Identification of Farnesoid X Receptor as a Novel Nuclear Receptor Sensing for Gallstone Diseases

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Abstract

Objective:-The bile acid-make active nuclear receptor farnesoid X receptor (FXR) acting an significant part in lipid then glucose metabolism, besides furthermore, it controls numerous lipid carriers complicated in stone disposition. We observed whether a useful single nucleotide polymorphism (SNP) in FXR (82 G>T) predisposed the gallstone development.

Material and Method:- The polymorphism were confirmed by polymerase chain reaction monitored by PCR amplicons were commercially sequenced from both (forward and reverse) termini according to education manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea)., in two group match by age≥20 : patient with gallstone (n=140) and stone-free control (n=140).

Result :- For Sequencing of the 195 bp region within the *NRIH4* (*FXR*) gene Within this locus, twenty samples were included in the present study that had shown to amplify the *NR1H4* (or *FXR*) genetic sequences in the chromosome number 12. The latter gene is responsible for encoding on nuclear receptor subfamily 1 group H member 4 (NR1H4). The alignment results of the 195 bp samples revealed the presence of one SNP occurred in this position in sample no. 10 within the analyzed twenty samples in comparison with the referring reference DNA sequences The sequencing chromatogram of the observed substitution SNP, as well as its detailed annotations, were documented, and the chromatogram details of the observed SNP were shown according to their positions in the PCR amplicon, in which samples no. 10-20 had shown this (T82G)variation.

Conclusion:- the education presented that the variation allele of the communal FXR 82G>T polymorphism was significantly associated with stone formation in Iraq patients. The association is possibly concluded the effect of the FXR 82G >T polymorphism on the expression of the efflux carriers for lipid trails in hepatic.

Key word :- *FXR , PCR , Gallstone, polymorphism, sequencing analyzing.*

Introduction

Gallstones are aggregation of hard substance that forms inside gallbladder [1], when there is disequilibrium in the constitution of bile such as more cholesterol, accumulated amount of pigment material and/or decreased amount of bile acid [2]. Gallstones may also result from dysfunction of gallbladder contraction [3]. Nuclear receptors (NRs) are ligand-activated transcription factors with important roles in different aspects of human physiology and development of gallstone [4]. FXR regulates the expression of various genes involved in bile acid, lipid and glucose metabolism, by binding to DNA either as a monomer or an heterodimer with a common partner for NRs, Retinoid X Receptor (RXR). FXR is highly expressed in the liver, intestine, kidney and adrenals [5]. Two known FXR genes exist, the *Fxr*α and *Fxrβ*. *Fxr*α gene in humans encodes four FXRα isoforms (FXRα1, FXRα2, FXRα3 and FXRα4) as a result of the use of different promoters and alternative RNA splicing. Given the importance of the FXR in bile acid and in regulating the expression of the gene that are involved in gallstone formation, we examined the association between the functional SNP FXR 82G>T and gallstone disorder.

Material and Method

Object in this analysis include two groups matched by age ≥20 years ,consecutive symptomatic patients (*n*

= 140) with gallstone disease (GD), and healthy stone-free control subjects (*n*= 140) confirmed by abdominal ultrasonography.

DNA extraction and genotyping

DNA was isolated from whole blood by Wizard® Genomic DNA Purification Kit Staining . FXR genotyping was performed as previously described [6]. Briefly, PCR was carried out in a reaction mixture of 25 µL containing 0.5 units of Taq DNA polymerase and 5 µL of template DNA with a concentration of 50 to 150 ng/µL, using primers 5'- GAGCCAGTGAACAGAAACCC -3' (sense) and 5'GTGAGAGAGGACAGAGGTTG -3' (antisense) [7]. Amplification reaction consisted of 30 cycles, with Initial Denaturation 95°C for 3 minute ,annealing 61°C for 30 second, Extension 72°C for 1minute ,to yield 194bp of product FXR.

