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Research Article

Investigation of CASR Gene Polymorphism in the Patients with hypothyroidism Disease in Babylon Province

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ABSTRACT
The calcium-sensing receptor (CaSR) is a calcium (Ca2+) sensitive G protein-coupled receptor implicated in various biological processes. In particular, it regulates Ca2+/Mg2+- homeostasis and senses interstitial Ca2+ levels and thereby controls downstream signalling cascades. The results of PCR-SSCP for CASR gene illustrated that two different haplotypes according to the numbers of bands in the CASR gene including 6 and 7 bands. While, these haplotype was detected between two groups; in hypothrodisum patient groups and control, the results indicate that was association between 6 and 7 bands in patients as compared with a control group. **Conclusion:** PCR-SSCP Is a good investigation technique to detection *Casr* gene polymorphisms in patient with *hypothyroidism*

Keywords: hypothyroidism, CASR, *SNP*, SSCP, PCR, rs1801725.

INTRODUCTION

Parathyroid glands regulate parathyroid hormone(PTH) secretion through the calcium-sensing receptor(CASR), CASR gene role in the cell membrane is controlled by two alternative promoters (P1 and P2), positioned before either exon 1A or exon 1B of the CASR gene[1]. both of them encode to the same CASR protein and their functional differences are still unknown causes of primary hypothyroidism comprise goiter due to iodine deficiency and too much iodine in the individuals with thyroid disease. a number of drugs can also cause hypothyroidism including lithium carbonate, para-aminosalicylic acid, thiourea drugs, sulfonamides, phenylbutazone and others. Decades previously congenital hypothyroidism was a frequent cause of mental retardation and severe disability in affected children[3]. The calcium-sensing receptor (CaSR), a G-protein coupled receptor (GPCR) family member, is universally expressed, but mostly in the parathyroid gland and the renal tubule. It enables CaSR-expressing cells to sense alteration in the level of blood calcium and to normalize its concentration, by regulating parathyroid hormone (PTH) secretion and kideny calcium excretion. The CaSR is capable to bind numerous ligands, to act together with multiple G-proteins, and to control highly divergent downstream signalling pathways and cell fate, through epigenetic and miRNA[3,16].

Calcium is an necessary nutrients that are acting a very important function in many functions, enzymes-mediated processes and blood clotting. Additionally, it provides skeletal severity by the virtues of its phosphate salts, calcium insufficiency has been recognized for numerous years to risks the skeleton in the long term. Lately, concern has also grown that calcium provide in excess of the necessities might raise cardiovascular danger[11].

The human CASR gene localize on chromosome 3q and has 8 exons, the first (1A and 1B) encoding alternative 5′-untranslatesd regions splicing. The CASR promoters are responsive to 1, 25-dihydroxyvitamin D, proinflammatory cytokines (TNF-alpha, IL-1beta and IL-6) and the transcription factor glial cells missing-2 (GCM2), irregular CaSR function affects the development of both calciotropic disorders, and non-calciotropic disorders, such as cardiovascular disease and cancer[4]. numerous disorders of calcium sensing take place from inherited or acquired abnormalities that 'reset' the serum calcium concentration upwards or downwards. The CASR gene might also be involved in tumourigenesis, mainly in the colon, breasts and the prostate, as well as in cardiovascular and inflammatory diseases, including both digestive and respiratory[5].

MATERIAL AND METHOD

Sampling

Twenty blood samples were collected from patient with primary hypothyroidism whom visit marjan hospital /Babylon /Iraq and twenty samples as control.

DNA Extraction

Genomic DNA from whole blood cells was extracted and purified using Extraction and purification Kit from Favergen company (Taiwan).

The targeted sites of DNA were amplified using specific primers: One primer was used for identify CASR (rs1801725), obtained from Bioneer, IDTDNA(USA). Primer: Forward sequence was 5-

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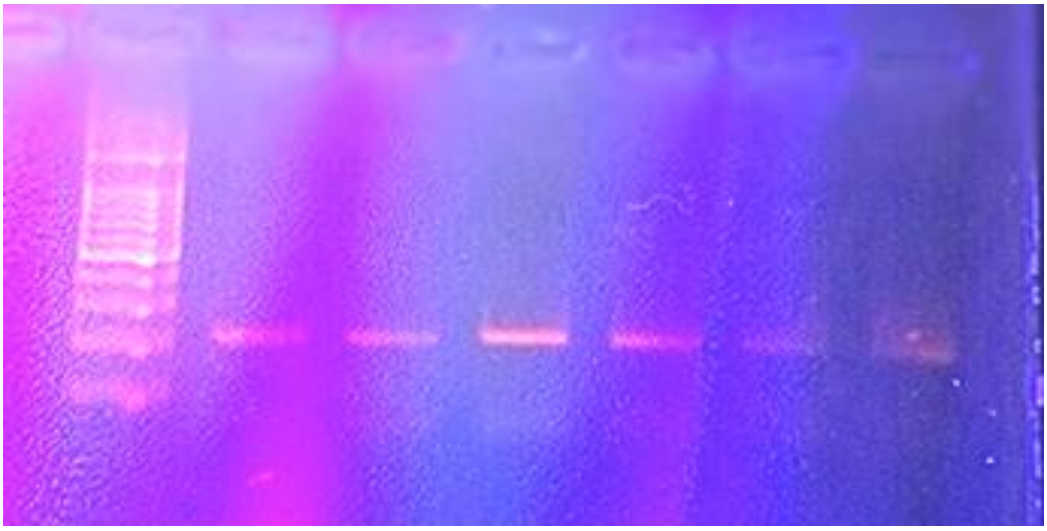

