Study of cytotoxic- T lymphocyte antigen 4 gene polymorphisms in immune thrombocytopenic purpura

Thesis
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By
Heba Mostafa Ahmed Mohammed
(M.B., B.Ch)

Supervised by

Prof. Dr Shahira Amen Zayed
Professor of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Prof.Dr Somaya Mohammad ELgawhary
Professor of Clinical and Chemical Pathology
Faculty of Medicine, Fayoum University

Dr. Hanan AL-Husseini Mohamed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University
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List Of Abbreviations

- aCL Anti cardiolipin
- AIHA Autoimmune haemolytic anaemia
- Ala Alanine
- ANA Anti nuclear antibody
- APCs Antigen presenting cells
- CBC Complete blood count
- CD Cluster of differentiation
- CI Confidence interval
- CMV Cytomegalovirus
- CO Carbon monoxide
- CT Computed tomography
- CTLA-4 Cytotoxic T lymphocyte antigen -4
- CX3CR1 CX3chemokine receptor 1
- DCs Dendritic cells
- dsDNA Double stranded DNA
- EDTA Ethylene diaminetetraacetic acid
- Foxp3 Forkhead family transcription factor p3
- GD Graves' disease
- GITR Glucocorticoids induced tumor necrosis receptor

- GM-CSF Granulocyte macrophage colony stimulating factor
- GO Graves ophthalmology
- GP Glycoprotein
- H pylori Helicobacter pylori
- HCV Hepatitis C virus
- HDMP High dose methylprednisolone
- HIV Human immune virus
- HLA Human leucocytic antigen
- HO-1 Hemeoxygenase -1
- HSCT Haematopoietic stem cell transplantation
- ICH Intracranial haemorrage
- IDO Indoleamine dioxygenase
- IFNɣ Interferon gamma
- IgA Immunoglobulin A
- IgG Immunoglobulin G
- IgM Immunoglobulin M
- IL Interleukin
- ITP Immune thrombocytopenic purpura
- IvIg Intravenous immunoglobulin
- KIR Killer cell immunoglobulin like receptor
- LA Lupus anticoagulant

- LEF1 Lymphoid enhancer factor-1
- M-CSF Macrophage colony stimulating factor
- MHC Major histocompatibility complex
- MMR Measles, Mumps, and Rubella
- MRI Magnetic resonance imaging
- MS Multiple sclerosis
- NFAT Nuclear factor of activated T cells
- NK Natural killer
- OR Odds ratio
- PaIgG Platelet associated immunoglobulin G
- PCR Polymerase chain reaction
- PEG-rhMGDF Pegylated recombinant megakaryocyte growth factor
- RA Rheumatoid arthritis
- RBCs Red blood cells
- REs Restriction enzymes
- RFLP Restriction fragment length polymorphism
- rhTPO Recombinant thrombopoietin
- sCTLA-4 Soluble cytotoxic T lymphocyte antigen -4
- SD Standard deviation
- SLE Systemic lupus erytheromatosus
- SNP Single nucleotide polymorphism
- SNPs Single nucleotide polymorphisms

- Ss Systemic sclerosis
- TCR T cell receptor
- Th T helper
- Thr Threonine
- TNF- ά Tumer necrosis factor alpha
- TPO Thrombopoietin
- Treg T regulatory
- UC Ulcerative colitis
- UTR Untranslated region
- UV Ultra violet
- VLA-4 Very late antigen -4
- WBCs White blood cells

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Abstract

Idiopathic thrombocytopenic purpura (ITP) is an acquired autoimmune disease with many immune dysfunctions. Cytotoxic T lymphocyte antigen 4 (CTLA-4) is a T-lymphocyte surface molecule that can down modulate and terminate immune responses. Recently, several studies have confirmed that some polymorphisms of this gene can influence its expression level, therefore speculating that they might be associated with autoimmune diseases. In order to investigate the role of the *CTLA-4* gene in ITP, we investigated A49G polymorphism of the *CTLA-4* gene in 30 ITP patients and 20 healthy controls through polymerase chain reaction (PCR)-restriction fragment length polymorphism. No significant differences were revealed in genotypes and allele distributions between the patients with ITP and the controls.

Keywords: CTLA-4, ITP, T lymphocyte, PCR-RFLP, Polymorphism .

