Review Article

CTLA4 Gene Variants in Autoimmunity and Cancer: a Comparative Review

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ABSTRACT

Gene association studies are less appealing in cancer compared to autoimmune diseases. Complexity, heterogeneity, variation in histological types, age at onset, short survival, and acute versus chronic conditions are cancer related factors which are different from an organ specific autoimmune disease, such as Grave's disease, on which a large body of multicentre data is accumulated. For years the focus of attention was on diversity and polymorphism of major histocompatibility complex in respect to human diseases specially the autoimmune diseases, but in recent years, access to other human gene sequences prompted investigators to focus on genes encoding the immune regulatory proteins such as the co-stimulatory, adhesion molecules, cytokines and chemokines and their receptors. Among them, CTLA4 (CD152) has been in the centre of attention for its pivotal role in autoimmunity and cancer. Although not fully understood, CTLA4 with no doubt plays an important role in the maintenance of the immune response by its expression on activated and regulatory T cells. CTLA4 (Gene ID:1493, MIM number:123890) has many variants and polymorphic forms, some present in regulatory positions, some in 3' UTR and the most important one in the leader sequence (+49 A/G). As a pivotal regulatory element of the immune responses magnitude, CTLA4 could be considered as a two-blade knife, for which only the optimal expression ensures an effective, but at the same time, safe immune response. It can accordingly be speculated that CTLA4 alleles associated with extraordinary expression could make a person more susceptible to tumor growth and/or progression. On the other hand, alleles associated with a compromised CTLA4 expression/function may accelerate the formation and/or manifestation of inflammatory autoimmune disorder. I hypothesized a spectrum of the functional dichotomy of CTLA4 SNPs diverging from autoimmunity to cancer. To examine these hypotheses, results from previously published investigations on CTLA4 polymorphisms together with the work done by our own group are discussed in details. Because the most published data are about the polymorphism at position +49, I concentrated on this position; however the data regarding other SNPs are also included for comparison. To support the significance of CTLA4 gene variation in these two major human diseases evidences from organ transplantation are also included. As

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will be discussed in the manuscript, our work and reports by others from a normal population perspective support the hypothesis that individuals inheriting a GG genotype at position +49, for which lower CTLA4 expression has been extensively suggested, are more susceptible for developing autoimmune disorders and those with AA genotype, with an existence of a state of self-tolerance, may have a higher chance of developing cancer. CTLA4 SNPs may accordingly be considered as a crucial element, along with other known or yet unknown mechanisms, in keeping the immune balance in predisposed individuals to cancer and autoimmunity. Although an spectrum line can be drawn between autoimmunity and cancer by considering published data regarding CTLA4 +49 polymorphism, the extreme functional dichotomy of this SNP appears to be more complex and difficult to understand, but there is no doubt that the future investigations will resolve most ambiguities.

Keywords: Autoimmunity, Cancer, CTLA4, Immune Balance, Polymorphism

INTRODUCTION

Human *CTLA4* gene (Gene ID:1493,MIM number:123890) is located on chromosome 2 with a nucleotide size of about 6.2 kb consisting of 4 exons. The first exon of *CTLA4* gene, encodes the leader sequence peptide while the second one encodes the extracellular immunoglobulin like domain containing the binding site. Exon 3 and 4 are responsible for producing the transmembrane and cytoplasmic domains, respectively (1). *CTLA4* hnRNA transcript undergoes alternative splicing, resulting into three final mRNA isoformes in human; (a) the surface full-length CTLA4 (flCTLA4) including exons 1, 2, 3 and 4, (b) soluble CTLA4 (sCTLA4) lacking exon 3 (transmembrane domain) and (c) a transcript lacking both exons 2 and 3 (binding as well as transmembrane domains). CTLA4 (CD152) is not expressed on resting T cells but its expression is up-regulated following an antigen challenge or by a mitogen stimulation of naive T cells in-vitro. Expression of CTLA4 persists much longer on memory T cells than on naive activated T cells. In addition to the activated T cells, CTLA4 is also expressed on T regulatory subsets and most of its immuno-regulatory roles reported so far have been originated from the functional and immunological properties of this T cell population. There are several reports confirming the expression of CTLA4 on other cells from hemopoietic and non-hemopoietic lineages (for review see reference 2) and *CTLA4* gene has been detected in all mammals and also in birds (3).

CTLA4 exerts its immunomodulatory effects through competition with CD28 for binding to B7.1 (CD80) and B7.2 (CD86) molecules in an affinity based manner. In addition to its gene sequence homology with CD28, *CTLA4* gene is located in the vicinity of CD28 gene on chromosome 2, separated by only 130 kb sequence (4). Based on sequence homology seen in *CTLA4* and CD28, it has been postulated that these two genes have been evolved as a result of gene duplication (4). The main function of CTLA4 is the negative regulation of T-helper cell effector function by inhibiting proliferation and cytokine synthesis. The best documented evidence for the function of CTLA4 is supported by observations in CTLA4 deficient mice in which massive lymphoprolifrative disorder with lymphocyte infiltration in multiple organs is seen (5). The CTLA4 regulatory function has been suggested to be induced as a result of

interaction between TCR and MHC peptides and transduction of negative signals via the cytoplasmic tail of CTLA4 molecules. Another possibility is the halting of the activating signal induced by CD28 as a result of competition for binding to B7.1 and B7.2 molecules (6).

CTLA4 GENE POLYMORPHISM

The important and fine function of CTLA4 in inducing immunemodulatory and hemostatic signals in the ongoing immune response poised this molecule under evolutionary pressure to go in the process of point mutation and polymorphism within the population and along the species. Two different and opposite scenarios played by regulatory T cells expressing CTLA4 in autoimmune diseases and malignancies, classified the significant position of CTLA4 in safeguarding the immune response in a well balanced self tolerance and protection against invader microorganisms. Such a situation for this molecule is another aspect which makes CTLA4 the target for transcriptional modification resulting in several polymorphisms. Several polymorphisms in CTLA4 gene have been reported at positions -1722, -1661, -318, +49 and in 3, un-translated region (UTR) at position +6230; generally known as CT60. In this review the frequencies of CTLA4 gene variations in the spectrums of two major human disorders namely the autoimmune diseases and cancer are compared.

