (IDT). PCR reaction was work in 25 µL of a

To identify the polymorphism of *rs10046* gene in the 3' untranslated region T>C in products of PCR was determined by PCR-RFLP, PCR reaction was conducted in 10 µL of a reaction mixture containing 5 µL of PCR product, one µL of enzyme (Nco I) (500U), 0.2 µL of BSA and 3.8 µL of Buffer. Then the reaction

Statistical Analysis

Statistical analysis was done utilizing Microsoft Office (SPSS vs. 19) which include the following (mean ± standard deviation-test, correlation coefficient; P-value ≤ 0.01 was been significant. In all studied groups the total prescient values for the outcomes were performed according

reaction mixture containing 1.5 µL of DNA, five µL of PreMix Master Mix, 1 µL of (10 Pmol\ µl) of each primer, 16.5 µL of distilled water. Amplification program was one cycle at 94°C for 3 minute, 35 cycles at 94°C for 45 second, 60°C for 45 second and 72°C for 45 second, one cycle at 72°C for 10 minute. The amplified product was subjected to 2% agarose gel electrophoresis with 1.0 µL red stain dye and 5 µL ladder, and visualized under UV (Agarose Gel Electrophoresis System MGu-502T-pk, USA) after Red safe staining.

mixture was mixed and then spins down and incubated at 37°C for 20 hours in incubation. After that 5 µL of the reaction mixture were run on 1.5 % agarose gel stained with 2.0 µL of red stain dye and 5 µL ladder and visualized under UV (Agarose Gel Electrophoresis System MGu-502T-pk, USA) after red safe staining.

to biostatistics via Daniel in 1987. and the statistical analysis system- SAS was utilized. The Pearson χ^2 criterion ($p \leq 0.01$), 95% confidence intervals (CI) and odds ratio (OR) tests were employed to compare the polymorphisms of frequencies of *CYP19A1* gene between the groups.

Results and Discussion:-

Table (1) showed the mean ±SD of aromatase activity levels

expressed as ng/mL in serum of 60 healthy subjects (males and females)

and 60 active acromegaly patients subjects (males and females). These results showed that there was a highly significant decrease in aromatase

activity levels of male and female acromegaly when contrasted with control group in both sex ($P \leq 0.01$).

Table (1): Serum aromatase activity levels in studied groups:-

Parameter		Controls	Patients	t-test	p-value
		Mean± Standard Deviation	Mean± Standard Deviation		
Aromatase activity ng/mL	M	11.209±0.364	2.836±1.095	-34.807	0.000*
	F	23.293±0.003	2.774±1.638	-51.648	0.000*

*The t-test insignificant at the 0.01 level or less, M: Male, F: Female.

The TT genotype was referred by a single band of 190 bp, while the CC genotype was referred by two bands of 169 and 21 bp, and the TC

genotype was referred by the existence of three bands, that is, 21, 169 and 190 bp as shown in Figure(1).

