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Tumour Necrosis Factor Alpha -308 Gene Locus Promoter Polymorphism: An Analysis of Association with Health and Disease

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SUMMARY

Tumour necrosis factor-alpha (TNF- α) is a potent immunomediator and proinflammatory cytokine that has been implicated in the pathogenesis of a large number of human diseases. The location of its gene within major histocompatibility complex and biological activities has raised the possibility that polymorphisms within this locus may contribute to the pathogenesis of wide range of autoimmune and infectious diseases. For example, a bi-allelic single nucleotide substitution of G (TNFA1 allele) with A (TNFA2 allele) polymorphism at -308 nucleotides upstream from the transcription initiation site in the TNF- α promoter is associated with elevated TNF- α levels and disease susceptibilities. However, it is still unclear whether TNF- α -308 polymorphism plays a part in the disease process, in particular whether it could affect transcription factors binding and in turn influence TNF- α transcription and synthesis. Several studies have suggested that TNFA2 allele is significantly linked with the high TNF- α -producing autoimmune MHC haplotype HLA-A1, B8, DR3, with elevated serum TNF- α levels and a more severe outcome in diseases. This review discusses the genetics of the TNF- α -308 polymorphism in selected major diseases and evaluates its common role in health and disease.

INTRODUCTION

Tumour necrosis factor- α (TNF- α) is a proinflammatory cytokine that produces left ventricular dysfunction, cardiomyopathy, pulmonary oedema and a variety of other diseases, when over-expressed [1-2]. Recent observations showed that levels of circulating TNF- α are elevated in patients with unstable angina, rheumatoid arthritis (RA) and autoimmune disorders [3]. Although exact clinical significance of these findings is uncertain, given that TNF- α produces negative effects, it has been postulated that the elaboration of this proinflammatory cytokine may contribute to the progression of the disease process. Important to the above hypothesis regarding the pathophysiological role of TNF- α in disease is the elucidation of the mechanisms by which this proinflammatory cytokine exerts its effects. Although experimental studies from a number of laboratories have begun to explore the basic mechanisms of TNF- α at the tissue and cellular levels, until recently polymorphism in the promoter region of the TNF was found to be linked with the genetic basis for these diseases.

Circulating TNF- α levels and their genetic associations between gene polymorphisms are involved in many diseases and clinical endpoints, e.g. rejection after heart, kidney and liver transplantation and with a wide variety of other diseases [4-6]. The reasoning behind the proposed involvement of TNF gene polymorphisms in diseases either/or disease manifestations is that they may influence *in-vivo* cytokine levels. This presumption is based on the observation that individuals differ with respect to the level of cytokine production after *in vitro* culture of their cells and that these differences may be attributed partly to single nucleotide (SNP) or microsatellite polymorphisms within its corresponding gene [7]. These associations have not always been robust, replicable and consistent, for example some studies could not show a relationship between TNFA -308 gene polymorphism and liver graft rejection [8]. The generally held view is that genetic factors may affect TNF- α levels and the location of TNF locus is within the Class III region of the human major histocompatibility complex (MHC; also known

as TNF β) on chromosome 6p21 conducive to its role in disease processes [9]. A number of SNP and other polymorphisms have been identified in the TNF locus, some of these have also been shown to influence the rate of transcription and protein production of TNF- α and TNF β associations with diseases [10]

In the light of these conflicting results we reviewed the literature of available studies to clarify the role of -308 G/A polymorphism of the TNF gene and the disease risk. There is explosion of information on the disease associations with genetic polymorphism and it is impossible to keep up with published literature. We have listed the main research studies on TNF -308 and disease associations with reference to geographical regions in Table-1 [6, 9, 11-40]. In text, we discuss only the main points and linkages published in the literature.

Biological Regulation of TNF- α Production and Function

The biological functions of the TNF- α are varied and complex, where on one hand it confers disease resistance and on the other causes pathological complications. Indeed, TNF- α plays contradictory role which may be related to genetic polymorphisms in the genes regulating its production and effect. In the acute situation, local production of TNF- α is clearly beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells, in particular neutrophils and macrophages, to translocate to sites of tissue damage and infection [41]. Furthermore, TNF- α activates phagocytes to engulf and clear infectious agents and cellular debris. However, systemic or protracted exposure to TNF- α may be harmful. High levels of circulating TNF- α are associated with toxic shock induced by bacterial endotoxins [42] and derangements of metabolism in surgery or trauma patients may be related to the cachetic properties of this cytokine. The induction of interleukin-1 and interleukin-6 production stimulated by TNF- α leads to elevated temperature, sleepiness and the release

of glucocorticoids [43]. These may be short-term value in combating certain infections, but their long-term effects are likely to be detrimental.

TNF- α itself can suppress the production of more TNF- α , an effect mediated through the TNFR1 and TNFR2 cell surface receptors for TNF- α . Cells from mice deficient of TNFR1 or TNFR2 produce substantially more TNF- α upon stimulation. Although TNF- α may be produced by many cell types, macrophages are the main source of this cytokine. TNF- α is produced as a membrane-bound 26kD molecule from which is released the soluble 17kD active TNF- α molecule by enzymatic cleavage. The enzyme involved is a metalloproteinase disintegrin called TNF- α converting enzyme (TACE). Remarkably, TACE also acts on membrane anchored TNFR2 protein thus controlling the amount of soluble circulating TNFR2. This adds another layer to the regulation of TNF- α function because soluble TNF receptor affects the activity of TNF- α [44].

Studies of TNF- α function in genetically manipulated mice have demonstrated that when homozygous TNF- α gene knockout mice (no TNF- α production) were infected with the bacterium, *Corynebacterium Parvum*, there was little or no initial response but the mice went on to develop a severe and fatal inflammatory reaction [45]. By contrast, normal mice developed an inflammatory response that resolved. This suggests that TNF- α has dual function, being proinflammatory in the initial infection and then anti-inflammatory or immunoregulatory in the later phases of the response. Heterozygous mice, those with only one active TNF- α gene were more susceptible to endotoxin-induced shock and certain infections. In fact, in one strain of genetically manipulated mice deficient in TNF- α production, an arthritic condition akin to ankylosing spondylitis developed [46]. Again, these observations are consistent with a dual role for TNF- α , acting as a proinflammatory agent to start with, but later having anti-inflammatory or immunoregulatory functions. Indeed, recent studies have shown that tumor necrosis factor alpha can autoregulate by activating PIAS1 [protein inhibitor of activated STAT1

(signal transducer and activator of transcription 1)] SUMO E3 ligase through a SUMO-dependent, inhibitor of kappaB kinase alpha (IKK alpha)-mediated phosphorylation event. Once activated PIAS1 is then recruited to the TNF- α gene promoter to repress transcription [47].