Detection of farsenoid X Receptor (FXR) gene polymorphism (82 T>G)

PCR Product using Promega Master Mix

PCR Product using Promega master mix was given sharp and apparent 195 bp band as figure (1) shown , therefore the study choose it in the rest of the work because of its high reliability

M 1 2 3 4 5 6 7 8 9 10 11 12 13

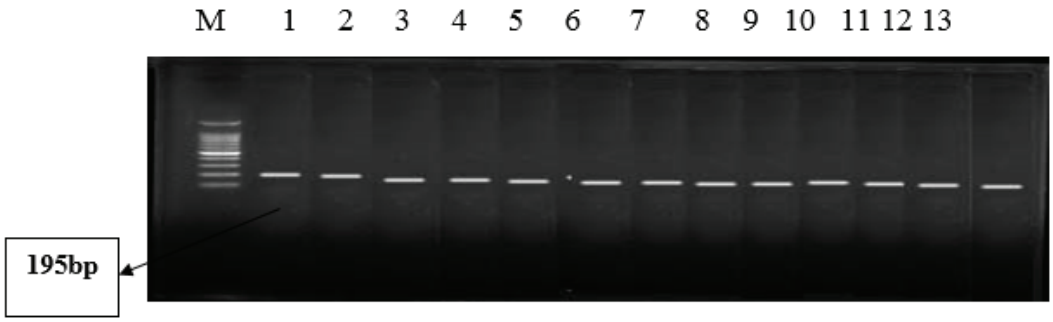

Figure (1): PCR Product using promega master mix on 1.5% agarose , 70V, and for 45 minute (5 µl of DNA loaded in each well) , lane M: 100 bp ladder Lane 1-13 : PCR product.

Sequncing Method

DNA Sequencing of PCR amplicons

The resolved PCR amplicons were commercially sequenced from both (forward and reverse) termini according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed DNA sequences of local bacterial samples with the retrieved neighboring DNA sequences of the NCBI Blastn engine, the virtual positions and other details of the retrieved PCR fragments were identified.

Interpretation of sequencing data

The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome.

Checking the novelty of SNPs

The observed SNP was submitted to the dbSNP database to check their originality. Each particular SNP was re-positioned according to its place in the reference genome Subsequently, the determination of the presence of previous SNP was performed by viewing its corresponding dbSNP position. Then, the dbSNPs position for the detected SNP was documented.

Sequncing Result

1.Sequencing of the 195 bp region within the *NRIH4* (*FXR*) gene

Within this locus, twenty samples were included in the present study that had shown to amplify the *NR1H4* (or *FXR*) genetic sequences in the chromosome number 12. The latter gene is responsible for encoding on nuclear receptor subfamily 1 group H member 4 (NR1H4). This protein functions as a receptor for bile acids, and when bound to bile acids, binds to DNA and regulates the expression of genes involved in bile acid synthesis and transport (https://www.ncbi.nlm.nih.gov/gene/9971). The sequencing reactions indicated that the exact identity after performing NCBI blastn for these PCR amplicons (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). Concerning the supposed 195 bp amplicons, NCBI BLASTn engine shown about 99% sequences similarity between the sequenced samples and the intended reference target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (GenBank acc. NG_029843.1), the approximate positions and other details of the retrieved PCR fragments were identified (Fig. 2).

