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Meta-analysis of CTLA-4 Gene A/G +49 Polymorphism and Susceptibility to Graves' Disease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cytotoxic T - Lymphocyte Antigen-4 (CTLA-4) gene located on 2q33 in human which plays an important role in the down regulation of CD28 interaction with the ligands on the surface of antigen-presenting cells (APCs). CTLA4 molecule is a susceptible gene for the severity of Graves' disease (GD). Recent research studies showed that the association between the CTLA4 exon-1 49A/G single nucleotide polymorphism (SNP) and the developing Graves' disease. So, the present study is planned to perform the meta-analysis to explore the association between the SNP49 and GD susceptibility in human beings to the society. SNP public databases and SNP databases showed that the genetic association of diseases such as obesity, diabetes, osteoporosis, asthma, hypertension, renal failure, heart diseases and thyroidism etc.

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Keywords: Mutation; DNA, hereditary; autoimmune disease; hyperthyroidism.

1. INTRODUCTION

Thyroid disorders are appearing to be the leading major endocrine disorders [1]. "Thyroid dysfunctions is the leading endocrine disorder in India and are most common in worldwide. It is estimated that about 42 million people in India are suffered from thyroid diseases. The common thyroid diseases in India are thyrotoxicosis, goiter, iodine deficiency disorders, hashimoto's thyroiditis, and thyroid cancer. Around 42 million people in India were affected by this thyroid diseases" [2]. Graves' disease (GD) [3] is a hyperthyroidism involving over production and secretion of thyroid hormones. The GD occurs with the development of autoantibodies which are directed against the TSH receptor on the thyroid cells. It mimics the action of TSH during the thyroid hormone production. The autoantibodies stimulate excessive secretion of thyroid hormones when it bounded to the receptor. In the condition of Hashimoto disease (Hypothyroidism) and Graves' disease (Hyperthyroidism), the thyroid gland is found to be become infiltrated with lymphocytes and is partly destroyed. If the thyroid gland is completely destroyed when the condition called myxedema leads to swelling of tissues around the face [4].

"GD is an autoimmune and multiple genetic factorial thyroid disease. The role of human leukocyte antigen (HLA) genes and CTLA-4 molecules are either facilitate or down-regulate the second signal to T-cells provided by the interaction between the two accessory molecules of CD28 and B7" [5]. Hence the study found that T cells and CTLA4 molecules are plays a crucial role in function of thyroid gland.

Single nucleotide polymorphisms (SNPs) are the common type of genetic variation among human genes. SNPs are occurred normally throughout an individual's DNA and almost once in every 1,000 nucleotides on average, there are approximately 4 to 5 million SNPs in genome. SNPs are found in the DNA between genes which acts as a biological markers. It is helping to the scientists to locate the genes that are associated with diseases and to predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins. It is also used to identify and track the inheritance of disease associated genetic variants within families. But, till more researches are needed on SNPs analysis (*Insilico*) in human genes in India. Hence the research is focused on the A/G SNP at position 49 in CTLA-4 gene among human population.

2. METHODOLOGY

2.1 Single Nucleotide Polymorphism (SNP) number search

The sequences used in this study were retrieved from the databases at the NCBI, EBI and SNP. Entrez and gene Ensembl are comprehensive sites and collective resource linking tools providing general information of gene structure, expression, and splice variants encoded proteins, regulatory elements and single nucleotide polymorphisms (SNPs). Online Mendelian Inheritance in Man (OMIM) database is used to establish or investigate disease association of gene of interest. UniProt provides a database for protein information.

The bioinformatics tools used in *Insilico* analysis of CTLA-4 gene A/G polymorphism at position 49 in exon 1 are GenBank, BLAST, CLUSTALW, OMIM, Domain search, SNP database and UniProt.

3. RESULTS AND DISCUSSION

Thyroid hormones levels are increased in Graves' disease called toxic diffuse goiter. The research study found that the disease presents in patients aged from 20–40 years old and male to female ratio is 1:8 with a significant familial propensity.The findings indicated that complex interactions between environmental, genetic and endogenous are involved in pathogenesis of GD. It also remains unclear about the interactions of susceptibility genes contribute to the pathogenesis and clinical severity of the disease.