FUNCTION OF SNPs

As will be discussed later, scientific literature is overloaded with respect to *CTLA4* gene polymorphism and predisposition to autoimmune diseases and to a lesser extent to cancer. A fundamental question not yet fully explained is the role mediated by these SNPs in disease pathogenesis, although one can find a SNP highly associated with a particular disease. There are limited data to describe the functional activity of *CTLA4* SNPs. Polymorphism at position +49A/G is the only polymorphism which changes an amino acid from alanine to threonine in the leader sequence which is later processed in the endoplasmic reticulum (ER), therefore postulating that this may affect the processing of CTLA4 in ER and result in a less efficient glycosylation and reduced expression of membrane CTLA4 protein (7,8). Ueda and co-workers (9) showed that the ratio of sCTLA4 to full-length isoform (flCTLA4) mRNA splice forms in un-stimulated CD4 T cells is 50% lower in +6230 (CT60) GG positive disease-susceptible individuals compared with AA protected individuals postulating a functional differences of CTLA4 protein expression in different individuals harbouring these two genotypes. Regarding +6230 (CT60), Atabani et al. showed that healthy individuals carrying AA genotype for this polymorphism have an increased number of T regulatory cells in their peripheral blood compared with those with GG genotype (10). It is worth mentioning that this polymorphism is in linkage disequilibrium with polymorphism at the leader sequence indicating that individuals with AA homozygote at position +6230 (CT60) almost carry a similar genotype with polymorphism at the leader sequences. Regarding -318 polymorphism at the promoter region, it has been suggested that higher promoter activity and consequently over-expression of membrane associated CTLA4 may be generated as a result of allelic or genotypic variations in this site (11). The functional

significance of two other polymorphisms in *CTLA4* promoter region are less characterized, however information from several studies suggest participation in the binding of cis-acting elements to the promoter (e. g. NF-1 and c/EBPbeta) and/or affecting the gene activity like -318 SNP (12-14). Here the frequency and significance of CTLA4 SNPs will be discussed in the main autoimmune diseases and also in some major solid cancers by focusing on the functional aspects of polymorphism at position +49 of CTLA4 leader sequence.

IMMUNE BALANCE TO PREVENT AUTOIMMUNITY AND CANCER: POTENTIAL APPLICATION OF CTLA4 SNPs

Cancer and autoimmunity represent a wide spectrum of diseases in humans for which the pathogenesis, spreading and progression is largely related to the role of the immune system; the first one is prone to hyper activation and uncontrolled proliferation of the host immune cells, and the second, that is autoimmunity, is in a state of suppression and an increased degree of self-tolerance, providing a favourable safe haven for cancer cells to survive and to expand. The balance and the tuning of the immune system is controlled by a complex of cellular networks and mediators to keep the state of tolerance at on position preventing self-reactivity at the same time to prepare the elements of the immune system defence mechanism ready to interact and overcome any non-self-components. There are several factors and conditions which physiologically influence this fine balance of health and disease, particularly the hormonal changes associated with growth and puberty, and aging that with no reservation has a major drawback on the ability of the immune system to be balanced for self-reactivity and at the same time is competent. In female cases the incidence of most autoimmune diseases is associated with changes in certain physiological circumstances associated with hormonal imbalance. On the other side, incidence of cancer with aging is a well-known epidemiological phenomenon blaming on gradual and chronic declining power of the immune system by age. With this background, the function and the role of key regulatory genes and molecules controlling the balance of the immune system in health and disease is highly significant. Diversity in the immune systems, both in humans and among other species, is fascinating and probably is regarded as the key factor of survival during evolution. The polymorphism and diversity of a regulatory molecule of the immune system such as *CTLA4* is important in this context.

As a pivotal regulatory element of the immune responses magnitude, CTLA4 could be considered as a two-blade knife, for which only the optimal expression ensures an effective, but at the same time, safe immune response. Extraordinary expression of *CTLA4* in a genetically and/or environmentally predispose individual to cancer; could make the person more susceptible to tumor growth and/or progression. From the other hand, a compromised *CTLA4* expression/function in another individual who, for instance, is genetically and/or environmentally prone to an inflammatory autoimmune disorder may accelerate the disease formation and/or manifestation. Considering the functional significance of SNPs in CTLA4 gene expression/function, observing the association of CTLA4 genetic variants in two opposite pathologic situations, like cancer and autoimmunity, could accordingly be expected. Accordingly I looked at the association between CTLA4 variation and these two different conditions to investigate

if CTLA4 SNPs can be considered as a crucial element, along with other know or yet unknown mechanisms, in keeping the immune balance in predisposed individuals to cancer and autoimmunity. Because the most published data are about the polymorphism at position +49, I concentrated on this position; however the data regarding other SNPs are also included for comparison. As already mentioned, +49 G allele are associated with less efficient glycosylation and reduced expression of membrane CTLA4 protein (7,8). Accordingly, individuals inheriting a GG genotype at position +49 is supposed to confer susceptibility to developing an autoimmune disorder and those with AA genotype, with an existence of a state of self-tolerance, may have a higher chance of developing cancer. I hypothesized a spectrum of the functional dichotomy of CTLA4 SNPs at which end one can see autoimmunity and cancer. To support the significance of CTLA4 gene variation in these two major human diseases evidences from organ transplantation are also discussed.

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with an incidence of 3 per 100,000 (15) in female patients. Like most autoimmune diseases, the aetiology of SLE is not known. Various host and environmental factors have been shown to have roles in the pathogenesis of SLE. *CTLA4* gene variations have been investigated in several reports in different ethnic populations resulting in inconsistent findings. Heward et al. (16) reported no association between *CTLA4* and SLE in Caucasian patients. Later, Aguilar et al. (17), Chua et al. (18), Lee et al. (19) and Liu et al. (20) confirmed Howard's findings indicating no association between SLE and *CTLA4* gene polymorphism. However, Ahemed et al. (21) reported an allele G of *CTLA4* polymorphism at the leader sequence to be associated with susceptibility to SLE. Most of these reports investigated the SNP at position +49 of the leader sequence. Later, more reports were published from different ethnic communities to search for an association between *CTLA4* and SLE not only targeting position +49 at the leader peptide but also screening the other SNPs located at the regulatory region and the 3'UTR. This approach and the increasing number of publications on *CTLA4* gene variants and SLE resulted in two meta analyses, one investigated 8 (22) and the other 14 already published papers (23) including seven European, six Asian, and one Mexican study. Both meta-analyses found a significant association between the risk of SLE and the GG genotype of exon-1 at position +49 in the leader peptide. The overall odds ratio for the *CTLA4* exon-1 +49 GG genotype was 1.287 (95% CI=1.058–1.564, p value=0.011). Therefore, this meta analysis indicated results contrary to those of previous sporadic publications with inconsistent results, demonstrating a clearly strong association of *CTLA4* gene at position +49A/G with SLE, particularly in Asian and with a lesser degree in the European population.