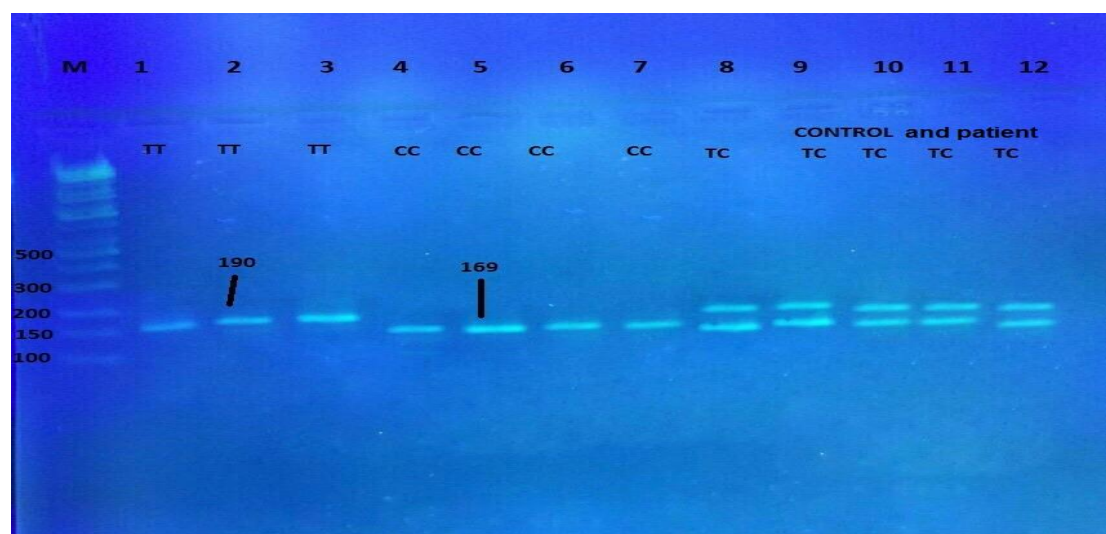


Figure (1): Genotyping of *CYP19 rs10046*: T>C by RFLP-PCR in control and patient samples: lane M, 500bp DNA ladder; lane 1-3, TT genotype (190bp); lane 4-7, CC genotype (169,21bp)); lane 8-12, TC genotype (190, 169and 21 bp)

Table (2) summarizes positive results of allele of RFLP in male and female of sample study (patients and control). Three male's patients from 30 male patients with present TT allele (25%) and no present with male

control. These results showed that there was highly significant in TT allele when compared with male control group ($p > 0.01$). While 24 male's patients from 30 male patients with present CC allele (72.73%) and

no present with male control. These results showed that there was highly significant in CC allele when compared with male control group ($p>0.01$) while 3 males patients from 30 male patients with present TC allele (20%) and all (30) male control with present TC allele (50.00%) these results showed that there was non significantly difference in TC allele when compared with male control group ($p>0.01$). And nine females patients from 30 female patients with present TT allele (75%) and no present with female control. These results showed that there was highly significant in TT allele when

compared with female control group ($p>0.01$). While 9 females patients from 30 male patients with present CC allele (27.27%) and no present with female control. These results showed that there was highly significant in CC allele when compared with male control group ($p>0.01$). While 12 females patients from 30 female patients with present TC allele (80%) and all (30) female control with present TC allele (50.00%) these results showed that there was non significantly difference in TC allele when compared with

female control group ($p>0.01$).

Table (2) Results of RFLP in male and female of sample study (patients and control)

The group	Sum of positive results of allele TT (190)		P-value	Sum of positive results of allele CC(169,21)		P-value	Sum of positive results of allele TC(190,169,21)		P-value
	Male	Female		Male	Female		Male	Female	
Patients	3 (25.00%)	9 (75.00%)	0.0001 **	24 (72.73%)	9 (27.27%)	0.0001 **	3 (20.00%)	12 (80.00%)	0.0001 **
Control	0 (0.00%)	0 (0.00%)	1.00 NS	0 (0.00%)	0 (0.00%)	1.00 NS	30 (50.00%)	30 (50.00%)	1.00 NS
P-value	0.0137 **	0.0001 **	---	0.0001 **	0.0125 **	---	0.0029 **	0.0029 **	

** ($P<0.01$), NS: Non-significant.

Table (3) summarizes the frequency distribution genotypes of *CYP19A1 rs10046* T>C polymorphism and allele frequency (according of RFLP results) in patients and control groups for all sex.

Sixty healthy individuals (100%) of total subjects were used in this study had present *CYP19A1 rs10046* T>C polymorphism amplification whereas the distribution of genotype TC was (60)100%, and 60 patients (100%)

had present *CYP19A1 rs10046* T>C polymorphism amplification, whereas the distribution of genotype TT was (12)20%, the distribution of genotype TC was (15) 25% and the distribution of genotype CC was (33) 55%. The frequency of homozygotes T allele in the present study of patients was

(0.33) which was lower than the frequency of homozygotes T allele of control was (0.50) while the frequency of heterozygotes C allele in the present study of patients was (0.67) which was higher than the frequency of heterozygotes C allele of control was (0.50)

Table (3): Distribution of gene polymorphism and allele frequency in patients and control (according of RFLP results)

Genotype	Patients	Healthy
	No. (%)	No. (%)
TT	12 (20.00%)	0 (0.00%)
TC	15 (25.00%)	60 (100%)
CC	33 (55.00%)	0 (0.00%)
Total	60 (100%)	60 (100%)
Chi-square value (χ^2)	8.923 **	15.00 **
Allele frequency		
T	0.33	0.50
C	0.67	0.50
Total	1 (100%)	1 (100%)
** (P<0.01).		