Figure 1: Agarose gel electrophoresis of CASR amplification products.
M; refers to DNA size marker lane 1 – 6 lane refers to the patterns of amplified products of CASR (221bp).

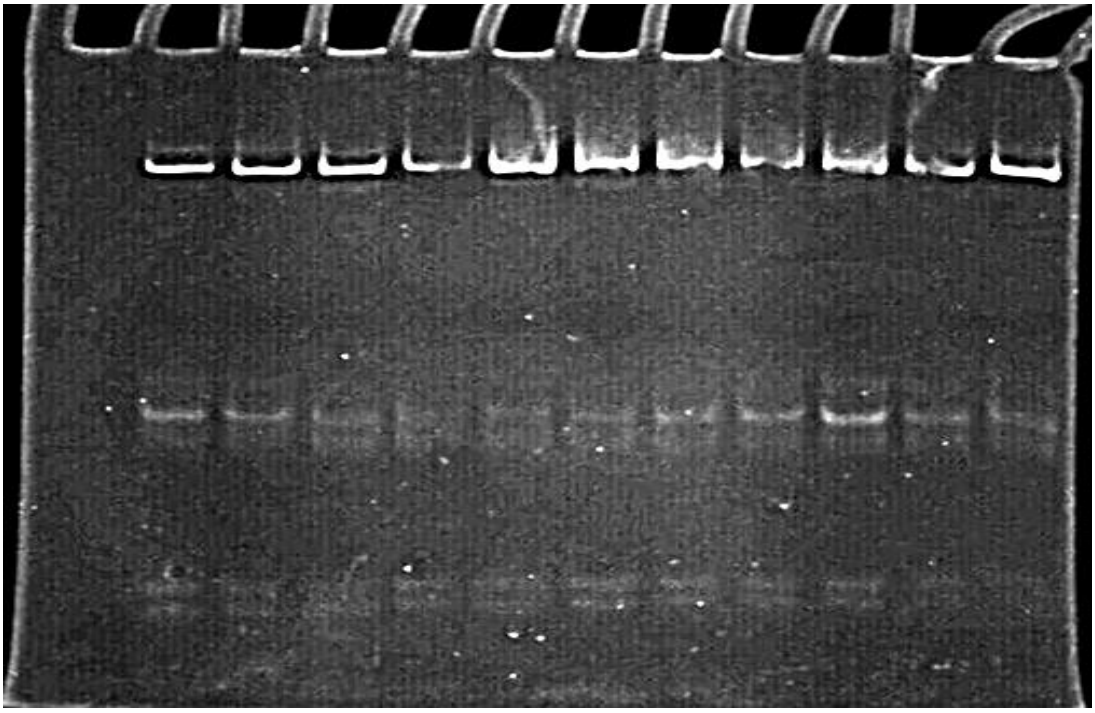

Figure (2): DNA polymorphisms of of CASR gene .
Selected lanes 1 –11 refer to the PCR-SSCP pattern of CASR . ″C″ refer to the control while ″P″ refer to the groups of patients.

AGCCCAGATGCAAGCAGAAG -3, and the reverse sequence was 5- CAGACCTGTTTCCTGGACGG -3.
PCR was carried out in 20µl reaction volumes containing 1 ul from reverse and forward primer, 12.5 ul of Green Master Mix, 3 ul of Genomic DNA and the volume of reaction was completed up to 20 ul by adding 2.5 ul of Nuclease free water. Amplification was carried out in a thermo-cycler (Biometra, Germany) programmed for two minutes at 94°C; for 30cycles 5 minutes each at 94°C, one minute at 61°C and one minutes at 72°C; and a final extension of 5 minutes. PCR products were electrophoresed using gel electrophoresis (Cleaver Scientific – UK) in 1% agarose at 75 V for 1 hour and visualized by ethidium bromide. Photos were taken using gel documentation system (Cleaver Scientific –UK).
The sharp and obvious bands were found after performing electrophoresis Regarding CASR and exon7 PCR fragment, where this fragment can amplify exon 7 successfully so, these amplifications are subsequently suitable for downstream SSCP tests.
All the obtained SSCP gels were aligned with each other to show how many haplotypes where, three types of SSCP band patterns were observed in SSCP gels. The single stranded (ssDNA) DNA bands, which occupy the upper portion of the gel and the double stranded (dsDNA), which occupy the lower portion of the gel were

Table 1: The DNA polymorphism distribution of CASR gene by the number of bands and their association with hypothrodisum patients and control groups.