RHEUMATOID ARTHRITIS (RA)

Rheumatoid Arthritis (RA) is a chronic and destructive autoimmune inflammatory disease with unknown etiology. Several genetic factors have been investigated in RA patients in different ethnic populations. Among these, class I and II of the human

leukocyte antigens (HLA) have been excessively investigated and it appears that the majority of RA patients are associated with HLA-DR4 antigen. Regulatory role of CTLA4 molecule in homeostasis of self tolerance prompted several laboratories to study the association of the *CTLA4* gene variants in RA patients. Similar to the above mentioned SLE results, these studies are represented as inconsistent findings. The lack of association between *CTLA4* +49A/G and rheumatoid arthritis was reported in two different populations (24, 25). Vaidya et al. (26), however, found a positive association of *CTLA4* genotype with RA in patients with the coexistence of other autoimmune diseases such as autoimmune thyroiditis and diabetes type 1. In a Spanish population, the strongest association between the sequence of *CTLA4* 3'UTR AT(n) repeats and RA was compared with their earlier analysis of the polymorphisms at position -318 and +49 and was reported by Rodriguez et al. (27). A report from a Japanese population indicates an association of G allele at position +49 of *CTLA4* in RA patients and such an association was prominently restricted in those patients carrying HLA-DRB1*0405 (28). A similar line of observation in a Spanish population but with a co-expression of allele G at position +49 of CTL4A leader sequence with HLA-DR3 alleles was reported by Gonzalez-Escribano et al. (29). In summary studies by Seidl et al. (30) indicated the strongest association between *CTLA4* mutation and RA in those patients harbouring HLA-DRB1 susceptibility allele, confirming a genetic link between the polymorphism in complex HLA system with a variation in a non-HLA locus. In addition to these reports and other so far published papers on the association of *CTLA4* gene polymorphism and RA, Lee et al. (31) and Vaidya et al. (26), two Meta analyses published in 2005 also reviewed this issue in a more comprehensive manner. In the Meta analysis of Han et al. (32) 14 published works were collected but after excluding 4 articles, they analysed data from 10 papers. Results of this Meta analysis indicated different patterns of association among European and Asian studies. The overall result of this Meta analysis indicates a significant association of *CTLA4* +49A/G in favour of G allele in the Asian population, in contrast to the increased but not significantly associated results in the European studies performed by Han et al. (32). The second Meta analysis was also performed by Lei et al. (33). In addition to performing a Meta analysis, this group reported the results of four *CTLA4* polymorphisms in Chinese RA patients, and demonstrated a significant association of G allele at position +49 in RA cases. Their Meta analysis included 6 articles published from Europe, 5 from Asian regions and one from Africa targeting only polymorphism at position +49A/G (33).Contrarily to Han et al. results, this Meta analysis indicated that allele G was to a lesser extent associated with RA in European population compared with Asians. However in both Europeans and Asians, RA was associated significantly with *CTLA4* +49 polymorphism in favour of allele G. In other words, in both ethnic populations, *CTLA4* variant may be considered as a susceptibility gene for the development of RA.

AUTOIMMUNE THYROID DISEASE (AITD)

Autoimmune thyroid Disease (AITD) is an organ specific autoimmune disease classified into two entities of Hashimoto thyroiditis with the major pathological sign of thyroid organ destruction and Graves'disease which produces anti-TSHR stimulating autoantibody and results in the enlargement of thyroid gland. Yanagawa et al. (34) and

Yanagawa et al. (35) were the first to report an association between *CTLA4* and Grave's disease. In their report, a series of investigations on the subject appeared from different populations worldwide. The finding of a non HLA gene with predisposition for autoimmune disease was considered as a new era in gene disease association. Apart from the investigation on the polymorphism at position +49, more works were published soon on other *CTLA4* sites in AITD including Hashimoto thyroiditis and Grave's disease. Heward et al. (36) studied *CTLA4* linkage with Grave's disease in a UK population which was later confirmed by other investigators (37-40). It was found that *CTLA4* gene or a closely associated gene (including CD28) confers susceptibility to Graves' disease particularly in female patients with protective HLA specificities (41), a finding which was in line with the data from the UK population irrespective of gender (42). *CTLA4* gene association seems not to be restricted only to Graves' disease or thyroiditis, rather it is common with other autoimmune thyroid diseases in general (43). In Japanese populations, Yanagawa et al. (41) showed that association of *CTLA4* gene conferring susceptibility for GD could be a genetic marker independent of the HLA system. In 2000, it was revealed that in addition to position +49A/G, a promoter polymorphism at position -60C/T confers susceptibility to AITD in a Korean population (44). In addition to the previously published reports regarding association of *CTLA4* polymorphism in the leader sequence with AITD, Kinjo et al. showed that even remission in Japanese AITD is associated with a variation at this position. This suggests that patients carrying G allele should receive a longer treatment than those with an A allele (45) .Our report on the association of *CTLA4* exon 1 in Iranian population was in line with the previously published works indicating that in addition to a significant association of G allele with GD patients, an association was also found with the GG genotype among the patient group (46). By searching through literature only one meta-analysis was found, the one in which our data was also included and consisted a total of 4848 cases with GD, 866 with HT, and 7314 controls for position +49 A/G. The group-level data for +6230 (CT60) included 3047 GD cases, 839 HT cases, and 3741 healthy controls. The overall conclusion of this meta analysis indicates that although there is an increase in the frequency of G allele at position +49A/G in GD patients compared to the control, but statistically significant association has been found at position C/T60 in the 3' un-translated region (47).

