

بسم الله الرحمن الرحيم





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جامعة عين شمس

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**A Genotyping and Posttranscriptional
regulatory Genetic Study Of The Cytotoxic T-
Lymphocyte Antigen-4 (*CTLA4*) Gene
Associated With Type 1 Diabetes Disease In
EGYPT**

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A Genotyping and Posttranscriptional Regulatory Mechanism study of The Cytotoxic T-Lymphocyte Antigen-4 (*CTLA4*) Gene Associated with Type 1 Diabetes Disease in EGYPT

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Summary

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Summary: Type 1 diabetes (T1D) is an organ-specific autoimmune disease characterized by T cell-mediated destruction of pancreatic islets. T cell proliferation is negatively regulated by cytotoxic lymphocyte antigen-4 (CTLA-4). CTLA-4 polymorphisms are associated with T1D in some but not all populations. We investigated the prevalence of the selected single nucleotide polymorphisms (SNPs) in the entire CTLA-4 gene sequence and di-nucleotide repeats polymorphism (AT)_n of 3'UTR region in the *CTLA4* gene with the 396 Egyptian patients ≤ 14 years old and 396 control subjects >24 years old, with a similar ratio of males to females in both groups. Genotyping study was performed using single strand conformation polymorphism (SSCP), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), Genescan, DNA sequencing techniques and haplotype analysis on the selected SNPs was performed. Moreover, we investigated the role of the (AT)_n of 3' UTR on CTLA4 expression, mRNA stability and protein expression via Real time PCR , Western blotting and Luciferase reporter gene assays. The final goal was to contribute to the understanding of the mechanisms that negatively regulate T cell activation by CTLA4 gene and contribute to the development of T1D disease among Egyptian population.

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ملخص الرسالة باللغة العربية

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عنوان الرسالة: التباين الوراثية وميكانيزم ما بعد النسخ للحمض الريبوزي النووي لجين (سؤال أيه 4) لمصاحب لمرض السكر النوع الاول في مصر.

ملخص الرسالة:

يعد مرض السكر النوع الأول مرضاً عضوياً مناعياً مزمناً نتيجة مهاجمة خلايا "تي" المناعية للبنكرياس في الجسم. ولقد وجد أن جين (سى تي ال أيه 4) هو المتحكم سلبياً في عمل خلايا "تي" المناعية عن طريق تثبيطها بعد أداء مهمتها المناوئة. أجريت العديد من الدراسات لهذا الجين على الشعوب والاعراق المختلفة المصابة بالمرض فوجد أن الطفرات الوراثية لهذا الجين تكون سبب لحدوث المرض ومصاحبة لشيوع مرض السكر النوع الأول، بينما هناك العديد من الدراسات على نفس النسق من شعوب واعراق مختلفة تناقض الامر وتنفي حدوثه نهائياً كإجراء دراسة على هذا الجين في الشعب المصري عن طريق:- أولاً دراسة معدل ذبوع انتشار بعض الطفرات الجينية الاحادية المختارة بعناية على كامل التتابع الجيني للجين مع الطفرة ثنائية التتابع المتكررة في منطقة 36 غير منسوخة جينياً من الحمض النووي على فئة من المرضى المصابين بالمرض من المصريين أعمارهم 14 عاماً في مقابل فئة من الاصحاء المصريين اعمارهم 24 عاماً بنفس معدلات المقارنة الاحصائية للجنس بحيث يتساوى عدد الذكور والاناث (1.1) استخدم تقنيات الوراثة مثل الرحلان الكهربى للتغير الفراغى للشق الوراثى أحادى التركيب وطريقة القطع بالإنزيمات محددة القواطع الجينية والماسح الجينى لتكرارات التتابع الجينى والمقياس الرباعى للتتابع الجينى للحمض النووى منزوع الأوكسجين مع الطرق الاحصائية لبيان مدى ارتباط الطفرات الجينية. ثانياً لقد تم دراسة تأثير منطقة التتابع الثنائى المتكرر في منطقة 36 غير منسوخة من الجين على كل من التعبير الجينى والبروتينى ومدى ثبات الحمض الريبوزى النووى المستسخ من الجين محل الدراسة باستخدام تقنيات متطورة مثل التفاعل البوليمريزى التسلسلى ذو القياس اللحظى ، وطريقة نموذج نقط ويسترن للبروتين، إختبار الجين المعيارى الضوئى اللوسيفيريزى. ولقد ساعدت نتائج البحث على فهم وتفسير ميكانزمات التحكم في خلايا "تي" المناعية بواسطة الجين محور الدراسة في كيفية حدوث مرض السكر النوع الأول في الشعب المصرى..

ABBREVIATIONS

AITD: Autoimmune Thyroid Disease
AH: Autoimmune Hypothyroidism
APC: Antigen Presenting Cell
ARE: AU-rich element
ASPs: Affected Sibling Pairs
BAC: Bacterial Artificial Chromosome
CHIP: Chromatin Immunoprecipitation
CMV: Cytomegalovirus
CTLA4: Cytotoxic T-lymphocyte antigen-4
DC: Dendritic Cell
EMSA: Electrophoretic Mobility Shift Assay
ER: Endoplasmic Reticulum
ERK: Extra-cellular-signal-regulated kinase
EST: Expressed Sequence Tag
FBAT: Family-based association test
FP: Fluorescence Polarization
GD: Graves' disease
HBAT: haplotype-Based association test
HLA: Human Leukocyte Antigen
HMG: High-Mobility Group
HtSNPs: Haplotype Tag SNPs
ICOS: Inducible Co-stimulator
IFN- γ : Interferon-gamma
Ig: Immunoglobulin
IL-2: Interleukin-2
INS: Insulin
LD: Linkage Disequilibrium
LEF1: Lymphocyte Enhancer Factor-1
MHC: Multi-HistoCompatibility
MS: Multiple Sclerosis
NOD: Non-Obese Diabetic
PBMCs': Peripheral Blood Mononuclear cells
PCR: Polymerase Chain Reaction
PFA: Paraformaldehyde
PHA: Phytohemagglutinin
PMA: Phorbol Myristate Acetate
RT: Reverse Transcriptase
rRNA:ribosomal RNA
SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM: Standard Error of the Mean
SLE: Systemic Lupus Erythematosus
SNP: Single nucleotide polymorphism
SNuPe: Single Nucleotide Primer Extension
SP: Signal Peptide
SPP: Signal peptide peptidase
SRP: Signal recognition particle
T1D: Type 1 Diabetes
T2D:Type 2 Diabetes
TCF1: T-cell Factor
TCR: T-cell receptor
TDT: Transmission Disequilibrium Test
TNF-a: Tumor Necrosis factor-alpha
TNF: Tumor Necrosis Factor
Treg's: Regulatory T cells,
VNTR: Variable Number of Tandem Repeats
18S rRNA:18 Svedbergs ribosomal RNA subunit

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