Nat. Volatiles & Essent. Oils, 2021; 8(4): 11431-11441

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Natural Volatiles &
Essential Oils

The Association Of ACP5 Gene (Rs2071484) Polymorphism With Osteoporosis In Patients Of Babylon Province/Iraq

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Summary

The aim of the study was to evaluate the association of ACP5 gene polymorphism rs2071484 with osteoporosis patients.This studyinclude 150 (20 men and 80 women)osteoporosis patients and 50 (19 men and 31 women) healthy as control group.Genotyping of rs2071484 of ACP5gene was carried out using the polymerase chain reaction-basedSingle strand conformation polymorphism (SSCP)technique.The result of this studyshowed ACP-5(rs2071484) was associated with osteoporosis in Iraqi patients and CC genotypes were high associated with osteoporosis disease by OR=4.47**.** In Conclusion: The ACP5rs2071484 polymorphism was associated with osteoporosis in Iraqi patients and CC genotypes were high associated with osteoporosis disease.

Keywords: osteoporosis ,ACP5, rs2071484 polymorphism, TRAP-5

Introduction

Osteoporosis is a metabolic syndrome of bones in which bones become so weak and incapable to support the body due to loss of both minerals and bone matric in equal proportions (Hinjorgoet al., 2008). It sets during the later years but it develops much earlier without cautioning. It is most rampant disease of aging that has affected more than 25 million people commonly women in all over the world. People are hospitalized due to osteoporosis each year in United States (Whitney and Rolfes, 2002).In normal process of bone turn over the rate of

boneresorption and bone formation is equal in which by acidification osteoclasts remove bone and by secreting osteoid into the resorption cavity osteoblasts build bone. The rate of bone turnover is increased due to elongation of the life span of osteoclasts and reduction in the lifespan of osteoblasts (Manolagas, 2000).Genetic influences are thought to contribute mainly to the acquisition of peak bone mass, occurring in young adulthood (Pollitzerand Anderson,1989) While some investigators have reported that bone loss may be genetically determined,(Kellyet al.,1993) others have found no evidence for genetic effects on bone loss, (Christianet al.,1989) even though there is good evidence that bone turnover is genetically determined.(Hunteret al., 2001) Many genome-wide linkage searches for quantitative trait loci (QTLs) that regulate BMD have been performed, but few of these studies have identified loci that meet the criteria for genome-wide significance, and there has been limited replication of linkage peaks between studies.(Ralston,2005).Several genes are associated with BMD and osteoporosis,The ACP5 gene provides instructions for making an enzyme called tartrate-resistant acid phosphatase type 5 (TRAP). The TRAP enzyme primarily regulates the activity of a protein called osteopontin, which is produced in bone cells called osteoclasts and in immune cells. Osteopontin performs a variety of functions in these cells. Two versions (isoforms) of the TRAP enzyme are produced: TRAP5a is found primarily in immune cells and TRAP5b is found primarily in bone cells called osteoclasts (Fagerlundet al.,2006;Janckilaet al.,2001).

Subjects and Methods

This study was carried out on patients attended to Bone Density unitin one hospital which is Marjan Medical City in Babylon Governorate. This study includes 150 females and males. They had been divided into twogroups, the first group included 100 patients (80 females and 20 males) with OP and thesecond group included 50 relatively healthy (females and males) ,the age wasof both groups was matched and ranged between (20- 80) years.Venous blood samples were drawn from patients and control subjectsby using disposable syringes . Five ml of blood was obtained fromeachsubject , 2 ml was placed into EDTA tubes and the remaining (3ml)pushed slowly into disposable gel containing tubes. Blood in the EDTAtubes was stored in(-20˚C) in order to be used later in genetic part of the study, while blood inthe gel containing tubes was allowed to clot at room temperature for 15minutes and then centrifuged at 3000 rpm for approximately 10-15.minutes , after that sera was obtained (Barbara and Anna,2012)andstored at -20˚C.

DNA Extraction

Genomic DNA from white blood cells (WBCs) for both OPpatients and control groups were extracted by using DNA extraction methods (Hashim and Al-Shuhaib,2020) summarized as follows:

1- Take 500 µl of the blood was transferred to an Eppendorf tube of 1.5 ml capacity. Up to1ml of washing buffer (Tris90%+Methanol10%) was added to the blood sample, mixed, and incubated at ambient temperature for 10min. ,or 5min. in a rotating mixer. The mixture was centrifuged at 10000 g for 2 min. After discarding the supernatant,1 ml of washing buffer was added again, and the tube was inverted several times to wash the pellet then centrifuged at the same speed for 10 seconds. The supernatant was discarded

2- The pellet was suspended with 200 µl of cell suspension buffer (EDTA) and add400 µl from extraction buffer and suspend well for distribution in the 60 Silesian for 15 min.