DIABETES

Type I diabetes is an organ specific autoimmune disease with a growing incidence worldwide but its aetiology has remained unknown. A list of predisposing factors have been indicated in most published work including genetic factors such as HLA and non-HLA gene systems, environmental factors such as diet and more recently the hygienic hypothesis which points to the importance of the interaction of the host immune system and the microbial challenges during early life for the maintenance and homeostasis of self tolerance and the prevention of autoimmune diseases (48). Among non HLA complex genes investigated in autoimmune diseases, *CTLA4* has been the focus of attention by several investigators particularly by those studying type I diabetes in different ethnic populations. An earlier report on a relatively large sample size of a Caucasian population from Belgium diabetes registry showed an association between *CTLA4* polymorphism in exon 1 G/A with type I diabetes (7,49). Similar approaches in

Japanese population resulted in inconsistent findings. Takara et al. (50) reported association between the *CTLA4* gene polymorphism and younger-onset type 1 diabetes with AITD, a finding which was not confirmed by Yanagawa et al. (34) revealing no association with type-I diabetes and *CTLA4* gene polymorphism at the leader sequence. Similar inconsistent reports were also published by other Japanese centres, which due to limited space are not mentioned here. Two more reports from Caucasian populations published consequently, clearly confirmed the association of *CTLA4* +49A/G and susceptibility to type-I diabetes (51, 52). Lack of association of the *CTLA4* +49 G allele with type 1 diabetes in a French population was reported, but the author hypothesized a *CTLA4* dimorphism and susceptibility to type-I diabetes by maternal HLA-DRB1*03 inheritance (53). Two other reports from two different populations along the Silk Road were also published with inconsistent results. Ma et al. (54) reported a clear association of both G allele and GG genotype conferring susceptibility and AA genotype conferring protection in Chinese population- a finding which was not confirmed in a Turkish population (55). Our own report on a population of Iranians, a population also living along the Silk Road showed that *CTLA4* +49 A/G polymorphism confers genetic susceptibility to type 1 diabetes, particularly in younger individuals (56).The lack of consistency of the association along the Silk Road makes it unlikely that the population admixture affected the distribution of this polymorphism, and the probable susceptibility to diabetes in these related populations. Kavvoura and colleagues' meta analysis is the only available published work, so far, that dissects the association of *CTLA4* gene polymorphism and type-I diabetes in 35 published articles (57). A total of 4,775 cases and 5,829 controls were included for this Meta analysis for the +49 A/G polymorphism. By calculating the odds ratios, it was suggested that a person carrying G allele has a 1.45-fold increase in susceptibility to type I diabetes- a finding that was statistically highly significant ($p<0.001$).

MULTIPLE SCLEROSIS (MS)

Multiple sclerosis (MS) is an autoimmune T cell mediated myelin degenerative disease with a variable age of onset from 30-40 and even in some rare cases over 50 years. Most autoimmune diseases are more common in females than males; a consensus that environmental factors should be regarded as the major etiologic cause. Search for genetic components implicated in the aetiology of MS started a long time ago by focusing on HLA systems (For review see 58). Later, non HLA genes including those encoding cytokines and regulatory T cell co-receptors were also considered. The particular non-HLA genes with potential disease association investigated to date include SNP's at IL-7R Alpha and IL-2R alpha chains (58). Knowing the major role played by T cells in the pathogenesis of MS, attempts to investigate the association of *CTLA4* variants with a putative molecule expressed on T regulatory cells in this autoimmune disease started in several laboratories worldwide. The first report on the association of *CTLA4* and MS was published by Ligers et al. (59) claiming a significant association ($p<0.05$) of homozygosity for the +49 G allele in a relatively large sample size of MS patients. On the contrary, Dyment et al. (60) and also Roxburgh et al. (61) found no evidence for an association or the susceptibility of *CTLA4* polymorphism in exon 1 and MS cases in UK population. Similar to other autoimmune diseases, controversy

observed in these reports triggered other investigators to study MS *CTLA4* gene variants in other ethnic groups including a Japanese population (62), a multi-centre analysis from Germans, Hungarian and Polish populations (63) and a South Australian population (64) Data were collectively presented as the absence of association between *CTLA4* exon 1 gene polymorphism and susceptibility to MS. Even a Meta-analysis reported by Bagos (65) did not present a clear evidence for this association. Our own published data (66) in an Iranian population was also consistent with these findings. However, a significant association was found between MS cases and polymorphism at position -1666 in the regulatory region. The overall conclusion on *CTLA4* association with MS indicates that in this T cell mediated autoimmunity, the polymorphism at the leader sequence may not confer susceptibility to MS, but the involvement of other *CTLA4* SNPs in the pathogenesis of MS cannot be ruled out.

SYSTEMIC SCLEROSIS (Ss)

Systemic sclerosis (Ss) is a chronic connective tissue autoimmune disease that affects both males and females. The disease is characterized by vascular and organ damages as a result of collagen deposition. Components of the immune system play a major role in pathogenesis of Ss of which involvement and infiltration of T lymphocytes of different subsets from gamma delta to Treg cells at the site of the lesion are exemplified (67). Although the role of B cells in Ss is not well defined, production of autoantibody in Ss has already been documented. In general a dysregulation in the function of the Immune cells in Ss has been notified. Long term follow-up of patients with Ss has revealed that these patients are at an increased risk of developing cancer. Distribution of cancer types in Ss patients vary. Lung, oesophageal, breast and haematological malignancies have been reported with a higher incidence. A recent review by Olson B, from northern Europe, shows that the rate of Lung cancer in male patients is about 1.5 and that of females is 1.4 %. Development of breast cancer has also been reported (68). In Olson's report smoking and alcohol were shown as main etiologic causes of malignancies in Ss patients. Genetic factors associated with Ss have been demonstrated. Polymorphisms in Immunoglobulin Fc receptors, cytokines and chemokines have also been reported (69,70). But recent and comprehensive report of the genome association study from a large European population indicates that a genetic element within CD247 may confer susceptibility to Ss (71). Very few reports have been published on the association of *CTLA4* and Ss. The first one was published by Takeuchi et al. (72) in a Japanese population indicating that contrary to other autoimmune diseases, the frequency of A allele at position +49 of the leader sequence was associated with Ss. Hudson and colleagues reported a lower frequency of AA genotype in Afro-American Ss patients compared with the control group (73). Our own group reported no association between +49A/G polymorphism and patients with Ss (74). However, we later analysed other polymorphic sites in *CTLA4* gene and found a strongly significant association with position -1722, -1661 and -318 in the promoter region (75). This report was later confirmed in a Japanese population (76) and in a group of Italian patients (77). In summary, the data published on the polymorphism of *CTLA4* gene and Ss patients indicate that not only allele G or GG genotype are not associated with susceptibility to Ss, but also increases in an A allele at the leader sequence, at least in a few reports, may

confer susceptibility. This provides evidence that polymorphism in the regulatory region may be important in disease initiation and progression.