3.1 Single Nucleotide Polymorphism (SNP) Number Search

CTLA-4 gene function, accession number, protein name, number, taxonomic identifier number, taxonomic lineage, total number amino acid, sequence status, disease condition, binary interaction, variant number, domain and SNP were found in Uniprot and SNP web database (Tables: 1 & 2).

The dry lab analysis shown that SNP in CTLA-4 gene causes the Graves' disease (GD). The present study also demonstrated that a SNP in CTLA 4 gene at position (amino acid) 49, the amino acid change is alanine (A) to threonine (G) nucleotide change and missense mutation is noted.

The multiple sequence alignment of CTLA4 molecule sequence was carried out by CLASTALW (Fig. 2). The evolutionary conservation at the position 49 (A/G) was observed and in which the missense mutation was identified. The results also revealed that A/G at the position 49 particular polymorphism is conserved across the species *Homo sapiens, Pongo abelii, Pan troglodytes, Gorilla gorilla gorilla* and *Hylobates moloch* (Fig. 2).

The A/G polymorphism of CTLA-4 is also found that in many other autoimmune diseases like Addison's disease, cardiovascular diseases, Obesity, Polycystic kidney disease, Polycystic ovarian disease and rheumatoid arthritis. The database analysis shown that CTLA4 49A/G SNP in exon-1 leads to the substitution of Ala with Thr in the signal peptide chain. Then it is reported to cause misprocessing of CTLA-4 molecule in the endoplasmic reticulum resulting in less effective glycosylation and reduced surface expression of CTLA-4 protein. According to Anjos et al. [6] and Takara et al. [7], the current study finding is also proved that the longer repeats of the UTR microsatellite are associated with reduced CTLA-4 inhibitory function [8,9].

"CTLA-4 plays an important role in the initiation and development of Graves' disease (GD) and it was found that CTLA-4 +49A/G polymorphism and genetic susceptibility to GD" [10]. Zhang et al*.* [11] was studied that "whether there is a significant association of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) gene polymorphisms with the susceptibility for Behçet's disease through meta-analysis". In Jiang et al*.* [12]. "Meta-analysis study suggested that the CTLA-4 exon1 +49A/G polymorphism is associated with the relapse risk of GD after ATDs withdrawal in Caucasians, not Asians. It was also compared with the AA genotype, Caucasian patients with GG genotype have 3.264 times risk of relapse. A more aggressive treatment such as radioactive iodine or thyroidectomy or longer time treatment of ATDs should be recommended in Caucasian patients with the GG genotype". Therefore, the study also adopted meta-analysis method to assess the exact correlation between this polymorphism and GD risk.

"Nowadays, the research studies are well-known that T-lymphocyte antigen-4 (CTLA4) is a susceptible gene for Graves' disease (GD)" [13]. The present study is also performed the meta analysis to explored the association between the SNP49 and GD susceptibility in human beings. At presently there are several SNPs are available in the public databases and SNP is found to be widely used in the genetic association studies of various complex diseases such as obesity, diabetes, osteoporosis, asthma, hypertension, kidney failure and thyroidism. The allele frequencies are important in selection of SNPs for studying complex diseases. The cytotoxic T lymphocyte-associated molecules-4 (CTLA-4) gene is related to the relapse of Graves' disease (GD) after anti-thyroid drugs (ATDs) withdrawal. Jiang et al. [12] performed a meta-analysis to generate large-scale evidence on whether the CTLA-4 exon 1+49A/G polymorphism can predict the relapse of GD after ATDs withdrawal. Hence, the suggested that still more SNPs would be analyzed in future research studies in CTLA-4 gene among the human population for the welfare of society health.

