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EVALUATION OF EXON 17 OF INSULIN RECEPTOR (INSR) GENE AND ITS RELATIONSHIP WITH DIABETES TYPE 2 IN AN IRANIAN POPULATION

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Abstract. Mutations in insulin receptor gene cause the inherited insulin resistant syndrome, especially diabetic diseases. Here were optimized the conditions for sequencing of a partial fragment of INSR gene, exon 17. We sequenced fourteen fragments from a diabetic sample and a control group chosen from an Iranian population. In this study we analyzed eleven sequences of diabetic's patients by DNAMAN program (DEMO 8.0), deposited in Genbank with accession numbers LC055416, LC055417, LC055419, LC055421, LC055423, LC055424, LC055425, LC055426, LC055496, LC055497, and LC055498. From our control group, three sequences were deposited in Genbank with accession numbers of LC055418, LC055420, and LC055422. Results showed that there were variation between the sequences of exon 17 of INSR gene in diabetics and control group. Variations were observed in fragments at the beginning and the end of exon 17 among diabetics population. Moreover we found subjected SNPs between diabetic's patients that haven't been reported by other researchers. In this study we concluded that mutations in exon 17 of INSR gene contributed in diabetic diseases.

***Keyword**:* Insulin receptor gene, Exon 17, Diabetes, Sequencing, Control group

Introduction

Insulin receptor (INSR) gene is consisted of 22 exons spanning 120 kilobases on chromosome 19 [KADOWAKI *et al.*, 1990; LI *et al.*, 2006; MOELLER *et al.*, 1990].

Studies were showed that mutations in the insulin receptor gene cause the inherited insulin resistant syndromes, Leprechaunism and Rabson–Mendenhall syndrome [LONGO *et al.*, 1994].

These recessive conditions are characterized by intrauterine and post–natal growth restrictions, dysmorphic features, altered glucose homeostasis, and early demise.

The region of exons 17–21 encodes the tyrosine kinase domain of the receptor, which is necessary for insulin signal transduction.

Mutations in exons 17–21 are very important because they are the causes of insulin resistance and hyperinsulinemia [SINO *et al.*, 1990; HOFFMAN *et al.*, 1997; WARD *et al.*, 2007].

Researchers had shown that SNPs in exon 17 to 21 had a relationship with INSR β subunit gene in women with PCOS. Talbot and collab. [TALBOT *et al.* 1996] using Southern blot analysis showed that a major mutation in INSR contributed with PCOS.

Other authors [SIEGEL *et al.*, 2002; DIAO *et al.* 2004, LEE *et al.*, 2006], had reported that polymorphism in INSR induces mild changes in INSR function which may contribute to the development of PCOS.

PCOS in women were observed almost between 5–10 % of them when they reached to the age of puberty.

The insulin receptor itself may be a susceptibility gene for PCOS [TALBOT *et al.*, 1996; TUCCI *et al.*, 2001; WARD *et al.*, 2007; DUNAIF, 2012; KNOCHENHAUER *et al.*, 1998; SAN MILAN *et al.*, 2004].

Insulin receptor gene has twenty two exons and twenty one introns that are located on chromosome 19.

Domains of receptor tyrosine kinase on exons 17 to 21 are encoded to convey the message (signal transduction), which is necessary for function of insulin hormone.

Mutations in exon 17 cause insulin resistance and lead to hyperinsulinemy [XITA *et al.*, 2003; WEEDON *et al.*, 2003; LEE *et al.*, 2006].

In this research we studied the exon 17of INSR gene in diabetic patients and control groups, and based on variations among them, wwe tried to find a number of SNPs in diabetic patients that could be the cause of diabetic diseases in an Iranian population.

Material and methods

Sample collection

Sixty samples of diabetic patients and control groups were collected from an Iranian population (age 32.34 ±3.1 years old with a BMI of 26.23 ±3.32 for patients and 31.56±2.41 KG/m^2 mean ± SD for control group, approximately) recruited from endocrinology clinics in north of Iran.

(The BMI was calculated by dividing the weight in kilograms by squared height in meters).

DNA extraction

DNA was extracted with phenol–chloroform method using proteinase K according to the protocol of Sambrook [SAMBROOK *et al.*, 1998; BUTNARIU *et al.*, *2016*].