MYASTHENIA GRAVIS (MG)

Myasthenia gravis (MG) is regarded as an organ specific autoimmune neuromuscular disorder with major clinical presentation and muscle weakness as a result of dysregulation in signal transduction. MG occurs in two common types, namely the paraneoplastic form (associated with thymoma), and the non-paraneoplastic type accompanied with the production of either antibody to AChR or muscle specific receptor tyrosine kinase. The association of thymoma or a form of neoplastic disease with MG makes this autoimmune disease unique for investigation for understanding the role of CTLA4, as a major regulatory molecule, in directing the course of this disease. In MG patients, autoimmunity and neoplastic transformation are observed in the same individual where breakdown of tolerance triggers self-reactivity and production of autoantibody and at the same time the possibility of the existence of a state of immuno-suppression which triggers the initiation of the clinical presentation of thymoma. Research on the genetic association and also on the functional activity of CTLA4 in MG is still progressing. An earlier report from a Swedish group (78) indicated no association between *CTLA4* gene polymorphism at position +49A/G and also in the regulatory region at position -318. But interestingly, at the same time this group reported a lower expression of membrane CTLA4 on T cells isolated from MG patients compared with the healthy subjects- a finding that supports the regulatory involvement of CTLA4 molecule in the imbalance of T cell activation in MG patients (79). More recently, the same group demonstrated new genetic elements in *CTLA4* and susceptibility to MG by showing an increase in the frequency of the TC genotype at position -1772 in MG patients compared with normal individuals. In addition when MG patients with the paraneoplastic form was similarly analysed, an increased frequency of the TC genotype at position -1772 was observed in MG patients associated with thymoma compared with the normal subjects (80). In a small sample size and in a comparative study in infections and autoimmune diseases, no association between frequency of the allele and genotype in a Latin American population and healthy subjects was reported (81).

However, compelling evidence came from a recent report from a European cohort study clearly demonstrating that AA genotype at the leader sequence is associated with susceptibility in patients with MG a complete different feature of *CTLA4* polymorphism to what we have discussed so far for most autoimmune diseases, particularly those of organ specific disorders such as Grave's disease (82).

Considering the *CTLA4* gene association with autoimmune diseases in general, inconsistency of the results published, might have been arisen from the differences in the sample size, heterogeneity of the populations studied, differences in pathology of the diseases investigated and etc. Analysing such a relatively large controversial date published about *CTLA4* gene and autoimmunity and providing an explanatory systematic review on the likely reasons merits more attention.

CTLA4 GENE POLYMORPHYSM AND CANCER

Polymorphism in HLA and non-HLA systems in relation to disease susceptibility in cancer has been investigated but to a lesser extent compared to the accumulating data for the autoimmune disorders. Although the role of physical and chemical factors in initiating malignancies are indispensable with technological advances in biomedical area, it has been known that genetic factors and their dysregulation have become more prominent in the pathogenesis of cancer. In this context, any variation, modification and over- expression in the immune gene profiles may be interpreted as a function to influence tumour initiation and tumor survival. In this respect, regulatory T cells and the up- and down-regulation of its membrane associated molecule, the CTLA4, is of particular interest. With this background and similar to autoimmune disorders, the frequency of *CTLA4* polymorphisms with a particular focus on polymorphism in the leader sequence is discussed in some major solid malignancies.

BREAST CANCER

Breast cancer is the leading cause of female deaths due to malignancies worldwide. Incidence of sporadic breast cancer is well correlated with an increase in age. In western countries, the mean age of breast cancer is over 55 years. On the contrary, in Iran and other similar socioeconomic regions, the mean age is about 10 years less (83) indirectly hinting on the importance of epigenetic factors governing the pathogenesis of this disease. CTLA4, a regulatory molecule with a known negative regulatory role in the immune system, represents as the most important target to be investigated in cancer biology and cancer immunotherapy. To this end, several clinical trials are already underway using anti-CTLA4 antibody for the treatment of various malignancies (84, 85). Regarding polymorphism of *CTLA4* in breast cancer, our group was the first to investigate the association of *CTLA4* in breast cancer (86). In a case control study, we found that allele A and genotype AA of *CTLA4* leader sequence is significantly associated with the risk of developing breast cancer in Iranian patients. After this finding, we extended our work in a new cohort and larger sample size by focusing on *CTLA4* polymorphism in the promoter region and the regulatory sequences. In this work, a haplotype combination (TAC/TAC, -1722T, -1661A, -318C) was found to be increased in the control subjects. Although insignificant, it points out to the protective role in normal individuals carrying this haplotype combination from the promoter region (87). In addition to our work, three other publications, two from China, appeared with similar results. The observation by Li et al. points out to the significance of *CTLA4* regulatory region in the Chinese Han population (88). Increased tumour size was also reported in patients carrying AA genotype at position +49 (89), but the major work with a large sample size was published by Sun et al. (90). In this recommended work, a functional analysis of AA and GG genotypes, the two major genotypes of *CTLA4* at the leader sequence, was also carried out (90). The significance of this functional analysis will be further discussed in this review, but the first part of their study presents a significant association of AA genotype in Chinese populations with breast cancer confirming our earlier observation of the functional dichotomy of *CTLA4* at the leader sequence in autoimmune diseases (46) and cancer (86). Despite the lack of a large number of publications regarding breast cancer and *CTLA4* genetic susceptibility, the

data extracted from a few articles presented here represent a consistent trend pointing to the importance of CTLA4 in breast cancer. Over-expression of CTLA4 has also been documented in PBMCs of patients with breast cancer in our recent report (91). More recently, we have shown that patients with breast cancer harbouring AA genotype at the leader sequence polymorphism produce more soluble CTLA4 (sCTLA4) (92); indirectly emphasizing the importance of CTLA4 in exacerbation of immunsuppression during breast cancer progression. Our findings are, indirectly, in line with those of Sun et al. (90) demonstrating that people with AA genotype have more CTLA4 proteins to engage with B7 molecules and therefore induce more negative signals in immune cells- a favourable condition for tumour escape mechanism.

