# Polymorphism of Human (*CTLA4*) Gene and Insulin-Dependent Diabetes Mellitus Associated with Obesity in Iraqi Population

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**Abstract:** The cytotoxic T Lymphocyte-Associated Antigen 4 gene (*CTLA4*) encodes the T cell receptor involved in the control of T cell proliferation and mediates T cell apoptosis. The receptor protein is a specific T lymphocyte surface antigen that is detected on cells only after antigen presentation. Thus, CTLA4 is directly involved in both immune and autoimmune responses and may be involved in the pathogenesis of multiple T cell-mediated autoimmune disorders. In this research the work was to investigate whether this gene conferred susceptibility to IDDM in a Russian population. The researchers carried out family studies using two polymorphic markers at the *CTLA4* gene locus: codon 17 dimorphism and the dinucleotide microsatellite in the 3' untranslated region. The Ala17 allele of the *CTLA4* gene was preferentially transmitted from parents to diabetic offspring (p<0.0001) as shown by the combined Transmission/Disequlibrium Test (TDT) and Sib TDT (S-TDT) analysis.

**Key words:** Polymorphism, (*CTLA4*) gene, diabetes mellitus, transmission, diabetic, Iraq

## INTRODUCTION

The cytotoxic T Lymphocyte-Associated Antigen 4 gene (*CTLA4*) encodes the T cell receptor involved in the control of T cell proliferation and mediates T cell apoptosis (Morran, 1997). The receptor protein is a specific T lymphocyte surface antigen that is detected on cells only after antigen presentation. Thus, CTLA4 is directly involved in both immune and autoimmune responses and may be involved in the pathogenesis of multiple T cell-mediated autoimmune disorders.

The human *CTLA4* gene was mapped to chromosome 2q33 (Saleh *et al.*, 2008). It consists of three exons. The first encodes a V-like domain of 116 amino acids. An A to G substitution at nucleotide 49 in exon 1 results in an amino acid substitution (Thr/Ala) in the leader peptide of the protein (Dejkhamron *et al.*, 2007). The Ala allele has been shown to predispose the individual carrying it to the development of various immune diseases including Graves disease (Mei *et al.*, 2007), Hashimoto's thyroiditis (Oliver and Silman, 2009), Addison's disease (Oliver and Silman, 2009), rheumatoid arthritis (Becker, 2004; Boright, 2008), celiac disease (Bergholdt, 2009; Vidal *et al.*, 2008) and others (Abraham *et al.*, 2008). The Thr17Ala dimorphism and the polymorphic (AT) n microsatellite starting at position 642 of the 3' untranslated region flanking exon 4 of the *CTLA4* gene and located 5.3 kb 3' of the biallelic codon 17 were shown to be linked to and associated with Insulin-Dependent Diabetes Mellitus (IDDM) in Italian diabetic families (Van Den Berg *et al.*,

2007). Van Den Berg *et al.* (2007) led to refer to the susceptibility to diabetes associated with CTLA4 as IDDM12. Further evidence for an IDDM susceptibility locus on chromosome 2 in 2q33, the region containing the *CTLA4* gene has been provided from various ethnic groups including Caucasians (British, Spanish, French, Swedish and Belgian), Mexican-Americans and Asians (Korean, Chinese and Japanese) (Doo and Kim, 2010; Trayhurn, 2011). In contrast, a lack of association of the *CTLA4* gene with IDDM was observed in other Caucasian populations including Sardinians (Mangge *et al.*, 2011), Germans (Bell *et al.*, 2005), Danish (Fawcett and Barroso, 2010; Lee, 2009) and U.S. whites (Ohman, 2001; Langberg, 2010). The researchers therefore, decided to investigate whether this gene conferred susceptibility to IDDM in a Russian population. The researchers carried out family studies using two polymorphic markers at the *CTLA4* gene locus: codon 17 dimorphism and the dinucleotide microsatellite in the 3' untranslated region.

## MATERIALS AND METHODS

**Subjects and DNA extraction:** Whole blood samples was obtained from 20 Iraqi patients affected by IDDM (10 male and 10 female, age ranged 4-25 years) and also obtained from healthy men were used to determine the PCR specificity and sensitivity. In each case, 4 mL of whole blood was collected into an EDTA-tube; the samples were stored at -20°C until further processing. DNA was extracted from the samples by wizard genomic (DNA

purification kit, Promega) according to the isolating genomic DNA from whole blood protocol. DNA extracted from 300 μL whole blood in each case. The volume of the extracted DNA solution was usually 100 μL were stored at -20°C.

**PCR amplification and genotyping:** A 152 bp fragment containing the +49 A/G polymorphism in exon 1 of CTLA4 was amplified using a forward primer (CTLA4-5: 5'-AAGG CTCAGCTGAACCTGGT-3') and a reverse primer (CTLA4-4: 5'-CTGCTGAAACAAATGAAACCC-3'). The forward primer was designed with a single base mismatch for the last nucleotide which corresponds to the +47 position to introduce a base change in the sequence of the PCR product according to (Morran, 1997) (Primers set supplied by alpha DNA Company).