Genomic DNA was isolated in both groups (diabetic patients and control group) and used to determine the functions of exon 17 in INSR gene.

For all groups we designed one pair of primers as follows:

INSR exon 17 Forward

Tgtaaaacgacggccagtccaaggatgctgtgtagataag

INSR exon 17 Reverse

caggaaacagctatgacctcaggaaagccagccccatgtc

These primers were applied in a PCR program and the reaction for getting a sharp band on the gel electrophoresis was as follows:

PCR amplification for Human insulin receptor gene – exon 17:

Template DNA (100ng/µL)	3.0 µL
Forward primer (10pmol/ µL)	0.5 µL
Reverse primer (10pmol/ µL)	0.5 µL
dNTP mix (2.5mM each)	1.0 µL
10X buffer	2.5 µL
$MgCl_2$	2.5 µL
Taq enzyme (3U/ µL)	0.3 µL
Water	15 µL
Total Reaction volume	25.0 µL

PCR Cycle condition:

94°C	95°C	55.5°C	72°C	72°C
5 min	30 sec	40 sec	30sec	10 min
	40 cycles			

PCR products were run on 1.5 percent gel electrophoresis to determine the quantity and quality of PCR products. PCR products were purified by High Pure PCR Product purification kit (cat no. 234.32, Cinagene Company).

Purified samples of suspected cases of diabetes and control samples were sent for sequencing (It should be noted suspicious samples of these patients were selected on the basis of a questionnaire).

Results and discussion

The PCR products were separated by electrophoresis in a TBE agarose gel containing ethidium bromide using standard protocols.

The desired PCR product band was visualized in a medium or long wavelength (e.g., ≥300 nm) UV light, and excised quickly to minimize the exposure of DNA to UV light.

The PCR product was transferred to a 1.5 ml micro centrifuge tube for sequencing.

Only samples with good concentration were selected and subjected to sequencing.

In Figure 1, sequences of exon 17 INSR gene in control group with accession numbers LC055418.1, LC055420 and LC055422.1 were analyzed by DNAMAN program.

According to our result, except for the first 24 nucleotides, there was high homology between sequences (100 %).

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Banat's Journal of Biotechnology
2016, VII(13)