Apart from thyroidism, Zheng et al. [14] reported "an association between cytotoxic T-lymphocyte associated antigen 4 gene polymorphism and susceptibility to asthma in different populations [15-18]. They were also performed a meta-analysis of 19 published case–control studies to obtain a reasonably accurate estimation of the relationship between *CTLA4* polymorphism and asthma". Zheng et al*.* [14] meta-analysis results suggested that "the +49A/G polymorphism in CTLA-4 was an important risk factor for asthma susceptibility in Asian populations, children, and atopic patients. So, the current study was investigated and found that the relationships

Table 1. SNPs in CTLA-4 gene

Gene	Domain	Mutation	Amino acid position	Wild type	Mutant	Allelic frequency
CTLA-4	Ig super family	Missense	49	A	G	0.23

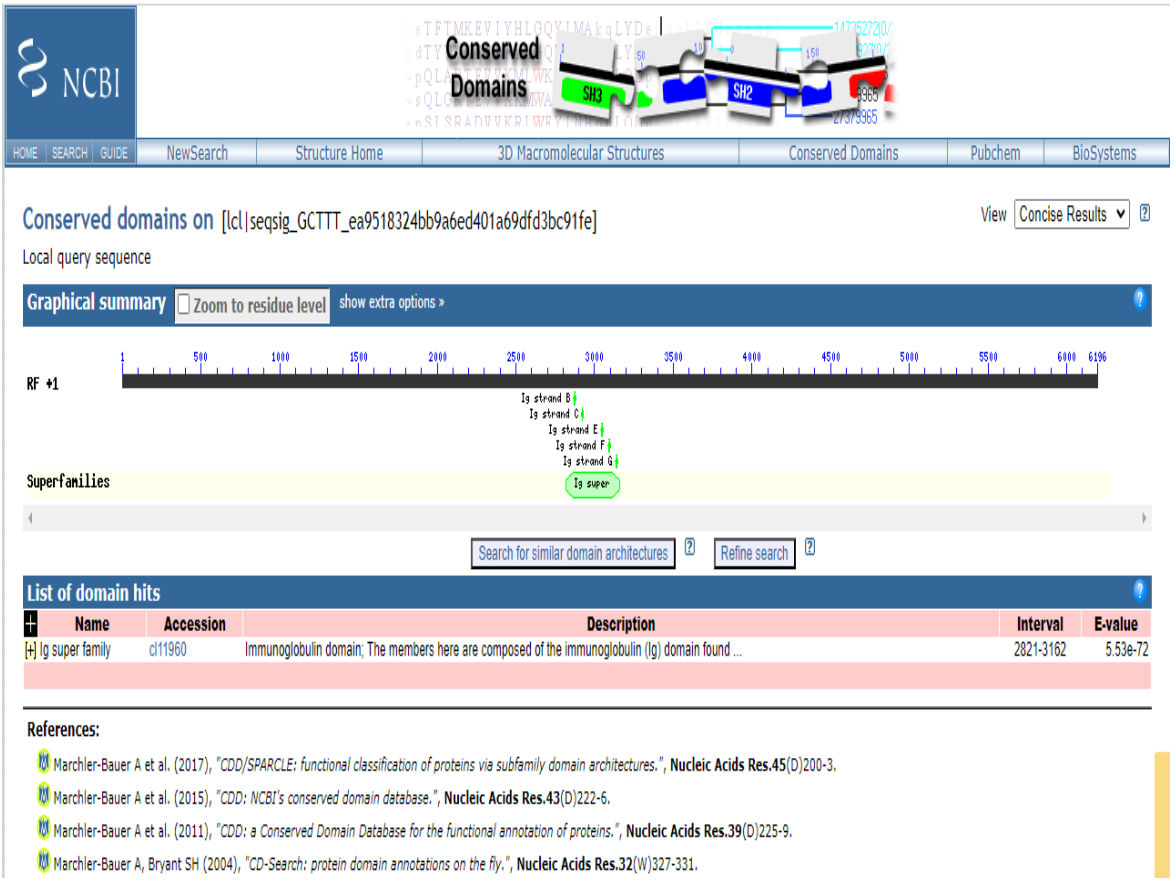

Fig. 1. Domain search

between GD and A/G single nucleotide polymorphisms (SNPs) from CTLA-4. That's why, another detailed study for investigation for relationship between GD and A/G single nucleotide polymorphisms (SNPs) from CTLA-4 will be a further study in future could be carried".