Genotype CASR	Patients	Control	P- Value	OR=(95%CI)
6 band[a]	2(10%)	9(45%)		
7 band	18(90%)	11(55%)	0.031*	0.13(0.02-0.74)
Total	20	20		

*P ≤ 0 .05.
S.E: Standard error.

observed. The variation of ssDNA in SSCP gels is relied to identify the genetic pattern of each amplified, and the condition for SSCP –PCR was 8 % Polyacrylmide gel electrophoresis power applied: 75 V, 20 mA for 160 min. After that gels visualized by ethidium bromide. Photos were taken using gel documentation system (EBOXCX – UK).

PCR-SSCP Techinque

The PCR-SSCP method includes subsequent steps, it was tried to resolve our PCR products on minigels and it was found that the optimum SSCP gel concentration to resolve these bands is 8%. Very simple and rapid silver staining method was relied on[13,14].

Statistical analysis

All the statistical analyses were done with the SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL), *P*- values <0.05 were considered statistically significant.

RESULTS

The figure (1) showed CASR gene amplification product. The figure (2) showed polyacrylamide gel electrophoresis of SSCP-PCR of CASR gene (221) bp amplified product. All the obtained SSCP gels were aligned with each other to show how many haplotypes where, two types of SSCP band patterns were observed in SSCP gels. The single stranded (ssDNA) DNA bands, which occupy the upper portion of the gel and the double stranded (dsDNA), which occupy the lower portion of the gel were observed. The variation of ssDNA in SSCP gels is relied to identify the genetic pattern of each amplified, and the condition for SSCP –PCR.

DISCUSSION

The consequences of CASR gene illustrated that two different haplotypes according to the numbers of the CASR gene including 6 and 7 bands. While, Conversely, these haplotype was detected between two groups; in hypothrodism patient groups, the results indicate that was association between 6 and 7 bands in patients as compared with a control group.

study of genetic polymorphisms associations and illness states or alteration in physiological and other variables can offer clues into illness pathogenesis, which can be explored further by experimental approaches. There have been a number of study investigative associations of CaSR polymorphisms with variables such as serum calcium concentrations, PTH levels, severity of primary hyperparathyroidism, calcium excretion, renal stones, fractures, bone mineral density, and risk of colon cancer[10].

Three polymorphisms are known intracellular tail of the receptor: Ala986Ser, Arg990Gly and Glu1011Gln were wholly studied. The Ala986Ser SNP was the foremost frequent polymorphic alternative in numerous populations, the study found that 986Ser (T) allele is considerably related to thyroid disease[6]. A variety of study have determined association degree between 986Ser (T) allele and high levels of serum Ca, in agreement with the findings from the opposite studies, they found Ca concentrations were significantly higher in people carrying 986Ser (T) allele and was recommended the inhibitory action of CASR on tubular calcium reabsorption and parathyroid hormone emission be depressed in subjects carrying 986Ser (T) allele. An additional study had been revealed the CASR receptor is sensitive to serum calcium for its location on the basolateral membrane of tubular cells. Also, CASR modulates calcium reabsorption according to the serum calcium levels. Therefore, the increase of plasma Ca let alone elevated Ca excretion in kidney patients carrying 986Ser (T) allele[12,15].

In agreement with this, mutations in the *CASR* gene have been revealed to reason abnormalities in blood calcium ion (Ca levels), the presence of an activating and inactivating mutation of *CASR* gene cause hypo or hyperthyroidism correspondingly .Three single nucleotide polymorphisms cause non conservative amino acid changes have been described on exon-7, encoding the intracellular domain of CASR protein. The the majority familiar SNPs, substitution of alanine to serine at codon 986 is associated with increased the serum calcium concentration and reduced calcium excretion[7]. Another vital polymorphism of *CASR* gene is Arg990Gly positioned in exon-7 and associated to symptom in patients with and will not urinary organ stones. consequences of *in vitro* investigation indicate that the Arg990Gly polymorphism will make to a useful gain for the *CASR*[8]. Other study illustrate CLDN14 play an significant role in the regulation of renal Ca excretion. CLDN14 is also regulated by CASR signaling, CLDN14 expression would be increased by the activating mutation of CASR gene and causing thyroid gland illness and nephrocalcinosis[9].

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