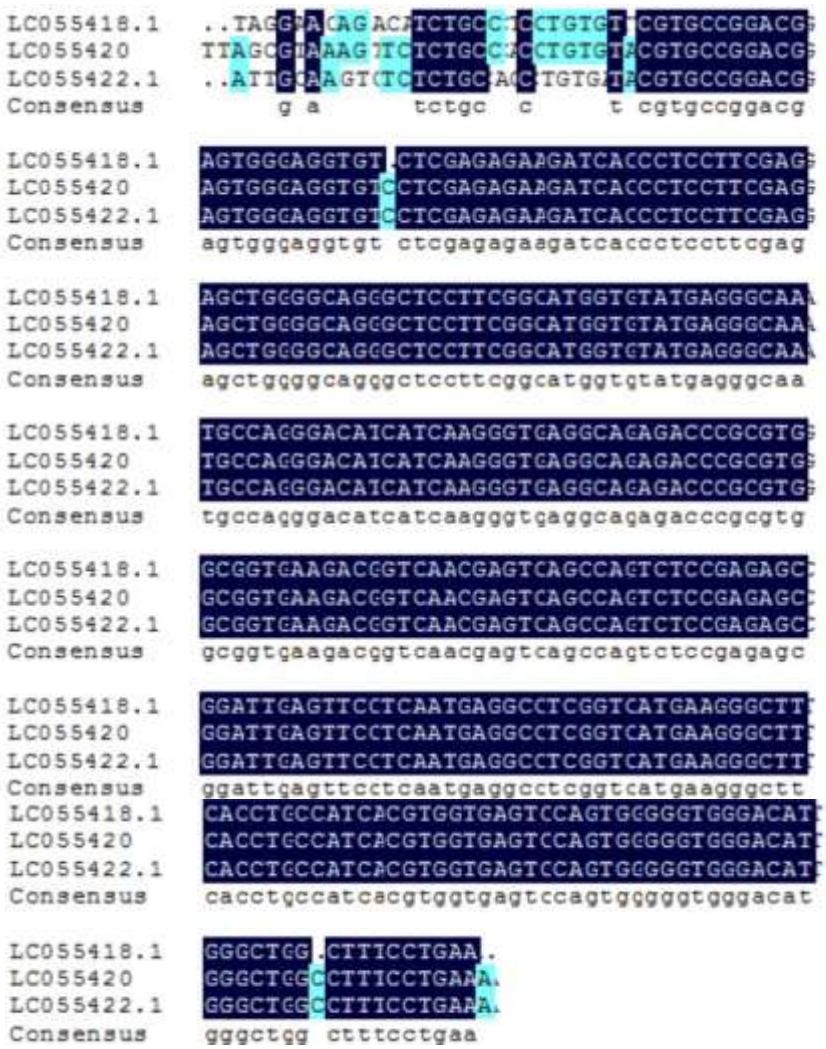

Figure 1: Results between the sequences of exon 17 in INSR gene in control group accession nos. LC055418.1, LC055420 and LC055422.1 by DNAMAN program. According to our results, except for thefirst 24 nucleotides, there was high homology between sequences (100 %).

In Figure 2 sequences of exon 17 in INSR gene were compared by DNAMAN program in diabetic patients with accession numbers of:

LC055416,
LC055417,
LC055419,
LC055421,
LC055423,
LC055424,
LC055425,
LC055426,
LC055496,
LC055497,
LC055498.

Based on our results, except for first 21 nucleotides and the last 12 nucleotides at the end of exon 17 INSR gene, other region of exon 17 has high homology between them.

To understand the variety and number of SNPs in both our control group and diabetic patients, results were compared by DNAMAN program (Figure 3).

According to our results, we observed ten SNPs from nucleotide of 23 of exon 17 between all sequences in this study.