To measured 95% confidence intervals and odds ratios for acromegaly risk, difference comparisons of *rs10046* alleles additionally analyzed C verse T alleles as seen in Table (4). The odds ratios of acromegaly subpopulations for TT verse CC and TC verse CC genotypes, as well as TT verse TC genotype were evaluated. The odds ratio for TC verse CC patients for acromegaly patients was 1.19 (0.96–1.62, 95% CI) and for TC verse TT patients was 1.35 (0.89–1.60, 95%

CI). So , the TT and CC genotypes tended to be correlated with an increased risk of acromegaly in this group as referred via odds ratios >1, although these differences were significant, while the odds ratio for TT verse TC subjects was 0.42 (0.97–1.63, 95% CI) So, the TC genotype tended to be for a non significantly increased acromegaly risk correlated with the TC genotype, as indicated by odds ratios <1 in this group, while the odds ratio for healthy control for TT verse TC and for CC

verse TC subjects was 2.00 (0.96–1.61, 95% CI) . So, the TC genotype tended to be correlated with decreased acromegaly risk in this group as

indicated via odds ratios >1, although these variance were significantly as shown in table (4).

Table (4): Odds ratios, 95% confidence intervals and p-values for the correlation of *rs10046* genotypes in *CYP19A1* with acromegaly risk

Allele	NO. Of patient (%)	Compared Genotypes	OR	95% CI
CC	33 (55.00%)	TC vs. CC	1.19	(0.96-1.62)
TC	15 (25.00%)	CC vs. TT	1.35	(0.89-1.60)
TT	12 (20.00%)	TT vs. TC	0.42	(0.97-1.63)
Chi-square value (χ^2)	8.923 **			
p-value	** (P<0.01).			
Allele	NO. Of healthy (%)	Compared Genotypes	OR	95% CI
TT	0 (0.00%)	TT vs. TC	2.00	(0.96-1.61)
CC	0 (0.00%)	CC vs. TC	2.00	(0.96-1.61)
TC	60 (100%)	CC vs. TT	0.00	(0.96-1.61)
Chi-square value (χ^2)	15.00 **			
p-value	** (P<0.01).			

Analyses of acromegaly samples verse controls of the indicated genotypes are shown; 95% CI: 95% confidence intervals, OR: odds ratios.

This research concludes to the aromatase enzyme activity measurement in serum for acromegaly patients and healthy controls and compares the results to their alleles. The results detected in aromatase activity were a significantly decreased ($P \leq 0.01$) in acromegaly relation to the control group, Table (5). Showed the

activity of aromatase was a significant decrease ($p \leq 0.05$) in patients carrying (TC) allele of *rs10046* contrasted to individual that had same alleles in healthy control group. While between the two groups the activity of other alleles did not seen any significant.

Table (5): Comparison Aromatase activity between acromegaly & control according to Genotypes

SNP	Allele Genotype	Aromatase activity (ng/mL)			t-test	P value
		Mean ± SD				
		gender	Patient	Control		
rs10046	TT	M	3.45 ± 0.56	-	10.649	0.004 *
		F	5.18± 3.73	-	4.169	0.001*
	CC	M	3.06± 0.53	-	28.064	0.0000*
		F	2.904±1.4	-	6.216	0.0001*
	TC	M	0.396±0.11	11.303±0.28	-61.106	0.0000*
		F	0.527±0.28	23.293±0.002	-276.3	0.0000*

* Significant difference: (P<0.05)

This is the first study in the world investigated the correlation of *CYP19A1* gene rs10046 polymorphisms with the acromegaly. We accurately limited the study people to the Iraqi people in the areas of Baghdad city to keep up genetic homogeneity between the people furthermore to avert the confusing effects of environmental factors and interracial variations of genetic backgrounds, like living behavior. The current study investigates the aromatase activity and polymorphism of *CYP19A1* genes among 60 apparently healthy, and 60 active acromegaly patients from different regions of Baghdad metropolis, the association among the *CYP19A1* gene rs10046 polymorphism and various factors were studied. Our research seen that there was associated between *CYP19* gene polymorphism

rs10046 with aromatase activity and related to acromegaly in Iraqi patients. There was statistically significant association between the aromatase activity and the existence of allele since whereas deficiency activity of aromatase it demonstrates the increased presence of allele or that allele is satisfactory allele and individual with this disease and thus when the appearance TC allele to patients is making sure that patients allele when there is a decrease of enzyme activity in spite of the emergence of the same allele in healthy and therefore can distinguish between of patients and healthy by measuring the activity of the enzyme. Our comprehension in human biology the role of aromatase has been promoted via a few individual situation statements of patients with complete aromatase deficiency⁽¹⁴⁾. In