The PCR amplification was performed in a total volume of 25 μL containing 5 μL DNA (conc. 20 ng), 12.5 μL Go Taq green master mix 2X (green maschuiter mix is a premixed ready to use solution containing Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA template by PCR supplied by promega (Promega corporation, USA). About 1 μL of each primer (50 pmol/1 μL) and up to 25 μL with nucleases free water.

The thermal cycling was as follows: denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 57°C for 1 min and 72°C for 1 min with final incubation at 72°C for 7 min using a thermal cycler (Gene Amp, PCR system 9700; Applied Biosystem).

The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after ethidium bromide staining. The substitution creates a BstEII restriction site in the A allele and this will be confirmed by incubated the PCR Amplified products (12 μL) with 5 U of BstEII (supplied by Promega corporation, USA) per reaction at 60°C over night. Digested products were electrophoresis on a 3.0% agarose gel. The digested A allele yields a fragment of 130 bp and the G allele yields an intact 152 bp fragment.

**RESULTS AND DISCUSSION**

The Ala17 allele of the *CTLA4* gene was preferentially transmitted from parents to diabetic offspring (p<0.0001) as shown by the combined Transmission/Disequilibrium Test (TDT) and Sib TDT (S-TDT) analysis. A significant difference between diabetic and non-diabetic offspring was also observed for the transmission of alleles 17, 20 and 26 of the dinucleotide microsatellite. Allele 17 was transmitted significantly more frequently to affected offspring than to other children (p = 0.0112) whereas alleles 20 and 26 were transmitted preferentially to non-diabetic sibs (p = 0.045 and 0.00068, respectively). A nonrandom excess of the Ala17 CTLA4 molecular variant (Maximum Logarithm of odds Score (MLS) of 3.26) and allele 17 of the dinucleotide marker (MLS = 3.14) was observed in IBD-affected sibling pairs. Figure 1 shows PCR product of (*FTO*) gene electrophoresis on agarose gel (2%), 45 min/70 V and the reaction was carried out as
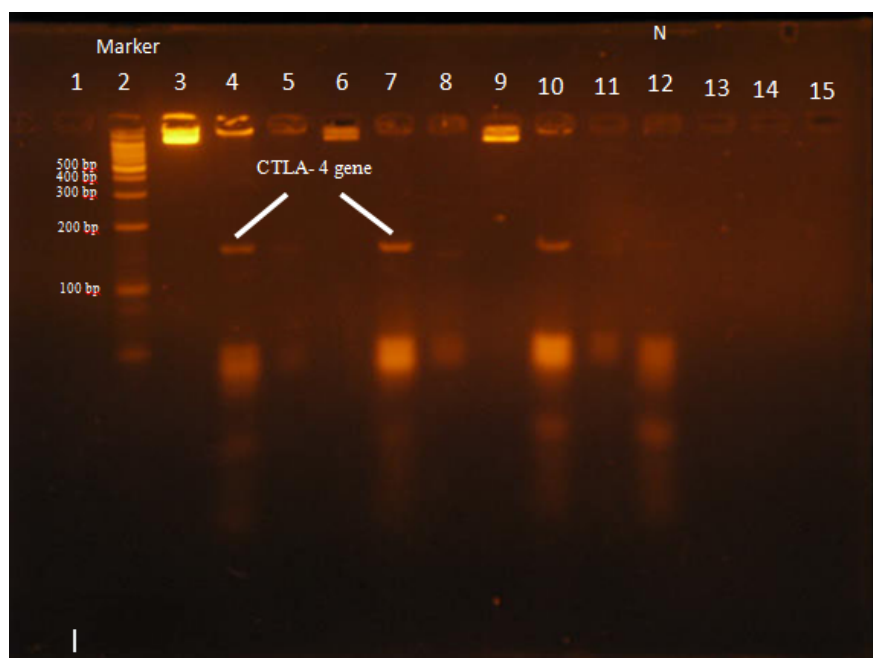


Fig. 1: PCR product electrophresed on agarose 2% 70 V/45 min

Table 1: PCR with FTO primes

| Step | Temperature (°C) | Time | No. of cycles |
|------|------------------|------|---------------|
| Denaturation 1 first loop | 94 | 5 min | 1 |
| Denaturation 2 | 94 | 30 sec | 30 |
| Annealing | 57 | 1 min | - |
| Extension 1 | 72 | 1 min | - |
| Extension 2 | 72 | 7 min | 1 |

shown in Table 1. From Fig. 1 Line 1 DNA marker 100 bp; Line 3, 6, 9 : PCR product; Line 10, 11: negative result Product 152 bp.

## CONCLUSION

A significant difference between diabetic and non-diabetic offspring was also observed for the transmission of alleles 17, 20 and 26 of the dinucleotide microsatellite. Allele 17 was transmitted significantly more frequently to affected offspring than to other children (p = 0.0112) whereas alleles 20 and 26 were transmitted preferentially to non-diabetic sibs (p = 0.045 and 0.00068, respectively).

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