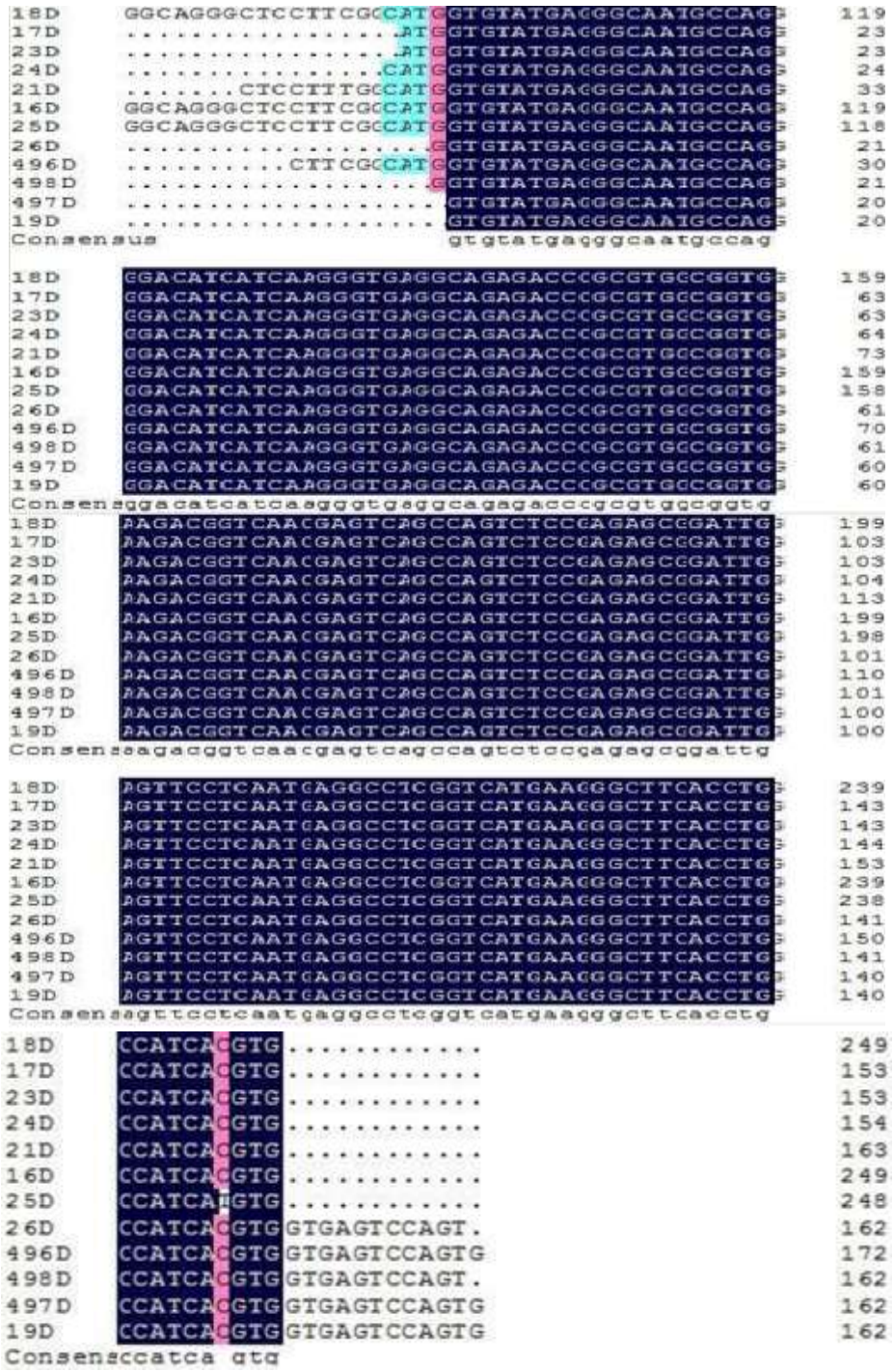

Figure 2: Results of the homology between the sequences of exon 17 in INSR gene in diabetic patients, accession nos. LC055416, LC055417, LC055419, LC055421, LC055423, LC055424, LC055425, LC055426, LC055496, LC055497, and LC055498 by DNAMAN program (D means diabetes and numbers are the end of the accession numbers). According to our results except for the first 21 and the last 12 nucleotides at the end of sequences, other regions of exon 17 has high sequence homology between them.

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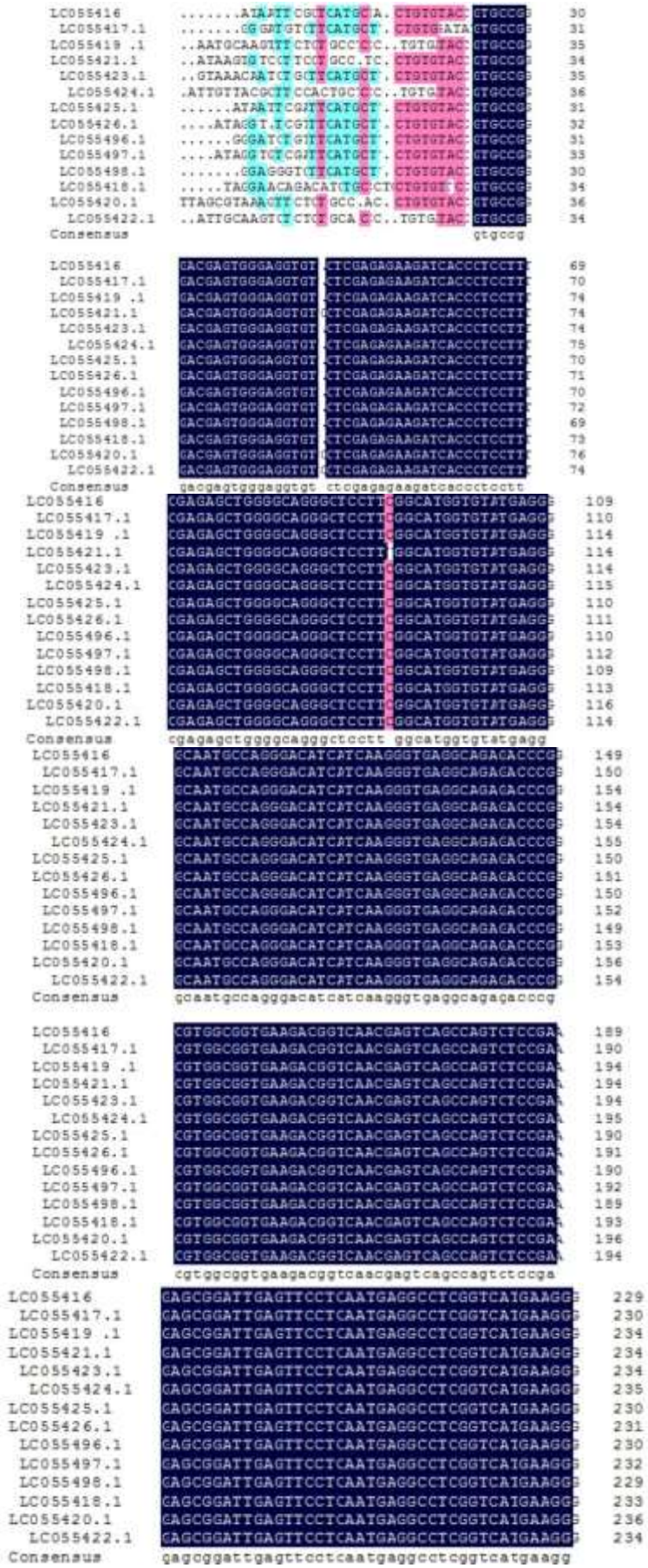