agreement with this suggestion .breast cancer, infertility and endometriosis studies have found that patients related with this polymorphism. The previous studies such as Lin L *et al.* (2007) explain that aromatase mutations can make in humans variable or "nonclassic" phenotypes. At puberty little residual aromatase activity may be adequate for breast and uterine development to occur, in spite of important androgenization in utero. That phenotypic variability may be affected further by modifying factors such as variability in coregulators, nonclassic pathways of estrogen synthesis, or differences in androgen responsiveness propose an important modulatory function for this enzyme in metabolic function and endocrine within the broader population⁽¹⁵⁾. And another study showed that estradiol levels circulating are genetically controlled and have been related to breast cancer risk. Since genetic variation in the *CYP19* gene contributes to variance in circulating hormone levels, it is tempting to speculate that genetic polymorphisms in this gene such as the *rs10046* SNP, a T-C variant in the 3' untranslated region, are potential candidates to have an impact on breast cancer risk ⁽¹⁶⁾. Based on our previous assessment, our analysis in this study evaluated whether inter-individual variation in the *CYP19A1* gene alters susceptibility to acromegaly conditions in Iraqi patients. The overall risk of either disease outcome was related to the individual genotypes since the risk of acromegaly associated with several gene variants differed according to

proliferation status. While other study by Ledan *et al.* (2014) shows that the variation in the *CYP19A1* gene alters capability to fibrocystic breast or breast cancer conditions in Chinese women. In our study, our analysis revealed a significant association between polymorphism *rs10046* and acromegaly related to deficiency in aromatase activity .in agreement with this suggestion .breast cancer, infertility and endometriosis studies have found that patients related with this polymorphism. The overall risk of either disease outcome was not related to any of the individual genotypes or block-specific haplotypes; although the risk of breast cancer correlated with several genes variants differed according to proliferation status. The observed increases in risk among breast cancer cases with, but not without, synchronous proliferative fibrocystic status indicate that *CYP19A1* variation may only influence breast cancer etiology in this particular subgroup of women⁽¹⁷⁾. The *rs10046* polymorphism has been studied in variant populations. A previous Chinese study provided evidence that the *CYP19 rs10046* polymorphism is associated with breast cancer risk among Chinese women⁽¹⁸⁾ and results from a study in a Spanish population also revealed an association between *rs10046* and breast cancer risk. In this study, the carriers of at least one C allele had an increased risk of developing breast cancer⁽¹⁹⁾, which is in agreement with other studies, where the frequency of the C allele is higher in cases verse controls^(20,21). In contrast, Kristensen *et al.* found that

the T-allele in *rs10046* of the *CYP19* gene is correlated with a high activity phenotype and that the TT allele of this polymorphism was correlated with increased risk of breast cancer⁽²²⁾. Comparable to that study, the result suggest that the TT allele has been a tend to be overrepresented in subgroups of patient correlated with advanced stage of cancer and positive cancer of lymph node. Based on our previous assessment, our analysis in this study found that the TT allele and CC allele of this polymorphism was correlated with increased risk of acromegaly. And another study showed the single-nucleotide polymorphisms in *CYP19A1* gene have been correlated with circulating estrogen levels; specifically *rs10046* (T), *rs727479* (T), and *rs4646* (G), while *rs700518* was found to be associated with increased aromatase expression^(23, 24). Another study by Long *et al.*⁽²⁵⁾ found an increased breast cancer mortality risk for five *CYP19A1* SNPs in a Chinese inhabitation. And this agreement with our study, while Fasching *et al.*⁽¹⁴⁾ found an inverse relationship for one *rs10046* SNP and Udler *et al.*⁽²⁶⁾ did not find correlations with *CYP19A1* SNPs in a NHW inhabitation. likewise the other studies by Vessela *et al.*(2000) showed that the examined *CYP19* polymorphisms (*rs700518*: A>G, *rs2236722*: T>C, [TTTA]_n repeat and *rs10046*: T>C) were not significantly correlated with the hazard of infertility and endometriosis or endometriosis with ovarian chocolate cysts in chinees women⁽²⁷⁾. Many prior studies from Korean people⁽²⁸⁾, Japanese people⁽²⁹⁾ and