3-Left in the room temperature the add 100 µl from sodium acetate and rotating mixture for 1 min.

4- The mixture was centrifuged at 10000 g for 10 min. take the supernatant centrifuged at 10000 g for 30 sec.

5-Washing for twice by 600 µl(70%ethanol+30%Tris) and centrifuged at 10000 g for 30 Sec.The previous step was repeated twice then dehydration at room temperature by centrifuged at 10000 g for 3 min for remove washing buffer, move The supernatant containing DNA was transferred to anew an Eppendorf tube of 1.5 ml capacity.

6- Added DNA elution buffer 100 µl and left for 5 min. then centrifuged at 10000 g for 1 min. and take the clear that containing the pure DNA.

Primer design

The design of PCR primers according to the protocol of (2) briefly as follow:

The primers were designed by the aid of NCBI-primer BLAST online software(http://www.ncbi.nlm.nih.gov/tools/primer blast/index.cgi?LINK_LOC=BlastHome) , at the same time the produced primers was checked for specificity for their target sequences by performing the BLAST against the human genome , then the primers pair was selected according to the demand criteria such as : product length , the similarity of melting temperature , primers length , specificity , etc. Then the mutations was interred according to the design demands .

The primer ability to form secondary structure was checked by the aid ofOligoCalconlinesoftware(http://www.basic.northwestern.edu/biotools/oligocalc.html) , the primer would be rejected if it had 5 bases or more able to form self-dimerization and/or it had 4 bases able to form hairpin .

Each primers pair was checked for dimer formation by the aid of "Multiple Primer Analyzer" online software from Thermo Fisher Scientific Inc.© , the sensitivity of the software was adjusted to the value 2 , the primer pair would be rejected if it made any dimers in this degree of sensitivity .

Table(1) Primersof ACP5 orTartrate-resistant acid phosphatase(TRAP-5)

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACAAGCTGGCTTAGGGAAGG	20	59.67	55.00	6.00	0.00
Reverse primer	TGTCCTGCTCCAGGGAAGT	19	60.15	57.89	5.00	1.00

PCR Amplification of ACP5 orTRAP-5 gene

One PCR fragment was selected for amplification, which supposed to cover 304bp of ACP5 or TRAP-5gene**.**

Optimization of ACP5 or TRAP-5 PCR Product.

Different annealing temperatures (gradient PCR) were used to optimize primer pairs which produced (304pb) of the TRAP-5 gene. A gradient of 55-67 C annealing temperature were used to optimize the PCR amplification**.**

Single strand conformation polymorphism SSCP.

The accurate analysis of genetic variation has major implications in many areas of biomedical research, including the identification of infectious agents , the diagnosis of infections, and the detection of unknown or known disease-causing mutations(Gasseret al .,2006).

Statistical Analysis

Analysis of the data was by using completely randomized design (CRD), Odd ratio The p-value ≤ 0.05 was regarded as significant. All analyses were performed using software of SPSS for Windows version20.

Results

DNA Extraction The result of DNA extraction was revealed in the figure (1)