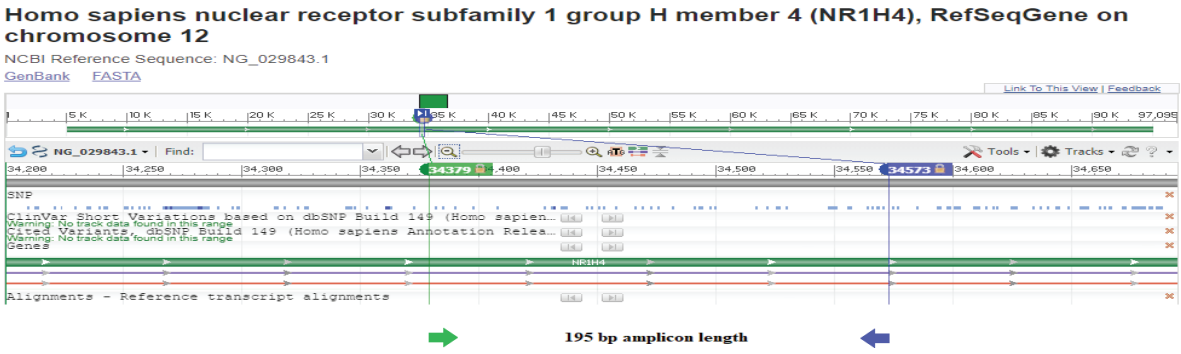

Fig. 2. The exact position of the retrieved 195 bp amplicon that partially covered a portion of the *FXR* gene within chromosome 12 (GenBank acc no. NG_029843.1). The green arrow refers to the starting point of this amplicon while the blue arrow refers to its end point.

After positioning the 195 bp amplicons' sequences within the *FXR* gene, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 195 bp amplified amplicon (Table 1).

Table1. The position and length of the 195 bp PCR amplicons used to amplify a portion of the *FXR* gene within chromosome no. 12 (GenBank acc. no. NG_029843.1). The grey colored sequences referred to the position of the forward and reverse primers, respectively.

Amplicon	Referring locus sequences (5′ - 3′)	length
DNA sequences within the FXR gene	*GAGCCAGTGAACAGAAACCCACCCTCTAAAAGTTCTTAAACTGGAAAAGTACTCCCCCAAAATGTTTATCTAAGAGACTGGTTTCCAGCTTACTAGGCAATTTGGCATTAAGAACTTTCCTCATAAACATTTACAAATATTCTGCTCCGTAATGAAGTTAATCAGTAAACCACACAACCTCTGTCCTCTCTCACT**	195 bp

* refers to the forward primer sequences

** refers to the reverse primer sequences

The alignment results of the 195 bp samples revealed the presence of one SNP occurred in this position in sample no. 10-20 within the analyzed twenty samples in comparison with the referring reference DNA sequences (Fig. 3).