The TNF gene is located in close proximity to the HLA-B locus in both humans and mouse. The 5' flanking region of the TNF gene contains multiple potential regulatory sites, including consensus sequences for the AP-1 and AP-2 sites, the cAMP-responsive element, and sequences similar to the kappa B sequences found in immunoglobulin and cytokine regulatory elements [48]. This sequence has been demonstrated to be responsive to LPS and TNF stimulation. The 3' untranslated region contains a sequence element affecting posttranslational control of TNF through mRNA stability and translation efficiency. The functional importance and interactions of these regulatory elements remain undefined. Experimental evidence suggests that phospholipase A2 and the lipoxygenase pathway may be central in the process of TNF induction in leukocytes. Down-regulation of TNF expression on the other hand is better understood. The inhibition of expression appears to result from high levels of cAMP, frequently induced through the action of PGE2. TNF secretion is separately regulated and may involve the action of G binding proteins [48]. In another study, posttranslational control of TNF-alpha release in LPS-stimulated alveolar macrophages exposed to the stress response was shown to be due to binding and sequestration by heat shock proteins (HSP) [49].

TNF- α Gene Polymorphism and Cytokine Production

The TNF- α gene is located on the short arm of chromosome 6 within the major histocompatibility complex, where genetic alterations in the TNF- α locus are now known to be involved directly in high TNF- α production [10]. Several polymorphisms have been identified inside the TNF- α promoter positioned at (relative to the transcription start site) -1031 (T \rightarrow C), -863 (C \rightarrow A), -857 (C \rightarrow A), -851

(C→T), -419 (G→C), -376 (G→A), -308 (G→A), -238 (G→A), -162 (G→A), and -49 (G→A), although those at positions -419, -163, -49 are rare in Caucasians (Figure-1). Among these variants, a polymorphism that directly affects TNF- α expression is located at nucleotide position -308. A single-base polymorphism within the promoter of the gene for TNF- α results in 2 allelic forms, one in which guanine defines the common allele (TNFA*1) and the other in which guanine is substituted by adenosine forms the rarer allele (TNFA*2) at position -308. The presence of the rarer TNFA*2 allele has been found to correlate with enhanced spontaneous or stimulated TNF- α production in both *in vitro* and *in vivo* [50].

SNP in the TNF- α gene itself are more likely to be of direct functional significance in terms of regulating TNF- α production, as there are many SNP within the TNF- α gene promoter. In particular, there is interest in those polymorphic sites in the regulatory regions of the TNF- α gene that coincides with the DNA motifs to which transcription factors bind. *In vitro* stimulation of TNF- α production by cells from -308*G/G homozygous individuals and G/A heterozygote individuals have produced conflicting results. Two studies have reported higher TNF- α production by cells from G/A donors than by G/G cells [51]. Other studies have reported no significant affect [52-53]. However, it is interesting to note that these studies used different LPS concentrations and the number of individuals with the G/A genotype studied was in most of the cases small, affecting the power of the study to detect any significant difference between the genotypes.

Gene reporter assays have been employed to investigate the -308 SNP and again, contradictory results were reported. It was suggested that the A allele does influence TNF- α gene transcription [8] while others have concluded that it does not [54]. There are many variables affecting the results of this type of experiments; including the length of the promoter sequence used, the presence or absence of the 3' UTR, the cell type used for transfection, and whether it is of human or non-human origin. Different

studies have used different approaches, thus making it difficult to draw a general conclusion. So far, evidence suggests that circulating TNF- α levels do not seem to correspond with the -308 TNF promoter polymorphisms. However, although circulating TNF- α level might be under a multifactoral regulatory process, local TNF- α concentration might be of greater importance and under more control by specific polymorphisms [55].

An analysis of the evaluation of the extended MHC haplotypes including the TNF region has been presented recently in which it was proposed that there are three ancient extended haplotypes from which the complex modern haplotypes were derived by mutation or crossover. In this analysis, the -308*G, -238*G haplotype was generally associated with low TNF- α production while either the -308*A allele or the -238*A allele were associated with high TNF- α production [56].

However, there are many biological steps, apart from the influence of the gene polymorphisms on TNF- α production, for which the control, production and release of TNF- α and its activity are regulated.

Genetic Associations between TNF -308G/A Polymorphisms and Disease

The polymorphism in the human TNF- α gene encoding high TNF- α levels may be important in the susceptibility or severity of diseases and in other inflammatory conditions as discussed below; .

TNF- α Genotypes in Parasitic, Bacterial and Viral Infections

In parasitic infections, the -308*A allele has been associated with a fourfold increase in risk for cerebral malaria and a sevenfold increase in risk for development of serious neurological consequences [57]. This association was shown to be independent of the inheritance of HLA antigens. A similar study confirmed the association with the -308*A allele in the population of Sri Lanka [58] whereas in the West Africans the -376 alleles are in strong positive linkage disequilibrium with the -238 alleles and in

strong negative linkage disequilibrium with the -308 alleles [59]. Furthermore, the -376*A allele was found a risk factor independent of the -308*A allele for the development of cerebral malaria. Taken together, these results suggest that there are two genetic effects operating independently, however, its mechanism is yet unclear.

The -308*A allele was also associated with the most severe outcome, mucocutaneous leishmaniasis [60]. Hence both the risk of infection and its course may be associated with polymorphisms in TNF gene. In multiple injured patients with severe sepsis the TNFA*2 allele acts as a predictor of severe post traumatic sepsis and increased levels of circulating TNF- α [61-62]. It is interesting to note that the -308*A allele has been associated with severe septic shock and death. Several studies have investigated the association between TNF alleles and viral infections. The -238*A allele was associated with chronic infection with hepatitis B virus. No similar findings were found for -308 polymorphisms [63]. Similar findings were also reported for hepatitis C virus infection, the -238*A allele being associated with chronic hepatitis while the -308 alleles were not [64]. A comparison of Cytomegalovirus (CMV) positive and negative healthy adults showed that the frequency of the TNF- α -308*A allele was slightly increased in the seronegative individuals [65]. However, this reached statistical significance only when the genotype of another cytokine that influences the inflammatory response, interleukin-1 receptor antagonist was taken into account.