GASTRO INTESTINAL (GI) CANCER

Gastrointestinal (GI) cancer is a general term used for gastric and colorectal cancers as well as other malignancies of the gastrointestinal tract. Infiltrating CD8+ and CD4+ effector T lymphocytes into GI tumor site are well known to be beneficial for the patients with GI cancers. Several strategies are now under development to manipulate immune regulatory cells as a promising immunotherapy approach in GI cancer. Through literature search, only a few publications were available on the association of *CTLA4* gene and colorectal and gastric cancers. The first one is an article by Cozar et al. (93) investigating the association of both colorectal cancer and renal cell carcinoma (RCC) with *CTLA4* polymorphism in Spain, reporting that despite a statistically significant association between AA genotypes at both +6230 (CT60) and +49 positions with RCC, no association is observed for colorectal cancer. Although, to data, only these data are published regarding *CTLA4* polymorphism and RCC there are a few more information regarding GI cancers. At the same time with the above mentioned study we reported the results of our work for the association of *CTLA4* with gastric and colorectal cancer (94). Similar to the investigations by Cozar et al. (93) and also the results of the work in Turkish population by Dilmec and co-workers (95), Solerio et al. (96)investigated colorectal and adenoma cancers in a Caucasian population. We did not detected any association for the allele or the genotype in our patients or controls, although we detected TACG haplotype (-1722T, -1661A, -318C, +49G) as a result of the analysis of SNP at the leader sequence and other SNPs at the promoter region with a more frequency in the control group (94). On the contrary, a report from Chinese Han population with GI cancer demonstrated a pattern similar to autoimmune diseases suggesting that *CTLA4* +49A>G polymorphism was associated with an increased risk of colorectal cancer (97). A similar pattern was also reported from China (98) in gastric cancer patients showing that GG genotype at the leader sequences and A/G heterozygote at -1661 of the promoter region were significantly associated with the patient group. Recently, Sun et al. (90) investigated 530 cases of gastric cancer and 530 controls from the same ethnic population for the association of *CTLA4* polymorphism at the leader sequence peptide. Result of this study revealed a significant association of AA genotype at the leader sequence and susceptibility to gastric cancer. Such a discrepancy in three reports, all from a single ethnic group makes their interpretation difficult. It is not clear if the cohorts of Qi et al. (97) and Hou et al. (98) included patients with a background of autoimmune pro-inflammatory condition such as

ulcerative colitis, although we have previously shown no association for *CTLA4* +49A/G polymorphism in Iranian patients with ulcerative colitis (99). The other differences are the large number of cases studied by Sun et al. (90) compared with the other studied groups (97,98). To reduce the controversy around *CTLA4* polymorphisn and GI cancer more data with large sample size is required for comparison from other ethnic groups

LUNG CANCER

Lung cancer is the most common type of cancer in industrial countries and is among the 10 most frequent cancers in Iranian populations; however, the incidence in Iran is reported to be lower than the world average. Only two published reports investigated the *CTLA4* polymorphism in lung cancer; one by our own group and the other one in a Chinese population with a larger sample size, focusing only on the polymorphism at the leader sequence. In our own work, although not having a relatively large sample size, no association between *CTLA4* SNP in the leader sequence or in the regulatory regions and the risk of lung cancer was indicated (100). However, the work by Sun et al. in 1032 cases of lung cancer and 1021 normal subjects of Chinese ethnic group pointed to a significant association with AA genotype polymorphism of *CTLA4* at the leader sequence and susceptibility to lung cancer (90). The discrepancy between these two reports, apart from the size of the samples, may be due to differences in histology and also as a result of the heterogeneity of lung cancer types. The majority of lung cancers are caused by environmental factors particularly smoking. It would be of great interest to compare the rate of smoking and the distribution of the histological type of lung cancer in Sun et al. report and ours.

CERVICAL CANCER

Cervical cancer is the best exemplified human cancer with a known infective etiology. Association of HPV infection and risk of cervical cancer is well known and thus a successful vaccine trail against this malignancy has been already provided in the market for the population at risk. Published works on the association of *CTLA4* variants and cervical cancer is limited to four publications. The first one was published by a Taiwanese group in 2007, reporting that polymorphism at position -318 at the promoter region may confirm susceptibility to cervical cancer for patients positive for HPV-16 (101). This group reported no association of polymorphism at the leader sequence and the risk of cervical cancer. This finding was confirmed in a Polish population, reemphasising the importance of -318 polymorphism and susceptibility to cervical cancer (102).

Results of our work from southern Iranian population supports the above observations in addition to the finding that A allele or AA genotype at position -1666 may confer protection for cervical cancer as the frequency of this allele and the genotype were significantly higher in healthy control group (103). Considering the sample size, the largest study published so far on cervical cancer and *CTLA4* polymorphism is the one published very recently by Hu et al. (104). They have analysed 719 cervical cancer patients and 719 control subjects from a Chinese population. Despite the lack of

association with *CTLA4* polymorphism in the leader sequence (+49A/G) observed in the above mentioned three reports, this group clearly demonstrated a significant association for AA or A allele at this position and reported susceptibility to cervical cancer (104). The association of AA genotype with cervical cancer was found to be even higher in younger patients, in those with lower age at menarche, in those with lower age at their first live birth and also in patients diagnosed as having squamous cell carcinoma. They did not report the rate of HPV infection in their studied group. By comparing this recent analysis and the previously published data, one can point out that factors such as having a large sample size and the homogeneity of population existed in the study of Hu et al. (104). No other confounding factors could be obtained from these studies and the interpretation of such data would be very difficult, unless new data with strong statistical power from other populations particularly from Caucasian registries would be published for comparison.

LEUKAMIA AND LYMPHOMA

Although leukaemia is known as the cancer of blood, and lymphoma; as the cancer of lymphatic system, both cancers affect the white blood cells. In leukaemia the neoplastic cells are either myeloid or lymphoid occupying the bone marrow, while in lymphoma the lymphoid cells undergo transformation in lymph nodes or other lymphatic organs. From CTLA4 point of view, the work by Monne and colleagues which deals with *CTLA4* gene polymorphism and susceptibility to non-Hodgkin's lymphoma (NHL) was among the earliest to report an association between *CTLA4* gene variants and cancer cases (105). In this article, Monne et al. reported the associations of the *CTLA4* +49 polymorphism with NHL in a Caucasian population, and particularly demonstrated that AA genotype was overrepresented in cases compared to the control subjects (105). Consequently a similar and consistent report was published by Piras et al. (106) indicating that a strong association exists between *CTLA4* +49A allele and NHL. Later, Nearman et al. (107) working on T-cell large granular lymphocyte leukaemia (T-LGL), a relatively rare case of hematologic malignancies, confirmed a similar finding and observed a statistically significant association of AA genotype polymorphism in the leader sequence of *CTLA4* with susceptibility to this chronic T cells leukemia. Consequently, no association was observed between the same positions in CLL in a Caucasian population (108). Contrary to the above reports, Cheng et al. (109) studied the gastric mucosa-associated lymphoid tissue lymphoma, either positive or negative for H. pylori infection. They reported a lower risk of gastric MALT lymphoma with *CTLA4* -318 C/T, whereas a higher risk was found with *CTLA4* +49 GG genotype (109).In this study a higher risk of H. pylori infection was reported to be associated with *CTLA4* GG genotype. It is not clear if GG genotype associated with gastric MALT lymphoma was a reflection of increased risk of H. pylori infection in individuals with GG genotype as reported earlier.