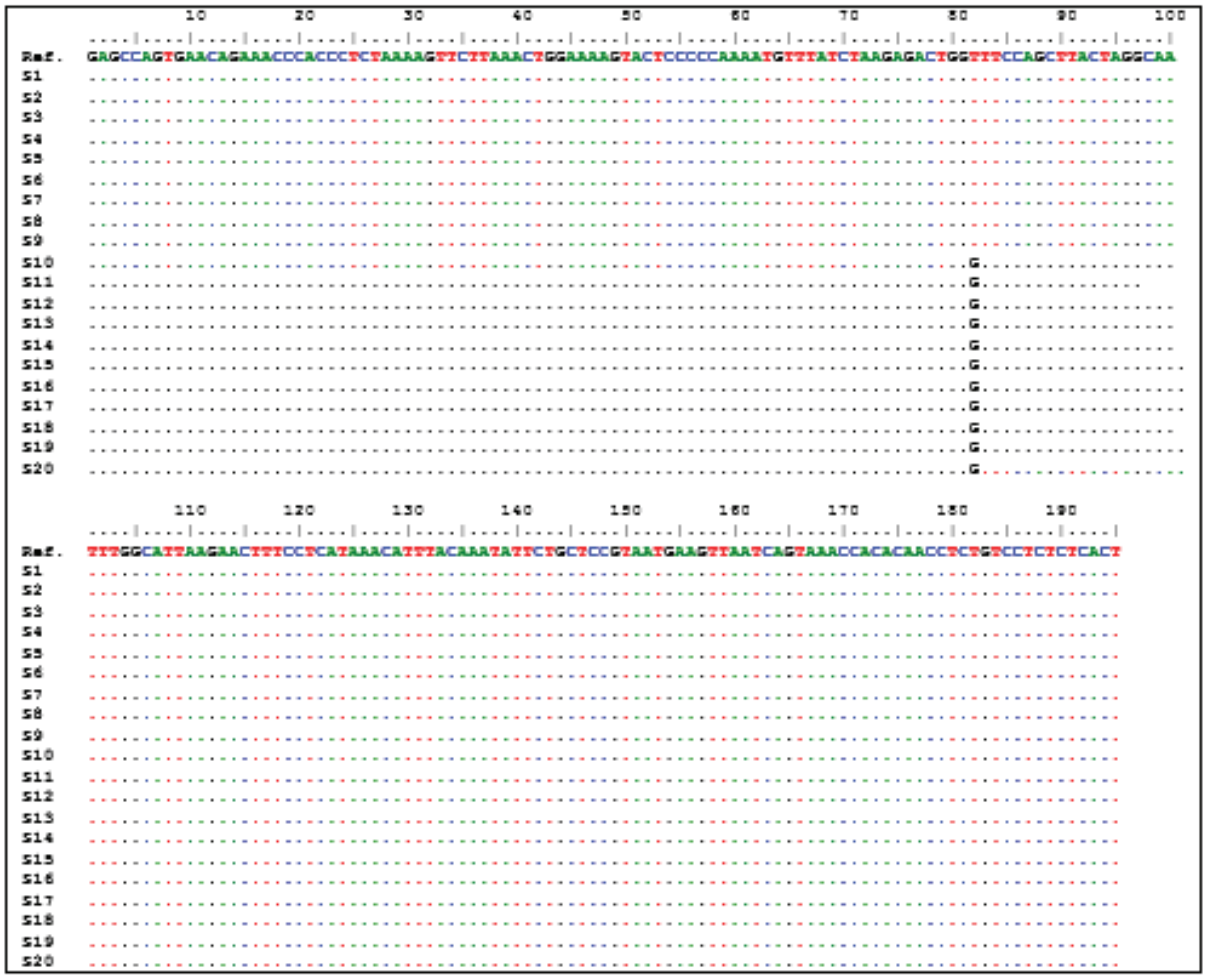

Fig. 3. DNA sequences alignment of twenty local samples with their corresponding reference sequences of the 195 bp amplicons of the *FXR* genetic DNA sequences. Each substitution mutation was highlighted according to its position in the PCR products. The symbol "ref" refers to the NCBI referring sequence, "S1-S20" refer to the samples 1 to 20, respectively

The sequencing chromatogram of the observed substitution SNP, as well as its detailed annotations, were documented, and the chromatogram details of the observed SNP were shown according to their positions in the PCR amplicon, in which only sample no. 10 -20 (S10-S20) had shown this T82G variation (Fig. 4).

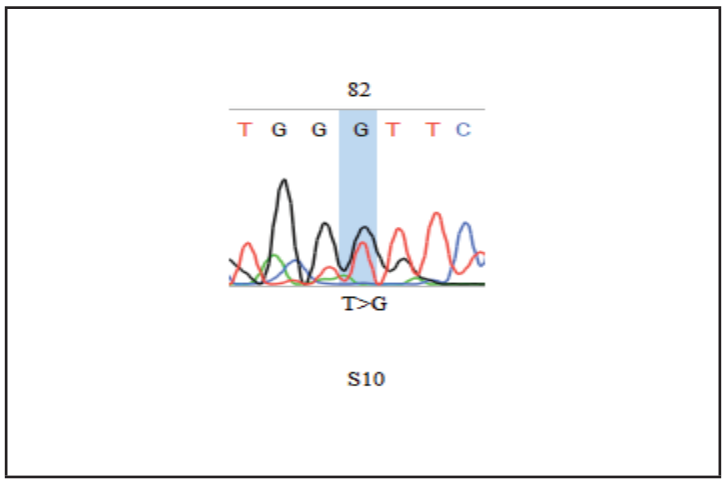

Fig. 4. The pattern of the observed substitution mutation within the DNA chromatogram of the targeted 195 bp amplicons within the *FXR* gene. The observed substitution mutations are highlighted according to their positions in the PCR products. The symbol ">" refers to substitution mutation.