TNF polymorphisms and Autoimmune Diseases

In autoimmune diseases, TNF- α is one of the most studied genes. As most autoimmune diseases are associated with HLA, results of TNF polymorphism studies could be interpreted by linkage disequilibrium with HLA or, *vice versa*.

Systemic Lupus Erythematosus: In systemic lupus erythematosus (SLE), there is an association with -308*A allele and also a strong association with the HLA-DR3 antigen. The association between SLE and TNF genotype might best be explained by the strong linkage disequilibrium between the -308*A allele and the HLA-A1, B8, DR3 haplotypes. The TNF-308*A was shown to be associated with SLE in this ethnic group independent of any HLA-DR associations [66].

Rheumatoid Arthritis: In rheumatoid arthritis (RA) joints, there is an abundance of TNF- α so it was predicted that TNF- α gene polymorphism is associated with the disease. Further analysis found no association between the -308 alleles and RA. There is an association between HLA-DR4 and RA but there is a lack of linkage disequilibrium between the TNF-308 polymorphism and this HLA antigen. More recently, other TNF-SNP polymorphisms have been shown to correlate with the severity of RA but not with the initial susceptibility to the disease [3]. In order to better understand the genetic background of juvenile rheumatoid arthritis (JRA) several studies have been carried out recently. A study by Date et al [67], demonstrated that the -857T allele of the TNF alpha gene which was related to high production of tumor necrosis factor alpha, was associated with systemic JRA and that the -857T allele may enhance the effect of the DRB1*0405/DQB1*0401 haplotype in predisposing to the development of systemic JRA. Ozen et al [68], studied a group of 51 Turkish JIA patients and a second group consisted of 159 JIA patients from the Czech Republic. Healthy individuals (93 and 100) from each country served as controls. In both JIA cohorts, the distribution of genotypes was not significantly different among the types of JIA. The G-->A -238 polymorphism did not have an effect on the patients' outcome in either group. The G-->A -308 polymorphism was significantly associated with a poor outcome in the Turkish group (P=0.005) but not in the Czech patients. Another recent study examined the association of multiple TNF SNPs with juvenile oligoarthritis by constructing and analysing SNP-

tagged TNF haplotypes [69]. A total of 144 simplex families consisting of parent and affected child, as well as 88 healthy, unrelated control subjects were available for study. In these individuals, 9 polymorphic positions of TNF were typed by a high-throughput genotyping method based on the SNaPshot assay. The results demonstrated that TNF locus is linked and is associated with juvenile oligoarthritis and that information on the htSNPs can be useful in genetic studies of diseases in which TNF may be of relevance. In addition, a study Schmeling et al [70] on Caucasian subjects on TNF-alpha promoter polymorphisms at positions -163, -238, -244, -308, -376 to determine the association with disease in 228 patients with JIA and 196 healthy individuals. Genomic DNA was isolated and a PCR fragment of about 500 base pairs of the TNF gene promoter were amplified by PCR. Detection of polymorphisms was achieved by a single sequencing procedure. The results demonstrated that TNF promoter polymorphisms may play a role in the pathogenesis of JIA. The TNF-238A allele seems to be associated with juvenile psoriatic arthritis. The TNF-308A allele is less frequently found in rheumatoid factor negative but not in rheumatoid factor positive polyarthritis and may therefore be associated with a more severe disease, while the more common TNF-308G allele may be protective.

As the pathophysiology of RA becomes better understood, new therapeutic strategies and agents have been developed [71]. The efficacy of early and aggressive treatment with disease-modifying anti-rheumatic drugs (DMARDs) has now been studied. DMARDs, such as methotrexate (MTX), sulfasalazine (SSZ) and leflunomide, have documented prevention of the structural damage of the joints [72-73]. Recently, biologic response modifiers targeting specific cytokines, such as tumor necrosis factor- α (TNF- α) or interleukin-1, have been introduced, and the results indicated the effective suppression of the RA activity. However, the outcome of the treatment with DMARDs in RA patients is known to vary among patients. Recent advances in genetics have clarified that these individual differences are based on genetic polymorphisms, and this knowledge has encouraged the application of

pharmacogenetics to the treatment of RA. The difference in the response has been reported in biologic response modifiers and that in the case with the tumour necrosis factor (TNF) blocking agents, 20-40% of patients have been described as non-responders [74]. A recent study by Padyukov et al [74] analysed whether polymorphisms of several cytokine genes are associated with the responsiveness to TNF blockade with etanercept. 123 patients with active RA were treated with etanercept and response rates were determined after three months using American College of Rheumatology (ACR)20 and disease activity score (DAS)28 response criteria. Genotyping was done for TNF (-308 TNFA), interleukin (IL)10 (-1087 IL10), transforming growth factor (TGF)beta1 (codon 25 TGFB1), and IL1 receptor antagonist (intron 2 IL1RN). The results indicated that 24 patients (20%) were defined as non-responders owing to their failure to fulfil any of the ACR20 or DAS28 response criteria. None of the recorded alleles was alone significantly associated with responsiveness to treatment. However, a certain combination of alleles (-308 TNF1/TNF1 and -1087 G/G) was associated with good responsiveness to etanercept ($p < 0.05$). In addition, a combination of alleles influencing interleukin 1 receptor antagonist (IL1Ra) and TGFbeta1 production (A2 allele for IL1RN and rare C allele in codon 25 of TGFB1 gene) was associated with non-responsiveness ($p < 0.05$). The study concluded that genetic polymorphisms, which may influence the balance of pro- and anti-inflammatory cytokines of relevance for the course of RA, are associated with clinical responsiveness to etanercept treatment.