Caucasian people⁽³⁰⁾ all of that showed no significant correlation of *CYP19* gene polymorphisms with the hazard of chocolate cysts or endometriosis. The individuals prevalence is not expressing the aromatase enzyme because of deletion of a homozygous gene which is reporting to be higher breast cancer in Japanese patients studied by Kaoru *et al.* (2004)⁽³¹⁾, showed that in estrogen biosynthesis the genetic polymorphisms *CYP19A1* are as well expected to affected the degradation or synthesis of estrogen, and, then, the breast cancer risk^(32, 33). In a survey by Paola *et al.*, (2015) the authors deals with the male patients with breast cancer and acromegaly. The breast cancer clinical course was closely concerning to the metabolic monitoring of acromegaly. The rapid metastatic lesion progression was temporally concerning to stopping treatment by pegvisomant and paralleled a rising in serum levels of IGF-1. Normalization of serum IGF-1 levels after re-starting pegvisomant impressively decreased the metastatic breast lesions progression of .Acromegaly control is compulsory in acromegalic patients with cancer and evaluated the function of AIP mutations, which occur in pituitary adenomas, in the common genesis types of cancer (breast, prostate, and colonrectal cancer)⁽³⁴⁾. The studies by Damineni *et al.* (2014) showed that the aromatase gene expression is regulated in a tissue promoter I.4 governs particular manner and in adipose tissue expression⁽³⁵⁾, while Belgorosky *et al.* (2009) suggested that the lower of aromatase activity

lead to the degree of masculinization of the external genitalia was greater at birth in females that exposure to an excess of androgens resulted in a changeable virilization of the mother and masculinization of the external genitalia since about 1% the activity of placental aromatase of wild type aromatase enzyme appears to be enough to prevent the mother virilization during pregnancy. Moreover, the absence of estrogen synthesis because of complete aromatase activity deficiency resulted in increase concentration of essential FSH^(36, 37). Based on previous results, we in our analysis expand and refines

Conclusion

This is the first study in the world investigating the correlation of *CYP19A1* gene *rs10046* polymorphism with the receptivity to acromegaly that's there was the TT genotype and CC genotype tended to be associated with an increased acromegaly risk and found the relationship between alleles of *rs10046* SNP and aromatase activity

previous results (including our own) by identifying a number of variables that predict and expected the presence deficits in function of aromatase activity in those patients In the current work ,we suggested once this correlation is confirmed by the biological importance of aromatase enzyme and illustrated through functional studies, this polymorphism could be a probable target for the early treatment and prevention of acromegaly. And in this study provide conclusive evidence that genetic variation in *CYP19A1* is a prognostic factor for acromegaly in Iraqi population

since whenever found the TT genotype or CC genotype or both and low aromatase activity that's mean there was associated with active acromegaly while the TC genotype-related with healthy but when the genotype found with deficiency of aromatase activity level that's lead to sign of acromegaly.

Acknowledgements

We thanks all person who provided their blood sample and approval for genetic analysis

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العلاقة بين التغيرات *rs10046* في جين *CYP19A1* ونشاط انزيم الاوروماتيز في المرضى العراقيين المصابين بمرض ضخامة نهاية الاطراف

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الخلاصة

ان نشاط انزيم الاوروماتيز في مرض ضخامة النهايات هو علامة هامة لتحديد السلوك الجنسي. هناك دلائل تشير الى وجود علاقة بين فعالية الانزيم والتغيرات في المنطقة الغير مترجمة للنهاية 3 منطقة تغير احادي النيكلو تيد SNP وتعرف بـ *rs10046* في جين *CYP19A1*. تم استخلاص الحمض النووي الدنا في دم كل من مجموعة المرضى والأصحاء باستعمال عدة استخلاص الحمض النووي الدنا و تم استعمال طريقة السلسلة المتعددة PCR لغرض مضاعفة الشريط الوراثي لجين *CYP19A1* في المنطقة احادية النيكلو تيد *rs10046*. تم قياس فعالية انزيم الاوروماتيز في مصل دم كل من مجموعة المرضى والأصحاء باستعمال تقنية بالانزيم الامتصاص المناعي المرتبط (ELISA). النتائج اظهرت وجود انخفاض معنوي في مستويات نشاط الانزيم لمرضى ضخامة النهايات ($P \leq 0.01$) وكذلك وجود زيادة معنوية عالية في أليل TT و CC بينما كان الفرق غير الهام في أليل TC عند مقارنته مع الاصحاء ($p > 0.01$) أيضا تميل الأليلات TT و CC إلى أن تكون مرتبطة مع زيادة خطر الإصابة بمرض ضخامة النهايات في هذه المجموعات كما هو محدد عن طريق نسب الأرجحية < 1 ، كانت هذه الاختلافات معنوية، في حين أن أليل TC تميل إلى أن تكون مرتبطة مع انخفاض خطر الإصابة بمرض ضخامة النهايات في هذه المجموعة كما هو محدد عن طريق نسب الأرجحية < 1 ، على الرغم من أن هذه الاختلافات معنوية.