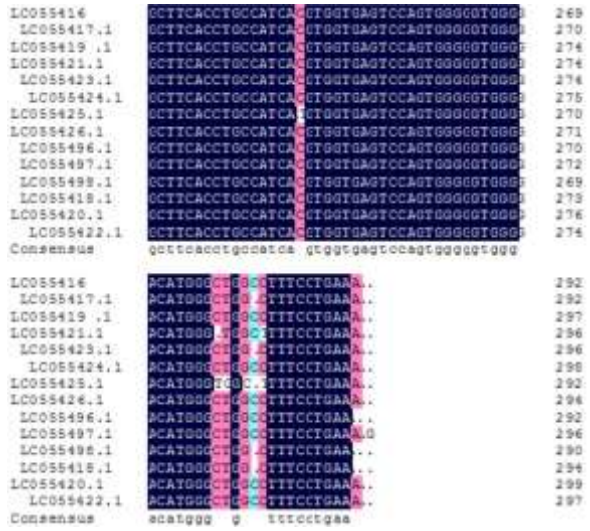

Figure 3: Results of comparison of exon 17 of INSR gene in control and diabetic groups, accession nos. LC055418.1, LC055420 and LC055422.1 (Control's group), LC055416, LC055417, LC055419, LC055421, LC055423, LC055424, LC055425, LC055426, LC055496, LC055497, and LC055498 (diabetic's group) by DNAMAN program. According to our results we observed ten SNPs from nucleotide 23 of exon 17 in all sequences in this study.

In Figure 4 we also compared a sequence of diabetic patients, accession no. LC055421, with INSR gene accession no. NG_008852.1.

Results only showed one SNP in position 16 of exon 17 in INSR gene.

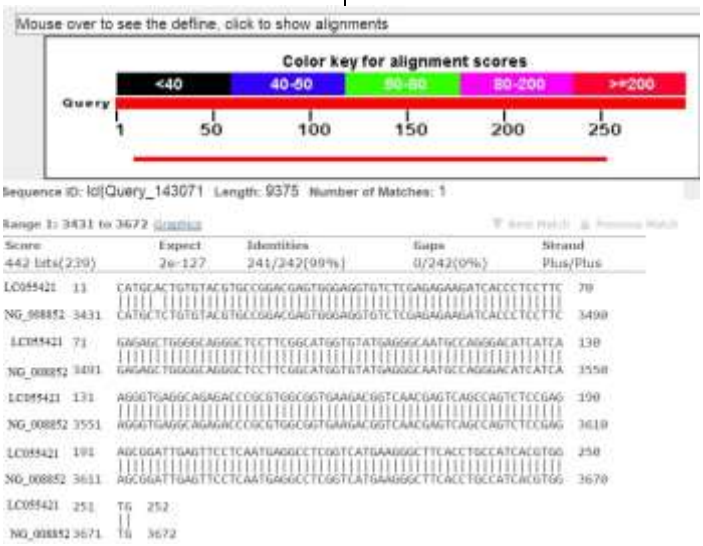

Figure 4. Comparison of sequences with accession no. LC055421 and NG_008852. Results showed that there were a SNP in position 16 of exon 17 in INSR gene.