Ankylosing Spondylitis: On the other hand, tumor necrosis factor-alpha also has a prominent role in the inflammatory process and bone resorption in patients with ankylosing spondylitis (AS). Indeed, a recent study by Woo et al [75], evaluated the markers of clinical efficacy and bone biochemical changes in Korean patients with AS treated with etanercept therapy. The authors demonstrated that in patients with AS, etanercept therapy may be effective at reducing disease activity and improving bone

biochemical markers and that matrix metalloproteinase 3 (MMP-3) may be a useful biomarker for monitoring etanercept therapy. Indeed, changes in MMP-3 had a high correlation coefficient with changes of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) upon etanercept treatment (CRP, $r = 0.446$, $p = 0.022$; ESR, $r = 0.449$, $p = 0.021$). In another study [76] by Maksymowych et al, where the authors determined whether a vertebral corner that demonstrates an active corner inflammatory lesion (CIL) on magnetic resonance imaging (MRI) in patients with AS is more likely to evolve into a de novo syndesmophyte visible on plain radiography than is a vertebral corner that demonstrates no active inflammation on MRI. MRI scans and plain radiographs were obtained from 29 patients recruited into randomized placebo-controlled trials of anti-tumor necrosis factor alpha (anti-TNF-alpha) therapy. New syndesmophytes developed significantly more frequently in vertebral corners with inflammation (20%) than in those without inflammation (5.1%) seen on baseline MRI ($P \leq 0.008$ for all reader pairs). They also developed more frequently in vertebral corners where inflammation had resolved than in those where inflammation persisted after anti-TNF treatment.

However, studies are divided as to whether TNF-alpha polymorphisms play a role on the pathogenesis of spondyloarthritis conditions. Earlier findings by Verjans et al [77] and Fraile et al [78] found no direct evidence to suggest TNF-alpha polymorphisms have an independent effect on AS susceptibility. In addition, Kaijzel et al [79] concluded in their study that an association between TNF-238G and AS is secondary to the HLA-B27 gene and that TNF-238 and TNF-376 alleles are not likely to be independently involved in the susceptibility to AS. In contrast, similar study by McGarry et al [80], showed -308 TNF-alpha gene allele-1 was significantly increased in patients with AS. Another study by Rudwaleit et al [81] showed that in HLA-B27 positive AS subjects, TNF-alpha gene 1/2 heterozygosity at -308 was associated with a higher percentage of TNF-alpha production and T cell numbers as

compared with TNF-alpha gene 1/1 homozygosity. Vargas-Alarcon and colleagues also demonstrated that TNF-alpha -308 promoter polymorphism was associated with the genetic susceptibility to undifferentiated ankylosing spondylitis [82] with a similar pattern was also observed in AS patients in a study by Shiau et al, in Taiwan [83]. A more recent study by Lu et al [84], demonstrated that HLA-A33-B58-Cw10 haplotypes associated with TNF-alpha promoter -308(G/A) polymorphism might play an important role in disease pathogenesis of AS in Chinese population partially related to increased TNF-alpha production.

The therapeutic options for patients suffering from severe forms of spondyloarthritis (SpA) have been rather limited in recent decades. However, there is now accumulating evidence that anti-TNF therapy is highly effective in SpA, especially in ankylosing spondylitis (AS) and psoriatic arthritis (PsA) and that this treatment seems to be even more effective than the same therapy in rheumatoid arthritis (RA) [85]. There is however, little evidence to suggest that TNF-alpha genetic diversity plays a role in the effectiveness of currently available anti-TNF treatments. Much of this information is discussed in a greater detail in the recent manuscript by Braun et al [85]. In brief, the anti-TNF-alpha agents currently available, infliximab (Remicade); Centocor), etanercept (Enbrel); Amgen) and adalimumab (Humira; Abbott), are approved for the treatment of RA in the US; infliximab and etanercept are approved in Europe. The situation in SpA is different to RA because there is an unmet medical need, especially in AS, since no therapies with disease-controlling anti-rheumatic drugs are available for severely affected patients, especially with spinal disease. Thus, TNF blockers might even be considered as first-line immunosuppressive agents in patients with active AS and PsA who are not sufficiently treated by non-steroidal anti-inflammatory drugs and sulfasalazine, if peripheral arthritis is present. For infliximab, a dosage of 5 mg/kg at intervals between 6 and 12 weeks was necessary to constantly suppress disease activity; this is also a major aim of long-term treatment. No dose-finding studies have yet been

performed. The standard dose of etanercept is 25 mg s.c. twice-weekly. No studies on adalimumab (standard RA dose 20 - 40 mg s.c. every 2 weeks) have yet been conducted in SpA. The efficacy of etanercept was first demonstrated in PsA and etanercept is now approved for this indication. A double-blind study has also been performed in AS, with similarly clear-cut efficacy [85]. There is preliminary evidence that both agents do also work in other SpA such as undifferentiated SpA.

Insulin dependent diabetes mellitus (IDDM) is strongly associated with the inheritance of the HLA DR3 and DR4 antigens. Studies of SNP have revealed weak association with the TNF -308*A allele, but in each study this could also be attributed to linkage disequilibrium with HLA-DR3 [86]. Similarly, the investigation of the TNF genotypes in Type II diabetes have demonstrated that 308 TNF- α gene polymorphism may contribute to CHD risk in patients with type-2 diabetes and it could constitute a useful predictive marker for CHD in type-2 diabetic women [25].

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system associated with HLA DR2 in North Europeans and North American Caucasians [87]. The TNFA-308 polymorphism was investigated by several groups, [88-89] none of these studies showed any significant association with MS.

TNF Polymorphisms and Transplantation

Biopsies from rejecting kidney or heart transplants show the presence of an inflammatory infiltrate of cells capable of producing TNF. Studies in kidney [14], heart transplant recipients [15] and liver [88] transplant recipients revealed an association between TNF A -308 polymorphisms and acute rejection. Death of heart transplant recipients as a consequence of irreversible acute rejection of their graft is exclusively confined to those of the TNF α -308*A genotype.

TNF- α Genotype in inflammatory conditions

Inflammation is a major component of a range of diseases. A TNFA -308*allele association is evident in the liver disease primary sclerosing cholangitis [89]. Equally, in primary biliary cirrhosis there was shown to be an association with the TNF -308* A allele shown in a group of Scottish patients awaiting liver transplants [90]. In addition, there are several inflammatory conditions of the gastrointestinal tract such as coeliac and crohn's disease [13] that show a positive association with the -308*A allele, although the last is very weak. By contrast, in ulcerative colitis the -308*A allele seems to have a protective effect [91]

TNF- α Genotypes in Cancer

The influence of cytokine genotype on susceptibility to cancer or its prognosis has been considered. The areas looked at have been cancers of the immune system such as Non-Hodgkin's lymphoma where the TNF-308*A and LT- α Nco1*1 alleles were found to be related to overall survival and progression free survival [92]. In chronic lymphocytic leukaemia again, the TNF-308 alleles were associated with the disease [93]. Hence high TNF production may promote the condition leading to the development of cancer, an indirect susceptibility factor for these diseases.