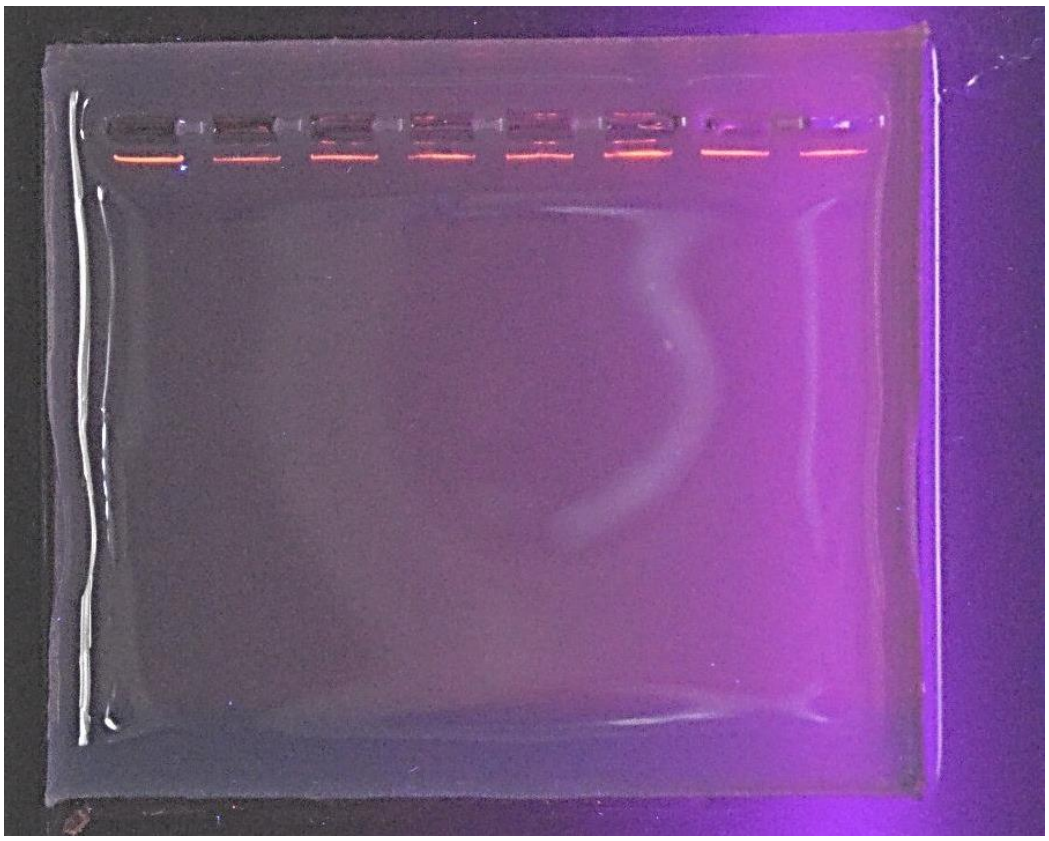
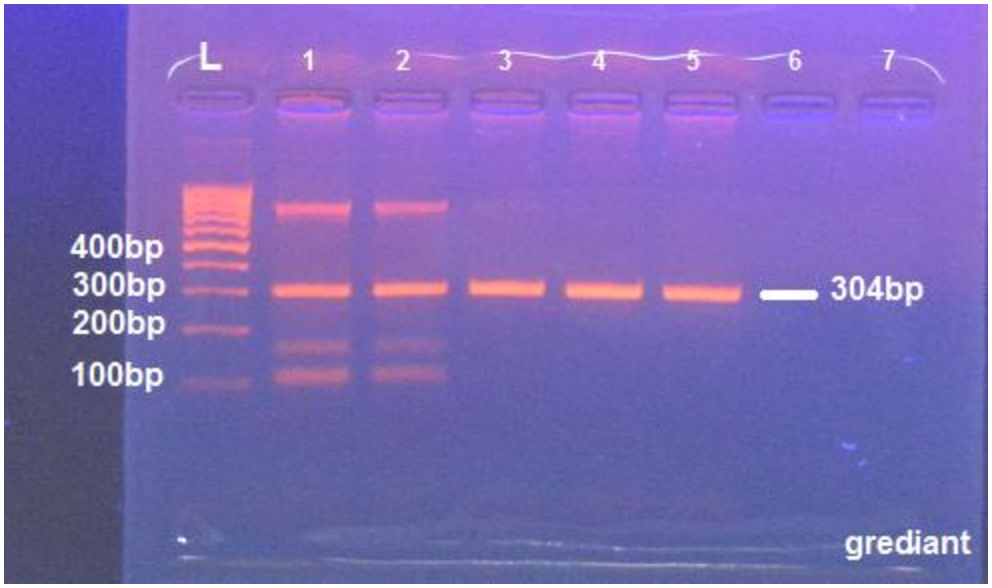


Figure (1)The electrophoresis pattern of DNA extracted from blood for patients with osteoporosis and control,1% agarose.

Determination of Annealing Temperature of ACP5Gene (rs2071484) (Gradient PCR).

different annealing temperature were record and select the temperature 63C° because it give best product and used for amplification all the samples.



Figure(2) show gradient PCR for targeted amplicon (304pb).DNA lader,

(55C° -58 C°-61 C°-64 C°-67 C°).