In this study we investigated single–nucleotide polymorphisms (SNPs) in exon 17 of insulin receptor (INSR) gene in patients with insulin resistance compared to a control group in an Iranian population. Thirty Iranian female patients with diabetics and thirty healthy Iranian women as controls were recruited.

All sixty samples were sequenced but just fourteen samples randomly selected for depositing in Genbank.

Our results showed a significant homology between diabetics and control group in exon 17 of INSR gene when compared by DNAMAN program.

SNPs analysis of exon 17 of INSR gene

This study describes the detection of single nucleotide polymorphisms in exon 17 of INSR gene between diabetic and control groups. Jin and collab. [JIN *et al.*, 2006] have reported a novel T–to–C

substitution at codon Cys1008 (position 3128 in the nucleotide sequence of NM_000208 of INSR). The novel SNP is within exon 17 of INSR, exactly at ATP binding site of the tyrosine kinase domain of INSR β–subunit, resulting in a missense mutation from cysteine to arginine. In current research we studied alignment of exon 17 in INSR gene accession numbers LC055420.1 and NM_000208 to find SNPs between both sequences. According to our results, we found SNPs in position of 3436 for NM_000208 and position of 16 for INSR gene accession no. LC055421.1 (Figure 4). This is an interesting result since Siegel and collab. found a SNP in position of 3128 for exon 17 of INSR gene that is different from our results. On other hand, studies have shown that absence of silent C/T SNP at codon His 1058 has a significant association with PCOS [SIEGEL *et al.,* 2002; WOOD *et al.,* 2003]. To understand the situation of variation between cases and control group, first we separated the subjects into two groups (healthy and diabetics groups). Figure 1 presents healthy group, and as we can see, except for the first 18 nucleotides, three SNPs were observed between the subjects of this group. Here we wanted to know the amount of variation between healthy group subjects collected from different regions of Iran. In Figure 2, exon 17 of INSR in diabetics group were compared by DNAMAN program; the results showed that except for the first 20 nucleotides and the last 12 nucleotides, there is only one SNP in this exon. Variations exist between diabetic group subject may be dependent of geographical regions in Iran. Finally, to understanding the number of nucleotide mutations, we compared both groups (cases and control group).

According to Figure 3, we observed SNPs in different regions of exon 17 in INSR gene. These results showed that in position 55 related to accession no. LC055420.1, LC055421.1 and LC055422.1, there is a single mutation in the form of addition of a C nucleotide, while in other sequences that were studied this nucleotide was absent.

Also we found SNPs in other position of exon 17 in INSR gene that were different between control and diabetic groups. This was a very interesting result because in no other research, like Siegel and Jin [SIEGEL et al., 2002, JIN *et al.,* 2006], such mutations in the same position have been reported. Our results were confirmed NCBI Network system (Figure 4). According to Figure 4, we observed a single mutation in position 11 of exon 17 INSR gene (accession no. LC055421 and NG_008852). Mutations of INSR gene have been reported by other authors [ATIOMO *et al.* 2009; YAMAMOTO *et al.,* 1990, LEGRO *et al.,* 1999, 2004]. They reported that mutation in the insulin receptor can cause a disease with a dominant pattern of inheritance as well. On the other hand, insulin resistance was associated with non–insulin–dependent diabetes mellitus (NIDDM) [MARCOVECCHIO *et al.,* 2005] and may be a central feature of a group of atherogenic metabolic variables sometimes referred to as syndrome X [CHITTENDEN *et al.,* 2009; FARQUHAR *et al.,* 2009; AZIZ, 2002; ODAWARA *et al.,* 1989].

The role of inherited defects of the insulin receptor in these conditions is unknown. Simultaneously, mutations in INSR have been linked to diabetic diseases. Moreover, we found out that the position of single nucleotide mutation between our Iranian population and other populations, such as Chinese population [JIN *et al.,* 2006; BUTU *et al.,* 2015], is different, which may be related to regional variables.

Conclusions

In this research we studied the sequences of INSR gene from Iranian populations. Results showed a variation between control group and diabetic populations. Particularly variations were observed at the beginning and the end of exons. Finally in our next projects we aim to study other exons of INSR for getting a complete picture of the relationship between INSR gene and diabetic diseases.

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