TNF- α Genotypes in Coronary Artery Disease

Coronary artery disease (CAD) is both multifactorial and polygenic in nature with atheroma formation, the pathological hallmark. CAD is an inflammatory process, with proinflammatory cytokines, such as TNF- α , having a major role in its pathogenesis [19]. Through its effects on lipid metabolism, insulin resistance and endothelial function, TNF- α is reported to be involved in the CHD. Whilst some reports

have suggested that -308 TNF- α gene polymorphism may contribute to CHD risk in patients, [4] others have found no evidence of influence on the CHD outcome [16].

TNF- α Polymorphisms and Restenosis in Coronary Artery Disease

Restenosis is still the main drawback of percutaneous transluminal coronary angioplasty (PTCA) [94]. It is thought to be multi-factorial process where recoil of the vessel, neointimal proliferation and thrombus formation are thought to play a role. Until now it has proved difficult to predict restenosis on clinical and procedural grounds, however, genetic epidemiology might provide more insights.

TNF- α has a broad spectrum of biologic activities and is predominantly known for its powerful proinflammatory effects [3]. In response to different stimuli such as mechanical injury of the arterial wall caused by stent deployment, a local inflammation is elicited that is characterised by adhesion and invasion of inflammatory cells. Tight control of gene activity and protein production may equilibrate the pro and the anti-inflammatory potentials of TNF- α and in turn prevent excessive inflammation and limit neointima formation [95]. However, imbalances in the regulation of this system may interfere with anti-inflammatory effects and stimulate proinflammatory activities that result in neointima formation and restenosis. The genes encoding TNF- α contain variable sites that may be associated with different responsiveness to regulatory signals. In particular SNP located in the promoter regions of the TNF- α gene (-308G/A) were found to differentially affect binding of nuclear transcription factors, transcriptional activity and/or protein production, [8, 52] characteristics that may be related to the unfavourable outcomes after coronary interventions.

Effect of TNF- α Promoter on Transcriptional Activation

Studies have suggested that the polymorphism at -308 has a significant effect on transcriptional activity in reporter gene assays and that this could explain the association between the high TNF- α phenotypes and the DR3 haplotype [8]. The molecular mechanism of this difference is not completely clear because no evidence was reported of a major difference in affinity of the DNA-binding protein(s) to the two allelic forms of the TNFA promoter. Perhaps as a result of difference in the DNA/chromatin structure at the polymorphic site, the interaction of transcription factors is enhanced leading to stronger transactivation of the TNF- α gene. Interestingly, a homologous sequence in the TNFA promoter (-254 to -230) has been shown to bind a transcriptional repressor and not AP2 [96]. It may be, therefore, that a novel protein binds to the polymorphic TNFA -308 site and evidence obtained from a study using both Jurkat and U937 cells has demonstrated binding of a novel protein only to the TNF2 allele [96]. A relatively recent study of TNF- α production from peripheral blood mononuclear cells stimulated with anti-CD3 and anti-CD28 has shown a higher TNF- α production phenotype in TNF2 carriers, and of two TNF haplotypes, differing only at -308, the TNF2 +ve haplotype produced significantly more TNF- α [74]. There is, therefore, evidence that the TNF2 genotype is associated with increased TNF- α productions *in vitro*.

TNF- α Gene Polymorphism Association in the Nutrition-Inflammation Axis

The success of the Human Genome Project and the powerful tools of molecular biology have ushered in a new era of medicine and nutrition. This new era of molecular nutrition—that is, nutrient-gene interaction can unfold in dichotomous directions. One could focus on the effects of nutrients or food bio-actives on the regulation of the gene expression (i.e. nutrigenomics) or on the impact of variations in gene structure on one's response to nutrients or food bio-actives (i.e. nutrigenetics). In a simple classification of nutritionally modifiable genes as being either constitutive or inducible, it is the second

order classification of these same genes as being either wild type or polymorphic that underlies the highly variable response of humans to a given diet (Figure-2). In order for TNF- α SNP to be a practical significance in the nutrigenetics paradigm, the SNP is likely to present with the number of characteristics such as exhibiting a high frequency in the general population of interest modifying or regulating proteins at the top of biological cascades or a rate limiting steps in intermediary metabolism and having attendant biomarkers that provide surrogate measures of clinical effect. At the present time there are only a few SNP that meet the criteria but from those that do we can learn a great deal.

CONCLUSIONS

Overall, TNF- α has been shown to be an important pro-inflammatory cytokine in disease and health. It is difficult to make general statements about the associations of TNF polymorphisms and TNF α production or pathology. The TNF polymorphisms are found in a region of great polymorphic variation and they are in linkage disequilibria with the HLA genes and with each other. Because of differences in the distribution of HLA alleles one might expect variation in associations between TNF polymorphisms and various conditions in different geographical areas. Indeed, there is a geographical variation in the frequency of the -308*A allele. It is present in approximately 5% of the population of South Africa compared with 30% in white Caucasians in the United Kingdom. Specimens from blood donors of regionally subdivided British population demonstrated significant differences exist among the subgroups of the population and it is argued that these may be involved in the invariable responses experienced in disease process [97].

Given the biological regulation of TNF- α and its role in the inflammatory process, it is perhaps surprising that the genetic influences on cytokine production have much influence on disease processes and their outcome. The associations between TNF- α genotype and disease are not absolute as suggested

by different conflicting studies (Table-1). Nevertheless, it is clear that the genetic regulation of TNF- α at sites of inflammation is important. Under circumstances where the release of TNF- α has been triggered the genetically endowed capacity for greater TNF- α production leads to more severe inflammatory reactions.

Given the relationship between TNF- α genotype and disease, the prognostic value of TNF- α genotyping is obvious, particularly in situations such as organ-transplantation where knowledge of the likely immune response to the graft may lead to modification of the immunosuppressive regimen. Other applications are readily foreseeable, too, so that genotyping for TNF- α polymorphisms is likely to be useful in many areas of medicine. To understand our personal uniqueness, nutrigenomics SNP is likely to open the door to the management and optimisation of our health through tailored nutrition. Clearly this is a lofty and distant goal; equally true is the fact that we have already started the journey towards it.