Introduction

Idiopathic thrombocytopenic purpura (ITP) is a bleeding disorder in which the immune system destroys platelets, which are necessary for normal blood clotting.ITP is sometimes called immune thrombocytopenic purpura.ITP occurs when certain immune system cells produce antibodies against platelets .The antibodies attach to the platelets. The spleen destroys the platelets that carry the antibodies. In children, the disease sometimes follows a viral infection. In adults, it is more often a chronic (long-term) disease and can occur after a viral infection, with use of certain drugs, during pregnancy, or as part of an immune disorder.ITP affects women more frequently than men, and is more common in children than adults. The disease affects boys and girls equally(***McMillan , 2007).**

Autoimmune disorders are the result of disturbed immune tolerance to self-antigens. After the presentation of an antigen in conjunction with human leucocyte antigen(HLA) molecules on the surface of an antigen presenting cell to a specific T-cell receptor (TCR), costimulatory signals are required for the T-cell responses. A number of co-stimulatory molecules are present on the T-cell membrane, which may have stimulatory or inhibitory effects on T lymphocytes. The cytotoxic T lymphocyte associated antigen- 4 (CTLA-4), also known as CD152, is expressed on T lymphocytes and inhibits the T-cell responses (**Teft et al, 2006).**

The CTLA-4 gene is located on chromosome 2 (2q33).Many single nucleotide polymorphisms (SNPs) have been identified in the *CTLA-4* gene that contains four exons and three introns. Among them, the −318C > T SNP (rs5742909) located in the promoter region, the 49A > G SNP (rs231775) located in exon 1, and the 6230A > G SNP (CT60, rs3087243) located in 3′-

untranslated region (3′UTR) have attracted more attention **(Chistiakov et al, 2006).**

The A49G polymorphism in exon 1 of the *CTLA-4* gene is especially important because it alters the structure of the CTLA-4 protein by causing Thr17Ala amino acid substitution. It has been suggested that this polymorphism reduces the inhibitory function of CTLA-4. The *CTLA-4* A49G polymorphism was found to be associated with autoimmune diseases such as insulin-dependent diabetes, rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple sclerosis, primary biliary cirrhosis, Hashimoto's thyroiditis, and Graves' disease **(Dallos &Kovacs , 2005).**

Aim of the work

The objective of this study is to examine the genetic association of A49G *CTLA 4* gene single nucleotide polymorphism in patients with idiopathic thrombocytopenic purpura.

Chapter 1
Immune Thrombocytopenic Purpura

Introduction

Immune thrombocytopenic purpura (ITP) is a clinical syndrome in which a decreased number of circulating platelets (thrombocytopenia) manifests as a bleeding tendency, easy bruising (purpura), or extravasation of blood from capillaries into skin and mucous membranes (petechiae) **(* Stasi et al,2008).**

Thrombocytopenia may be subdivided into four grades as follows: grade 1 with a platelet count of 75,000-150,000/μL; grade 2 with a platelet count of 50,000-<75,000/μL; grade 3 with a platelet count of 25,000-<50,000/μL; and grade 4 with a platelet count of <25,000/μL. The causative mechanisms of ITP are varied, making ITP a heterogeneous disorder. Thrombocytopenia may be caused by failure of or reduction in platelet production or by increased platelet destruction **(Sekhon & Roy, 2006).**

Classification of ITP

ITP is classified in to two types according to duration of disease :Acute (less than six months) and chronic (6 months or more) **(George et al, 1996) & (Cines & Blanchette, 2002).**

Acute ITP usually occurs within few weeks after a viral or bacterial infection in approximately two thirds of patients. It may also follow an immunization such as for measles, mumps and rubella (MMR) . The majority of children with acute ITP (70-90%) spontaneously attain complete remission within 12 months after the initial diagnosis **(Di Paola & Buchanan, 2002).**

The remaining 10-30% of children develop chronic ITP. Some children with chronic ITP improve with the passage of additional time past 6 months from diagnosis. Others, especially those who are more markedly thrombocytopenic, may continue to require repeated treatments, frequent

follow-up and modification of their lifestyle. Some children may continue to respond to, but require, ongoing treatment, while others may never have respond well to treatment(s) or have initially responded to a treatment but then subsequently become refractory to it **(Imbach et al, 1995)**.

The term chronic refractory ITP refers to patients with persistent thrombocytopenia, (i.e. platelet counts of 20 000/ μL or less) for more than 6-12 months and at least minor bleeding manifestation , it occurs in 2-10% children presenting with acute ITP . They may (or may not) have failed splenectomy, and generally require further treatment to increase and sustain their platelet count to avoid bleeding complications. Since management of these patients is complicated and challenging, it creates frustrations for the patient, family and physicians **(Impach et al, 1995).**

Thrombocytopenia is classified as primary, also referred to as idiopathic thrombocytopenic purpura, or as secondary to an underlying disorder . Both malignant and non malignant disorders can be associated with ITP. Within the non malignant disorders a great variety of conditions such as infections, immunodeficiency states, drug treatments (e.g. quinidine, heparin) and autoimmune diseases are included **(Cines & Blanchette, 2002).**

Incidence :

The annual incidence of ITP is estimated to be 5 cases per 100,000 children and 2 cases per 100,000 adults **(Fogarity & Segal, 2007)** , but these data are not from large population-based studies. Most cases of acute ITP, particularly in children, are mild and self-limited and may not received medical attention. Therefore, estimated incidences of acute ITP are difficult to determine and are likely to understate the full extent of the disease **(Segal &Powe, 2006).**