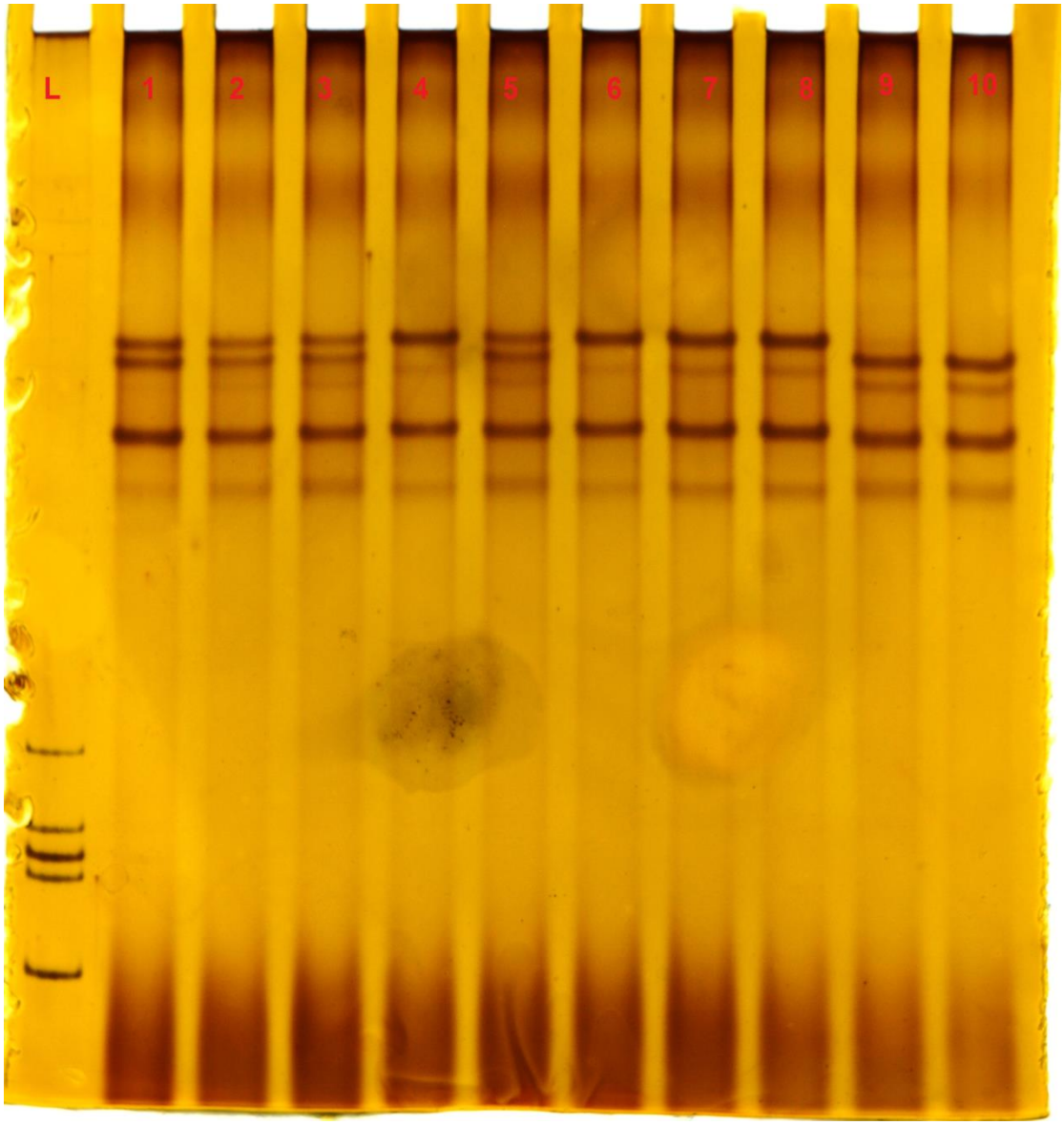


Figure (3) Electrophoresis pattern of PCR-SSCP by 4% polyacryl amid gel for PCR product (304bp)

Genotype of ACP5 or TRAP-5 gene polymorphism with Allele frequency:

The allelic frequency and allelic association of (rs2071484)with osteoporosis according to the result study showed there is no allelic association as listed in the table (2).

Table (2):The allelic frequency and allelic association of (rs2071484)with osteoporosis

	All subjects		control		case		OR (95% CI)	P-value
Allele	Count	Proportion	Count	Proportion	Count	Proportion		
T	160	0.53	53	0.53	107	0.54	1.020	0.934

							(0.631-1.651)	
C	140	0.47	47	0.47	93	0.46	0.980 (0.606-1.586)	

On the other hand the genotype association showed that CC genotype have a significant association with disease (p value 0.0026), in which the CCgenotype have odd ratio equal to 4.47 comparing to the other TT and TC genotype this indicate that C allele inherited as recessive pathogenic allele. The table below (3) show genotype frequency and association of (rs2071484) genotype with osteoporosis under different models of inhere tins. Individual that have CC genotypein which we found allelic C represent a recessive pathogenic allele have the portability to develop osteoporosis (4.5) time more than individuals with T/T and T/C genotypes.