SQUAMOUS CELL CARCINOMA (SCC)

Squamous cell carcinoma (SCC) is the cancer of squamous epithelium appears in several different organs including skin, oral cavity, airways, cervix, and genital as well

as gastrointestinal tracts. The ethiology of SSC is not well known but the role of a variety of risk factors have been characterized including smoking, nutritional regimen, genetic background, chronic irritation, exposure to radiation, viruses and etc. Although manifestations vary depending on the involved organ, the immunopathology of all SCC types share common characteristics with T cell as the central player in the game. In respect to the *CTLA4* polymorphism, Wong et al. (110) reported that although no association exists between cases and controls in respect to the allele or the genotype frequency at position 49A/G, a significant association was observed in patients with AA genotype, those younger , and those with more poor survival (110). Also a significant association of *CTLA4* at position -1661 A/G genotype and the risk of oral SCC in Caucasian population and not in the leader sequences was reported Kammerer et al. (111). Our report in the squamous cell carcinoma of the head and neck confirmed the lack of any association of either polymorphisms at the leader sequences or the promoter regions with SCC in cases and controls, but a significant decrease was found in the patient group for the A allele or the AA genotype at position +6230 (CT60) polymorphism (Unpublished observation). In a large sample sized analysis, Welsh et al. (112) investigated the association of non melanoma skin cancer with GG genotype at position +6230 (CT60), a genotype already shown to be highly associated with Graves' disease and diabetes. They found that GG genotype of CT60 polymorphism is associated with a decreased risk of both BCC and SCC compared with the AA genotype with a similar risk in a UK population. For nasopharyngeal carcinoma (NPC), a report with a relatively large sample size (457 NPC patients and 485 healthy controls) has recently been published investigating the *CTLA4* +49A/G. In this work it has been demonstrated that subjects carrying A allele or AA genotype at the leader sequence polymorphism have a significantly increased risk of NPC compared with those carrying G allele of GG genotype at this position; a finding which was pronounced in younger subjects as reported by Wong in SCC patients in the Taiwanese population (113).

EVIDENCE FROM TRANSPLANATION

CTLA4 gene and its potential negative regulatory function has also been the focus of attention by investigators in the field of transplantation immunology. For instance anti-CTLA4 antibody has been used as an immuno-suppression modality in various clinical trials (114) .In addition allogeneic transplantation provides a unique situation to study the functional aspect of *CTLA4* polymorphism particularly in studies with long clinical follow up. In this respect, the variation and polymorphism at *CTLA4* gene has also been the subject of studies in donor-recipient cases both in solid grafts and in bone marrow transplantation and in respect to the clinical outcome. Genetic studies have been carried out by several groups on the association of *CTLA4* gene polymorphism in kidney allograft transplantation where, in most cases, no association have been found between allele or genotype frequency in donors and recipients as a whole. Regarding clinical outcome and graft survival in kidney transplantation, a report confirmed that subjects receiving a kidney from a live related donor with an A allele or an AA genotype at position +49 of *CTLA4* may experience protection from acute rejection (115) .Similar findings were later reported by a group from Tunesia (116). In a lager cohort study of Caucasian patients with a 10-year follow up, Kusztal et al. (117) reported that recipients bearing a combination genotype of AA/LL (AA at position +49 in the leader sequence

and low AT repeat number (82 bp) at the microsatellite polymorphism in the 3'UTR of exon 4 (AT)(n)) of the *CTLA4* gene) had a markedly higher eGFR than those bearing the GG/HH genotype set (H for high AT repeats) after 12 months, 60 months and 96 months post transplantation. On the contrary, subjects with GG/HH genotype had a continuous decline in EGFR after 8 years post transplantation. These evidences all together point to a functional activity for *CTLA4* polymorphism in the leader sequences (+49 A/G) in an in-vivo system supporting sustainability of the immunological tolerance in allogeneic kidney transplantation in individuals with an A allele and AA genotype. Experience from bone marrow transplantation represents a mixed finding. Azaraian et al. (118) reported no association between +49A/G or +6230 (CT60) polymorphisms and acute GVHD, relapse, survival, or infections in allogeneic bone marrow transplantation. But they found that patients who received a graft from a donor with a GG genotype of+49A/G had a stronger risk of developing chronic GVHD compared with those having a donor with either AG or AA genotype. It was postulated that a more proliferative response, associated with a CTLA4 low phenotype, may cause such a clinical complication. On the contrary, a longer overall survival time was noted in patients received a graft from a homozygote G/G for the +49A/G polymorphism. This increased survival was interpreted (119) as a reflection of lower negative signals by the lower CTLA4 expressing donor T cells contributing to the known phenomenon of graft versus leukaemia- a reaction by donor lymphocytes against residual leukemic cells. Perez-Garcia et al. (120) investigated the role of +6230 (CT60) polymorphism and BM transplantation outcome in AML and found that the presence of at least one G-allele of CT60 polymorphism in the donors was associated with reduced overall survival (OS), higher relapse risk, and lower risk of acute graft versus host disease. This is the same allele which causes strong susceptibility to Grave's disease (47) which is in linkage disequilibrium with polymorphism at the leader sequences. This data indicate that *CTLA4* genotype with a known susceptibility to Graves' disease represents a lower state of tolerance in subjects receiving bone marrow from individuals carrying this genotype (121) reported that AA genotype of CT60 predicts the chance of acute GVHD, a finding well correlated with the observation of Pérez-García et al.(120) who reported G allele or GG genotype of CT60 polymorphism in the donor will reduce the chance of GVHD in AML cases. Similar line of evidence came from Azerian et al.(118) reporting that patients who received a graft with G allele or GG genotype at the leader sequence of *CTLA4* had a stronger risk of developing chronic GVHD in comparison with those with either AG or AA genotype; again reiterating the propensity of individuals with G allele or GG genotype at this position with either self reactivity or more cellular proliferative capacity.