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DECLARATION: We declare no conflict of interests

FIGURE LEGENDS

Figure 1: TNF complex on chromosome 6, showing location of microsatellites and SNPs (See reference 11)

Figure 2: Summary of the molecular nutrition hypothesis

Figure-1:

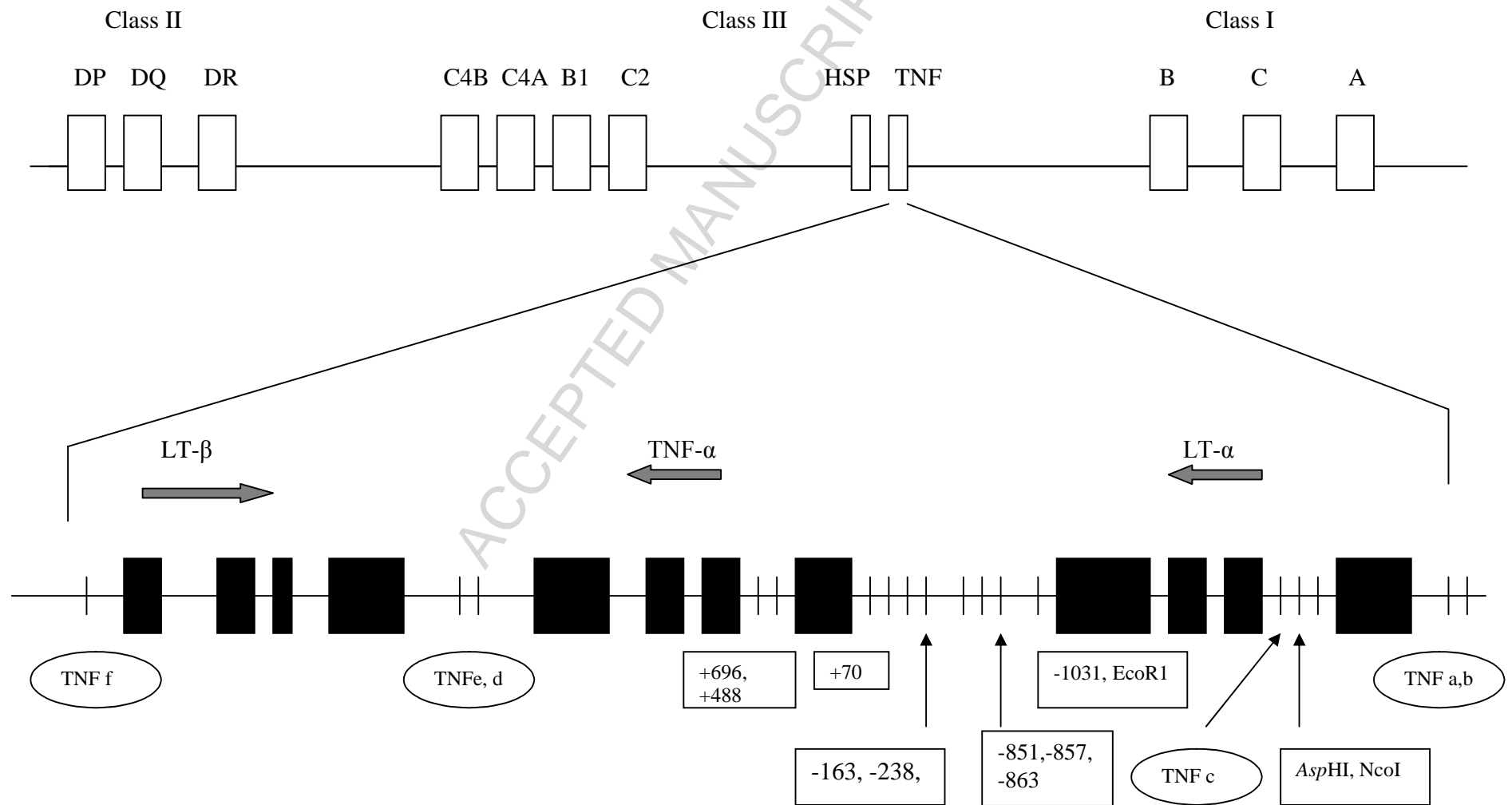


Table-1: Characteristics of published studies showing the associations between TNF- α genotyping and disease outcome.

Subcontinent/ Country	POPULATION/ REGION	DESIGN	DISEASE GROUP	NO of SUBJECTS	FINDINGS	ASSOCIATION	REFERENCE
AFRICA							
KENYA	Western Kenyan Native	Observational	Malaria	1,048 children	TNF2 homozygosity was associated with preterm birth, higher risk of death, high density of P.falciparum parasitemia, & severe anaemia	TNF-308 promoter polymorphism allele 2 is a risk factor for early childhood mortality and malaria morbidity in children	Aidoo M et al., 2001 ¹¹
SOUTH AFRICA	Native Blacks	Case control	Systemic Lupus Erythematosus	98	Reduced TNF-2 allele level to disease	TNF-308 promoter polymorphism is not an independent risk factor to SLE	Rudwaleit M et al., 1996 ¹²
EUROPE							
BELGIUM	Belgian Caucasian	Non randomised receiving Infliximab Treatment	Fistulizing and Non Fistulizing Crohn Disease	226 patients + 128 Healthy	Frequencies for TNF-308 were not different for responders and non responders	TNF-308 promoter polymorphism is not associated with clinical response to treatment in Crohns Disease	Louis E et al., 2002 ¹³
SWEDEN	Natives	Retrospective Analysis	Kidney Transplants	157 patients	Tendency towards a increased risk of rejection episodes and worse survival in TNF-308/2 recipients (P<0.02)	TNF-308/2 allele have significantly increased risk for early graft rejection	Wramner LG et al., 2004 ¹⁴
	Goteborg	Observational	Heart Transplant	70 patients	Increased CAV and mortality in Homozygous for TNFA and TNFB	Positive association with homozygous TNFA and TNFB in morbidity and mortality.	Ternstrom L et al., 2005 ¹⁵
	Gothenburg	Observational	Coronary artery bypass graft surgery	86 CABG patients	No difference observed with allelic frequency and TNF- α production	TNF- α gene polymorphism does not influence the postop inflammatory process	Westerberg M et al., 2004 ¹⁶
IRELAND	Dublin	Case control	Meningococcal Disease	183 patients + 389 controls	Frequencies for TNF-308 were not different in survivors and non survivors	TNF-308 promoter polymorphism is not associated with disease outcome	Balding J et al., 2003 ¹⁷
UNITED KINGDOM	Edinburgh/ Scotland	Case Control	Acute Pancreatitis	190 patients +	Frequencies for TNF-308 were not different in patients and controls	Genetic factors are not important in determining TNF- α secretion in	Powell JJ et al., 2001 ¹⁸