SNPs characteristics check

To elucidate the positions of the observed SNP with regard to their deposited SNP database of the sequenced 195 bp fragment, the corresponding position of the *FXR* gene was retrieved from the dbSNP server (https://www.ncbi.nlm.nih.gov/projects/SNP/). To find out the nature of the observed SNP, a graphical representation was performed concerning the *FXR* dbSNP database within chromosome 12 (GenBank Acc. No. NG_029843.1). By reviewing the dbSNP engine, it was found that this SNP was novel (Fig.5). However, this SNP was found to be located in an intronic position.

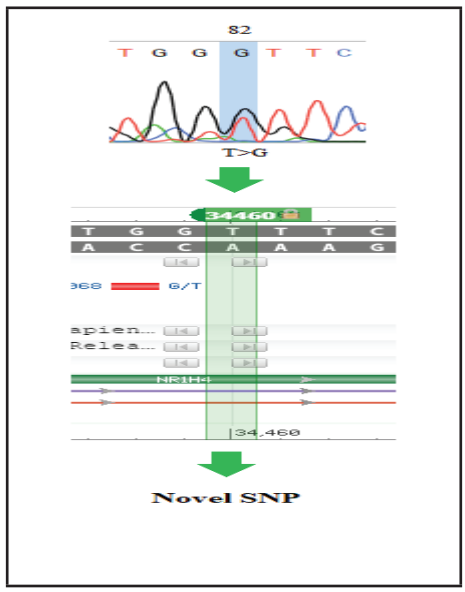

Fig.5. The SNP's novelty checking of *FXR* genetic single nucleotides polymorphism using dbSNP server. The identified SNP is marked with a green color.

Therefore, it was found that this observed variant was not previously known and positioned in the intronic region within the *FXR* gene. To summarize all the results obtained from the sequenced 195 bp fragments, the exact position of the observed variation was described in the NCBI reference sequences **.** The pattern of the observed SNP in the 195 bp amplicons in comparison with the NCBI referring sequences of the *FXR* gene within the chromosome 12 (GenBank acc. no. NG_029843.1).

Discussion

It has been recommended that variations in monitoring protein coding genes, such as FXR, may possibly donate to the general dissimilarity for gene expression in tissues then thus effect stone nature then replies [8,9]. This education is the first to report that the communal polymorphism (82 G>T) in FXR resulting in significantly condensed role of the gene was related with development of stone in Iraq patients through gallstone syndrome then that this suggestion remained important after regulating for the potential features.

To our information, there is no education reporting whether the gene for ABCG2, the main efflux carrier of , is a board of FXR, but then again unintended indication proposes that hepatic ABCG2 expression is controlled by some nuclear receptors, such as constitutive androstane receptor (CAR) then pregnane X receptor (PXR) [10], and the last is a board of FXR[11]. Consequently, it is likely that the condensed FXR action because of the 82G>T mutation may possibly decrease the ABCG2 expression by sound effects on PXR. It seems that the relationship between the FXR 82G>T polymorphism and stone development is additional apparent in issues homozygous for the wild-type alleles than in those with one or two duplicates of the variation allele of the FXR82G>T polymorphism. This consequence may possibly propose that the outcome of the FXR polymorphism on the gallstone is facilitated concluded a monitoring outcome on gene expression.

Ethical Clearance: Approval by scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the medical college.

Source of Funding : The funding body written out in full by self

Conflict of Interest : If any then mention it otherwise write it as nil

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