AUTOIMMUNITY VERSUS CANCER: THE POTENTIAL APPLICATION OF CTLA4 SNPs

The hallmark of the human autoimmune diseases is the hyper activation and uncontrolled proliferation of the immune cells while malignant diseases have been extensively been reported to be associated with a state of immune suppression and an increased degree of self-tolerance. Considering CTLA4 as one of the most crucial regulatory element of the immune responses magnitude, CTLA4 optimal expression

seems to be necessary for a safe, but at the same time, effective immune response when the immune responses are being formed in a predisposed individual to autoimmunity or when the immune responses have to be formed against malignant cells. It can accordingly be speculated that CTLA4 alleles associated with extraordinary expression could accelerate the formation and/or manifestation of malignant diseases, and alleles associated with lower expression may promote the progression of the inflammatory autoimmune reactions in the genetically and/or environmentally predispose individual.

A spectrum of the functional dichotomy of CTLA4 SNPs at which end one can see autoimmunity and cancer was hypothesised. By summarizing the known information about the functional aspects of the SNP at the leader sequence of CTLA4 explained in the earlier and recent works, the key points implicated as a result of this diversity are as follows: lymphocytes from patients with autoimmune diseases have more proliferative capacity compared with lymphocytes from healthy subjects (122,123). Normal individuals with GG genotype at position +49 have one third less CTLA4 membrane expression than individuals with AA genotype. Individuals with AA genotype have less proliferative activity in response to mitogen and IL-2 production. Moreover, they have a lower affinity to bind to B7 molecules compared with those carrying GG genotype (84). Therefore, harbouring a GG genotype in the leader sequence of CTLA4 may confer susceptibility in individuals and result in the development of autoimmunity, while individuals with AA genotype have a higher expression of membraneCTLA4 and a higher state of self-tolerance and may have a higher susceptibility in developing tumours. However, there is no doubt that, these possibilities are subject to other genetic and epigenetic factors. CTLA4 SNPs may accordingly be considered as a crucial element, along with other know or yet unknown mechanisms, in keeping the immune balance in predisposed individuals to cancer and autoimmunity. This extreme functional dichotomy of a single nucleotide polymorphism at CTLA4 leader sequence, in a way that draws a line between autoimmunity and cancer, appears to be more complex and difficult to interpret. As mentioned throughout this review, both in autoimmune diseases and in cancer, this rule is not consistent in every disease condition and is even less pronounced in cancer than in autoimmune diseases. A brief representation on the frequency of CTLA4 +49 A/G polymorphism in autoimmunity and cancer is illustrated in Table 1. Although not absolutely consistent, the Table horizontal line can be considered as a spectrum line, on one extreme autoimmune disease are positioned; most in association with G allele; for which lower expression of CTLA4 has been extensively suggested. On the other extreme most of the cancer types are being seen in association with A allele; representing genetic variation associated with higher CTLA4 expression. One can consider normal individuals in the middle of the spectrum with either of G or A alleles but not other genetic and/or environmental risk factors for cancer and autoimmunity, i.e. the effect of CTLA4 SNPs may come into effect when a person has already been proven for cancer or autoimmunity.

Table 1. A brief representation on the association of CTLA4 +49 A/G polymorphism with autoimmunity and cancer. Although not absolutely consistent, the table horizontal line can be considered as a spectrum line, on one extreme autoimmune disease are positioned; most in association with G allele; for which lower expression of CTLA4 has been extensively suggested. On the other extreme most of the cancer type are being seen in association with A allele; representing genetic variation associated with higher CTLA4 expression. Note other genetic and/or environmental background should never be ignored when talking about the association of a non-penetrate gene, like *CTLA*4, with these two types of human diseases. For more discussion refer to the manuscript.

	Autoimmune Diseases							**Cancer**						
CTLA4 +49 A/G allele association	SLE	RA	GT	Diabetes	MS	SC	MG	BC	Lymphoma	SCC	RCC	GIC	LC	CC
G	**+**	**+**	**+**	**+**	**+/-**	**+/-**	**-**	**-**	**-**	**-**	**-**	**-/+**	**-**	**-**
A	**-**	**-**	**-**	**-**	**-/+**	**-/+**	**+**	**+**	**+**	**+**	**+**	**+/-**	**-**	**-**

SLE: Systemic Lupus Erythematosous; RA: Rheumatoid Arthritis; GT: Graves' Thyroiditis; MS: Multiple Sclerosis; SC: Systemic Sclerosis; MG: Myasthenia Gravis; BC: Breast Cancer; SCC: Squamous Cell Carcinoma; RCC: Renal Cell Cancer; GIC: Gastrointestinal Cancer; LC: Lung Cancer; CC: Cervical Cancer.

In conclusion, a comparison was made between the frequency of CTLA4 alleles and the genotypes in two major human diseases, the autoimmune disorders and cancer. Attention was made mostly to a single nucleotide polymorphism in the leader sequence of this gene.Availability of a large number of publications and meta-analysis on CTLA4 polymorphism made this task easier in autoimmune diseases than in cancer, in which only a short review has recently been published describing significant variations of this gene in susceptibility to cancer, in addition to a limited research publications (124). CTLA4 has a unique position, being at the prickly edge of the controlling point of an immune response, with expression on T regulatory cells and activated T cells. Any variation in the gene or any dysregulation or aberrant expression of this gene at the protein level may, with no doubt, cause over reactivity in immune response or may induce suppression. With this reality in mind, over-expression of membrane CTLA4, as a result of a single base substitution, may generate a state of chronic suppression in cancer which will be a window for tumour cells to escape from the networks of immune surveillance. While in autoimmunity, an off signal within the CTLA4 signal transduction machinery, as a result of the reverse substitution (an alanine to a threonine), may cause lower expression of membrane CTLA4 protein. Therefore this may trigger the silenced auto reactive lymphocyte repertoire to wake up, proliferate and participate in the pathogenesis of the diseases. More reports on *CTLA4* polymorphism and disease susceptibility either in autoimmunity or cancer will probably be published in future, however we need to extract, from the enormous reports published on this gene, scientific information to reach a consensus on the practical approaches in reducing the risk associated with this single amino acid change that has a large clinical impact on health and disease.

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