				102 Healthy		patients with Acute Pancreatitis	
	Blackpool	Case control	Coronary artery disease	One vessel (58), multi-vessel (122), normal et coronaries (79), Healthy (250)	No significant difference in TNF- α 308 was found between the groups	No correlation found b.w TNFA 308 with the development of CAD.	Allen RA et al., 2001 ¹⁹
FRANCE	Pessac	Observational	Coronary artery disease	Male CAD (299)	Genotype – 308 G/A TNF- α , A allele increase in unstable / stable angina (P= 0.029) and not in MI	Confirmation of crucial role of TNF- α in unstable angina	Bernard Vet al., 2003 ⁴
GERMANY	Southern Germany	Case Control	Ankylosing Spondylitis	96 patients + 58 healthy	Reduced TNF –308/2 allele Expression in patients	TNF-308/2 is protective for AS in Southern German Population	Milicic A et al., 2000 ²⁰
	Marburg	Case control	Chronic obstructive pulmonary disease	113 COPD, 113 CAD, 243 Healthy	No significant difference in TNF- α 308, TNF-B, IL-6 and IL-10 b.w COPD and counterparts	No correlation found b.w TNFA 308 with altered/ abnormal inflammatory response	Seifart C et al., 2005 ²¹
	Bonn	Non randomised receiving immunosuppressive therapy	Aplastic Anaemia	56 patients + 117 healthy	Positive response to therapy among carriers of the TNF2 gene	TNF-308/2 is contributes to the pathogenesis and therapeutic response of AA	Demeter J et al., 2002 ²²
	Bonn	Clinical	Coronary artery bypass surgery	CABG on CPB 47; CABG off pump 36	Homozygous TNF-B2 allele had increase TNF- α levels	Strong association reported	Schroeder S et al., 2003 ²³
SPAIN	Zaragoza	Case control	Peptic and Duodenal ulcer	130 DU + 50 PU patients + 102 healthy	Reduced TNF-308/2 frequency in PU in contrast to DU	TNF-308 allelic distribution contributes to the pathogenesis of PU and DU	Lanas A et al., 2001 ²⁴
	Tarragona	Case control	Type 2 Diabetes	235 CAD patients DM –ve; 106 CAD DM +ve; 135 DM CAD –ve, 207 health	- Gender difference +ve. TNF-A/308 increased in CAD and with or without DM than controls (P= 0.0056)	308 TNF- α gene polymorphism contribute to CAD risk with DM	Vendrell J et al., 2003 ²⁵
	Madrid	Case control	Celiac Disease	157 patients + 305 Healthy	TNF-2 (A) was increased in patients than controls	TNF-308 allelic distribution contributes to the pathogenesis of Celiac Disease	Fernandez L et al., 2002 ²⁶

<i>ASIA</i>							
INDIA	Tamil Nadu/ Brahmin	Population based study	Schizophrenia	86 unrelated trios	Frequencies for TNF-308 A allelic were not associated with schizophrenic cases	TNF-308 promoter polymorphism is not an independent risk factor to disease	Handoko HY et al., 2003 ²⁷
CHINA	Chinese Han	Case control	Schizophrenia	314 patients + 340 Healthy	Frequencies for TNF-308 G/A allelic were not different in patients and controls	TNF-308 promoter polymorphism is not an independent risk factor to disease	Duan S et al., 2004 ²⁸
	Taiwanese	Case control	Type2 Diabetic Mellitus	261 patients + 189 Healthy	G-308 promoter polymorphism and high fasting plasma glucose levels adjusted for age, sex, BMI and diabetic status was found	TNF-308/2 genotype might be more susceptible to diabetic complications	Shiau MY et al., 2003 ²⁹
INDONESIA	Sulawesi; ethnic groups (Bugis, the Makassans and Torajans)	Population based study	Parasitic infection	150 Bugis, 168 Makassans and 58 Torajans	Significant differences exhibited at the TNF loci TNF-308/2 (p<0.05)	Unique allelic combinations with potential to influence cytokine secretion are present in Sulawesi.	Lamsis F et al., 2002 ³⁰
JAPAN	Tokyo & Nagoya	Case control	Schizophrenia	297 patients + 458 Healthy	TNF-308G>A polymorphism frequency of both the patients and control are low (1.5% and 0.8% respectively)	Japanese schizophrenics are unrelated to the -308G>A polymorphism of the TNF- α gene	Hashimoto R et al., 2004 ³¹
KOREA	Seoul	Population based study	-	80 individual from 20 normal Korean families + 133 unrelated healthy controls	TNF-308 A were found more frequently in Koreans (91.3%) than Caucasians (p<0.001)	TNF microsatellite haplotypes constitute a highly polymorphic system with possible association with disease process	Kim HK et al., 2000 ³²
MIDDLE EAST	Palestinian & Jordanian	Case control	Behcet's Disease	102 patients + 115 Healthy	TNF-308/2 allele polymorphisms was not raised in blind and non blind subjects	A primary role of TNF-2 allele was not associated with the development of ocular disease	Verity DH et al., 1999 ³³
<i>OCEANIA</i>							
AUSTRALIA	Australian Caucasian	Case control	Schizophrenia	65 patients + 151 healthy	Frequencies for TNF-308 A allelic were not associated with schizophrenic cases	TNF-308 promoter polymorphism is not an independent risk factor to disease	Handoko HY et al., 2003 ²⁷
	Busselton	Population Based study	Asthma	1004 subjects from 230 families	TNF-308/2 was more strongly associated with disease (p=0.002); showed association with bronchial	Functional allele TNF-308/2 might be responsible for an increased risk of asthma.	Moffatt MF et al., 1999 ³⁴