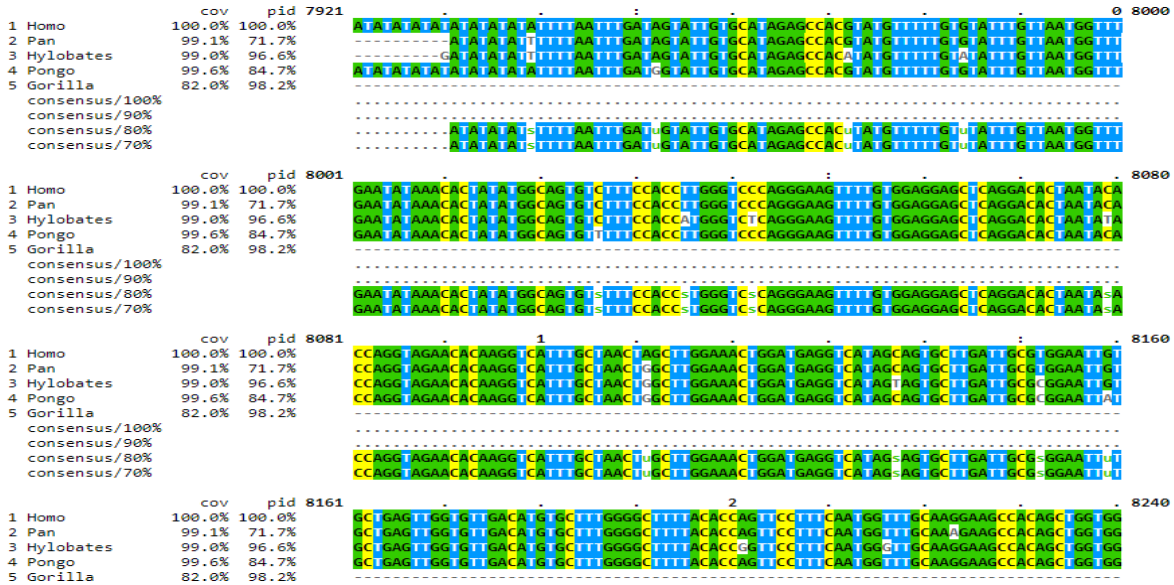

Fig. 2. Multiple sequence alignment

Note: *Highlighted with yellow colour is found to be conserved across the species*

Table 2. Uniprot and SNP web database of CTLA-4 gene

Assign Number	Alleles	Chromosome	Canonical Spdi	Gene	Functional Conseguence	Clinical Significance	Maf
rs231775 *[Homo sapiens]*	A>G,T	2:203867991 (GRCh38) 2:204732714 (GRCh37)	NC_000002.12:203867990:A:G,NC_000002.12:203867990:A:T	CTLA4	missense_variant,codingsequence_variant	benign,risk-factor	G=0.371744/109761 (ALFA) G=0.208333/45 (Qatari) A=0.23913/11 (Siberian)
rs199912925 *[Homo sapiens*	A>G	2:203871485 (GRCh38) 2:204736208 (GRCh37)	NC_000002.12:203871484:A:G	CTLA4	intron_variant,coding_sequence_variant,missensevariant	uncertain-significance	G=0.000025/4 (ALFA) G=0.000013/1 (PAGE_STUDY) G=0.000033/4 (ExAC)

Table 3. CTLA-4 gene sequence based prediction tools

Web Server	Effect
SNP database	Missense
Variant effect predictor	CTLA-4 gne
Uniprot/Swiss-prot	Single nucleotide polymorphism
(DM)2 – Domain Mapping & Disease mutation	Polymorphism
SIFT	Tolerated (Score: 0.08)
PolyPhen	Benign (Score:0.006)
PolyDoms	Nonsynonymous & Synonymous
MuSTAB	Disease

4. CONCLUSION

The research work is found that the relationship between *CTLA-4* polymorphisms (A49G, 1822 C/T and CT60 A/G) and Hashimoto's thyroiditis (HT) and Graves' disease (GD). So, the *insilico* analysis study concluded that C*TLA-4* A49G polymorphism is an important genetic determinant of the risk of HT and GD.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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