Table(3) genotype frequency and association of (rs2071484) with osteoporosis under different model of inhere tins.

Model	Genotype	control	case	OR (95% CI)	P-value
Codominant	T/T	7 (14%)	35 (35%)	1.00	<0.0001
	T/C	39 (78%)	37 (37%)	**0.19 (0.08-0.48)**	
	C/C	4 (8%)	28 (28%)	1.40 (0.37-5.27)	
Dominant	T/T	7 (14%)	35 (35%)	1.00	0.0049
	T/C-C/C	43 (86%)	65 (65%)	**0.30 (0.12-0.74)**	
Recessive	T/T-T/C	46 (92%)	72 (72%)	1.00	0.0026
	C/C	4 (8%)	28 (28%)	**4.47 (1.47-13.58)**	
Overdominant	T/T-C/C	11 (22%)	63 (63%)	1.00	<0.0001
	T/C	39 (78%)	37 (37%)	**0.17 (0.08-0.36)**	

Discussion

genetic traits are nonmodifiable factors for osteoporosis(Vijayakumarand Bu¨sselberg,2016) and approximately 75% of osteoporosis is heritable(Mendozaet al .,2012)Moreover, BMD, an essential biomarker for osteoporosis and osteoporotic fracture prediction is a highly heritable quantitative trait(Chen and Xia,2014) .It is evident that approximately 50% to 82%

of variations in BMD are of genetic origin (Liu et al.,2012). These genetic variations are also believed to be associated with menopausal status(Hunteret al.,2001). For instance, the total genetic percentage of spine BMD variance in premenopausal and postmenopausal women was 88% and 77%, respectively (Hunteret al.,2001).The current study has been discussed whether or not the association of ACP-5rs2071484 with osteoporosis disease in Iraqi osteoporosis patients in Babylon province.Many studies have investigated the role of mutations and the polymorphism in ACP5 or TRAP-5gene in the development of different diseases.Briggset al .,2011 showedin 10 patients with spondyloenchondrodysplasia with immune dysregulation (SPENCDI) from 8 families, identified homozygosity or compound heterozygosity for mutations in the ACP5 gene . In vivo testing confirmed a loss of expressed protein, and all 8 cases assayed showed elevated serum interferon alpha activity, with gene expression profiling in whole blood defining a type I interferon signature.Simultaneously and independently.

(Hayman et al.,1996)observed that mice with a targeted disruption of the Acp5 gene, or Trap, suffered from developmental deformities of the limb and axial skeleton and had osteoclasts defective in bone resorption, resulting in mild osteopetrosis.The rs2071484 polymorphism studied for the first time in Iraq on the osteoporosis patients (male and female) and this study showed there is significant correlation between the (rs2071484) polymorphism and the osteoporosis.

The allelic frequency and allelic association of (rs2071484)with osteoporosis according to the result study showed there is no allelic association.On the other hand the genotype association showed that CC genotype have a significant association with disease (p value 0.0026), in which the CCgenotype have odd ratio equal to 4.47 comparing to the other TT and TC genotype this indicate that C allele inherited as recessive pathogenic allele.Individual that have CC genotypein which we found allelic C represent a recessive pathogenic allele have the portability to develop osteoporosis (4.5) time more than individuals with TT and TC genotypes.This study showed significant correlation between gene and osteoporosis and this result agree withMehrunnisaet al .,2017 that showed there is high significant correlation between gene ACP5 and osteoporotic Indian postmenopausal women. This study showed significant correlation between ACP5 gene and osteoporosisthis gene is osteoclast specific gene and many factors such as hormones, interleukins, growth factors, nutrition, physical activity and lifestyle play a major role in the development,differentiation and maturation of osteoblasts and osteoclast precursor cells, suggesting that bone resorption is being influenced by both local and systemic factor.osteoporosis is a multifactorial disease in which

genetic and epigenetic factors along with lifestyle play an important role.osteoporosis in the women menopause status is the most vulnerable factor in women. 'eficienc\ of estrogen with simultaneous increase in the FSH level accelerates bone loss inducing osteoporosis.

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