					hyperresponsiveness (p<0.0001) and the use of inhaled or oral steroids (p=0.04)		
FIJI	Indigenous Fijian	Case control	Schizophrenia	134 patients + 92 Healthy	Frequencies for TNF-308 A allelic were not associated with schizophrenic cases	TNF-308 promoter polymorphism is not an independent risk factor to disease	Handoko HY et al., 2003 ²⁷
NORTH AMERICA							
USA	Alabama	Clinical Trial	Rheumatoid Arthritis	457 subjects with early RA	No association	TNF-308 promoter polymorphism is not an independent risk factor to disease	Hughes LB et al., 2004 ³⁵
	Boston	Prospective Observational Study	Asthma	708 children	No association	TNF-308 promoter polymorphism does not influence asthma susceptibility	Randolph AG et al., 2005 ³⁶
	Dallas	Observational	Sepsis	159 patients with burns	TNF- α -308 A allele imparted 1.8 fold increase of developing sepsis	Strong association of TNF- α -308 polymorphism with sepsis	Barber RC et al., 2004 ³⁷
	California	Prospective Observational Study	Sickle-cell anaemia	230 children with SCA	The combination of TNF -308 GG homozygosity was associated with a particularly strong predisposition to large vessel stroke in SCA	TNF -308 play a role in predisposition to specific stroke subtypes in children with SCA	Hoppe C et al., 2004 ³⁸
	San Diego	Case control	Asthma	Asthmatic (236) and non asthmatic(275)	TNF- α -308 A allele was correlated with asthma(P<0.04)	↑ risk of asthma with TNF-a 308 * A	Witte JS et al., 2001 ⁹
MEXICO	Mastizos	Case control	Rheumatic heart disease with Mitral valve lesion and disease	87 patients + 101 healthy	MVD showed increased frequency of -308 (AG) and (A), while MVL showed increased frequency of -308 (A)	Distribution of -308 polymorphism was similar in MVD and MVL	Hernandez-Pacheco G et al., 2003 ³⁹
	Mastizos	Case control	Systemic Lupus erythematosus	51 patients + 55 healthy	TNF-308 and genotype distribution failed to show any difference between disease process and healthy	TNF-308 does not play a any role in SLE	Zuniga J et al., 2001 ⁶
SOUTH							

<i>AMERICA</i>							
BRAZIL	Native	Prospective Observational Study	-	295 men + 389 women	Increased hs-CRP serum levels in individuals harbouring TNFA2 allele (p<0.05)	Positive association of disease with the presence of TNFA2 allele	Araujo F et al., 2004 ⁴⁰

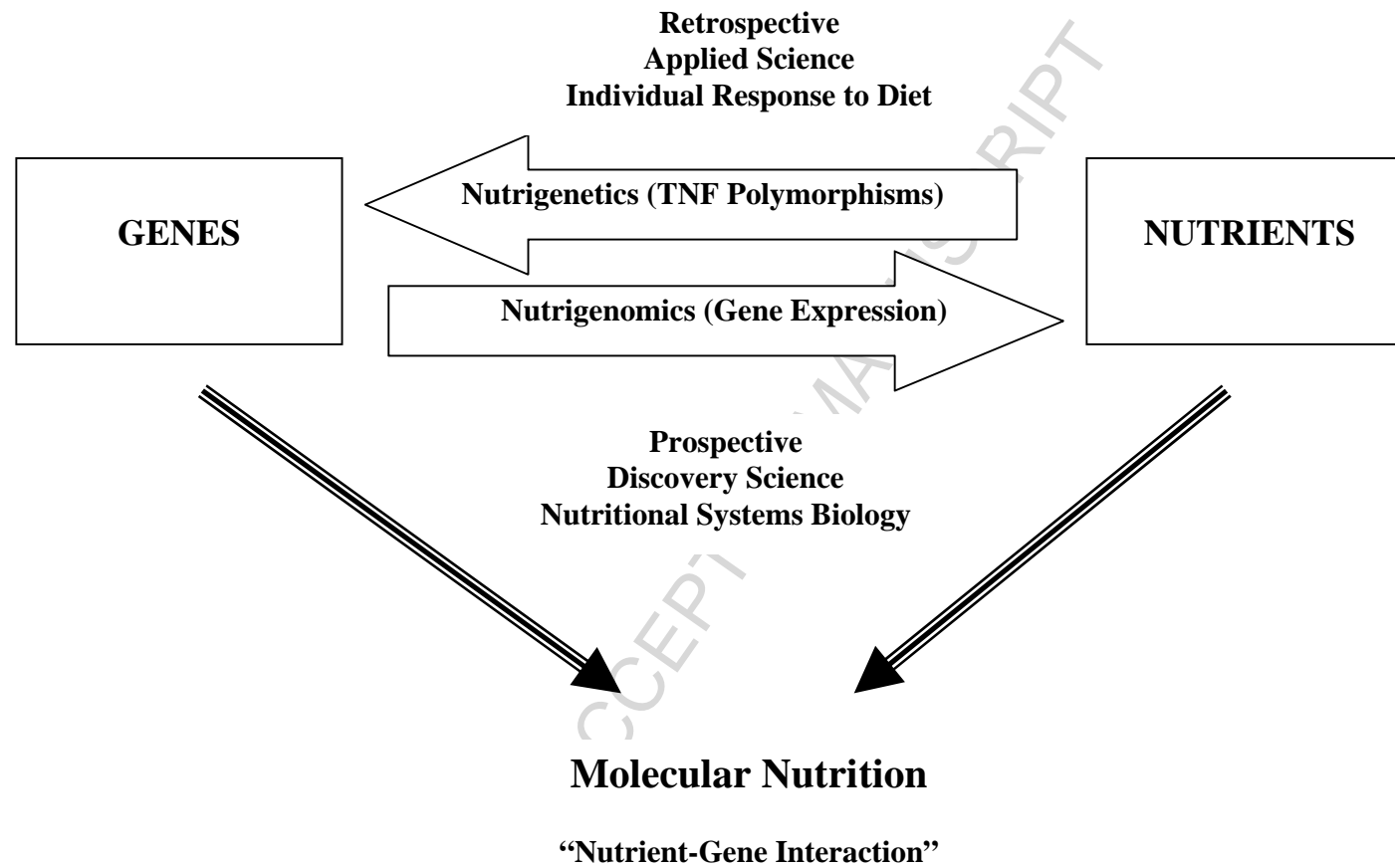


Figure-2: