METHYLENETETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM IN PSORIASIS IN CHINESE HAN POPULATION

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1. INTRODUCTION

Psoriasis is chronic dermatological immune mediated disease, that has a characteristic feature of well-defined erythematos plaques and papules that are capped with very large, loose scales that have a silvery appearance. The disease has a very unpredictable course.\(^1,\)\(^2\)

One of the most characteristic features of psoriasis is that the epidermal layer especially the outer most layers of the skin that act as protective barrier from any external stimuli are defective. The other feature, other than the defective epidermal barrier is the high rate of turn over that causes an additional harm to those who have psoriasis.\(^3,\)\(^4\)

As a result of the contribution of these two mechanisms the quantity of water loss leading to dehydration of the skin and a higher vulnerability to skin infections occurs all of which contributes towards the increased disease morbidity.\(^5\)

Psoriasis is a one of those type of dermatological disorders that exists in a variety of forms, but the note worthy fact is that all forms have one or the other forms of genetic abnormality.\(^12\) The heritable susceptibility for psoriasis till date has not been thoroughly evaluated, but one of the well known associations that independently contributes towards the morbidity to the disease is the methylenetetrahydrofolate reductase gene abnormalities that causes very high levels of plasma homocysteine.\(^6,\)\(^7\)

Psoriasis is a disease in which the morbidity not only affects the skin it also has its untoward effects on various other systems of the body ranging from the peripheral vascular system to the nervous system.\(^8\) The abnormality responsible for the morbidity of the disease is the very high levels of plasma homocysteine; which itself is considered as an independent risk factor for CVS disorders.\(^9,\)\(^10\) The elevation of the levels of plasma homocysteine can be attributed either to acquired or genetic factors.\(^11\) Among the disorders that are inherited relating to the enzyme deficiency. Methylenetetrahydrofolate reductase deficiency is one of the common ones that leads to elevated levels of plasma homocysteine and is a known risk factor for psoriasis.\(^12\)
The methylenetetrahydrofolate reductase gene defects not only are known an association with psoriasis but also with elevated homocysteine levels that is responsible for the pathogenesis of morbid events, among all the genetic changes that are seen the deletion of C allele of the methylenetetrahydrofolate reductase gene is the least studied though it is associated with serious effects.

**EPIDEMIOLOGY**

The data that is available on the epidemiology of the disease states that the disease is distributed universally and is seen in all nations of the world. Griffiths et al in their study that was published in the year 2017 stated that in countries that are nearer the equator like the Asian countries with a lower latitude have a lower prevalence of the disease.\(^1\)[13]

**PATHOGENESIS**

The specific pathogenic mechanism of the disease is still a mystery. Adaptive immunity is considered as the main force in the development of psoriasis.\(^14-16\) The presence of copious number of lymphocytes and dendritic cells in the tissues of the psoriatic and efficiency of drugs targeting the adaptive immune system have led to this conclusion.\(^14-16\)

The cells of the immune system namely the keratinocytes and T lymphocytes are said to play the role a major contributor in its pathogenesis.\(^14-16\)

The cells of the skin termed as the keratinocytes are known to have a role in the immune response of the cutaneous system by means of expression of cytokines are found to have an amplified expression of the cytokine interleukin-23.\(^14-16\) This interleukin has a very important role to play in the activation of the memory T cells and is needed to generate the interferon gamma which helps in the continuation of the inflammatory process.

The presence of these T lymphocytes has also been confirmed by immunophenotyping and hence they have been implicated in the causation of the disease.\(^11,17,18\)

It is well known that the T cells following their activation are known to release the cytokines that belong to the type 1 variety that cause the keratinocytes that are present in the skin to hyperproliferate by stopping the process of apoptosis.\(^17,18\)

The stimulus from the tumor necrosis factor alpha is responsible for the release of cytokines from T type lymphocytes and macrophages. The chemokine discharge from the macrophages, coupled along with the appearance of adhesion molecules on the cells of the endothelium of the blood vessels in the tissue in combination contributes to the pathogenesis of the disease. This pathogenic mechanism also makes a major contribution to the cardiovascular morbidity of the disease vascular endothelial cells. The inflammation leads to oxidative stress that may have systemic consequences since high levels of oxidants stimulate the formation of atherosclerotic lesions.\(^17,18\)

**THE SITES AFFECTED BY THE PSORIASIS**\(^19,20\)

The disease can affect practically any part of the body, but still the most common sites that are affected by the disease are as follows: -

1. The scalp layer of head,
2. The skin over the knees and elbows,
3. The lower part of the back,
4. The face,
5. The hands and the feet,
6. The nails,
7. The region of the genitals in both males and females,
8. The folds of the skin.

**CLASSIFICATION**

The classification of psoriasis based on the sites and severity the disease is classified into two main types.\(^21-23\): -

1. The localised form of psoriasis
2. The generalised form of psoriasis
   - The localised form of psoriasis the areas affected are as follows.\(^21,22\): -
     a. Psoriasis that affects the folds exclusively,
     b. The seborrhic variety of psoriasis,
     c. Psoriasis of the scalp,
     d. Psoriasis of the hands and feet – the palmo plantar variety,
     e. Psoriasis plaque variety that affects the limbs exclusively,
     f. Psoriasis plaque variety that affects the trunk exclusively.
   - The generalised form the areas affected are as follows \(^21\)-\(^23\): -
     a. Gutatte variety of psoriasis,
     b. Generalised plaque psoriasis,
     c. Erythrodermic variety of psoriasis,

There are 7 varieties of psoriasis that the researches have delineated based on the clinical appearance and association of the lesions with various trigger factors.\(^24\): -

i. Plaque Variety of Psoriasis,
ii. Inverse Variety of Psoriasis,
iii. Erythrodermic Variety of Psoriasis,
iv. Palmoplantar Variety of Psoriasis,
v. Psoriatic Arthritis,
vi. Nail Psoriasis,
vii. acute pustular variety of psoriasis

**CLINICAL FEATURES**\(^25\)

Chronic plaque psoriasis typically is symmetric and bilateral. Lesions begin as papules and eventually coalesce to form plaques. Plaques have silvery scale covering which are well demarcated. Plaques exhibit the Auspitz sign that refers to the bleeding occurring after the removal of scale lesion of psoriasis.\(^26\) Koebner phenomenon (lesions induced by trauma) The candle
The extensor surfaces (elbows and knees) commonly are involved, as well as the lower back, the scalp, and the nails. The nail changes include onycholysis, that is the separation of the nail from its bed; pitting of the nails, oil spots that are either yellow or brown spots caused by cellular debris under the nail), and dystrophy of the nails. Most of the patients eventually develop nail involvement, although very rarely the nail findings precede skin findings in about four percent of patients that are affected with psoriasis. Aged patients, those with chronic disease, extensive skin lesions, or joint involvement have more nail involvement.\[25-31\]

COMPLICATIONS

Though psoriasis is known as a very disabilitating and a chronic disease, it rarely affects the life span of the affected person. None the less especially Chronic plaque psoriasis has associated with it a variety of co-morbid conditions that range from simple benign lesions that cause no harm to severe life endangering malignancies.

The diseases often found to accompany psoriasis are as follows: -

Non – psoriatic arthritis, Psychological and emotional disturbances from the disfigurement that is caused by the lesions, Cardiovascular events like myocardial infarction and acute coronary events. The other psychartic disturbances that are often seen with the disease are depression, anxiety, sexual dysfunction, poor self-esteem, and suicidal thoughts may coexist with psoriasis, even in patients with less severe disease\[32\]. Malignancy, Cohort studies have shown that there is an increased risk of cutaneous malignancy associated with psoriasis, especially of the non-melanoma category especially the lymphomas.\[32\]. This risk of cutaneous malignancy is much higher in those patients who are affected with more severe disease, but it is not clear whether disease severity or treatment accounts for the increased risk.\[33\]

Psoriatic arthritis is considered as an inflammatory, seronegative arthritis that has an a variable course. The arthritis commonly is asymmetric and involves the fingers and toes. The estimates of the prevalence of psoriatic arthritis vary widely, but the authors of one study\[30\] at a higher referral centre found that one third of patients with psoriasis had arthritis and that, in two thirds of those with arthritis, skin lesions preceded arthritis.

THE MANAGEMENT OF PSORIASIS

The Treatment of the disease can be grouped into two categories

1. Non Pharmacological interventions
2. Pharmacological interventions

(a). systemic and
(b). topical

GENERAL APPROACH

When we treat a patient with psoriasis it has to be remembered that the patients and their family remembers have to be counselled that this disease is a disease which is never completely cured and that should have a realistic expectation from the treatment they get. The management and a more comprehensive treatment of the disease hence involves a multi disciplary.\[34\] The treatment itself should focus on the improvement of symptoms and to postpone the worsening of the disease at the related complication not disappearance, of lesions. the other important fact to be remember that with only the use of topical medications it is not possible to completely treat the lesions of the disease and expect them to be cured miraculously Complete clearing of lesions usually is not possible in patients who use only topical therapy, and overuse of topical therapies results in more side effects.

Caution has to be taken when we treat the high risk groups like pregnant women and children as they are more prone for adverse effects.\[35\]

TOPICAL THERAPIES\[36\]

The Topical medications that have proven to be beneficial in the management of the disease are as follows:-

Topical steroids, Topical derivatives of vitamin D, Topical retinoids, Topical immunosuppressants, Topical anthralin, and Topical coal tar ointment.

Topical Steroids

The potent topical steroids like the the mild potent fluocinonide ointment, mometasone ointment and the very potent clobetasol, halobetasol propionate ointment to a very great extent improve the lesions of psoriasis especially the plaques. The short duration averaging about four weeks of treatment was a major limitation.

Skin atrophy, striae, and tachyphylaxix is the main side effects of topical steroids. Only weak that is the nonfluorinated topical steroids should be used on the face and in skin folds. Longer duration usage of these drugs and higher potency and occlusive dressings had increased side effects.

Vitamin D Derivatives

The action of Vitamin D and its products is by inhibition of keratinocyte proliferation and keratinocyte differentiation improvement. Some studies found that calcipotriene has efficacy as topical steroids and more effective than placebo in psoriasis.\[37,38\] Ratger than the topical agent alone the calcipotriene used in combination with topical steroids improves psoriasis. The main side effect of calcipotriene is perilesional skin irritation and rarely it can require the cessation of treatment.\[38\]. Skin irritation can be reduced by combination of calcipotriene and topical steroids.
**Topical Retinoids**

The topical retinoid tazarotene has shown improvement in chronic plaque psoriasis. Success rate of tazarotene at 12 weeks on elbow and knee lesions showed 65% with tazarotene 0.1% with fluocinonide. Combination with steroid and tazarotene 0.1% is better tolerated than tazarotene alone. The overall treatment response at 12 weeks is better with tazarotene and steroid group compared to tazarotene placebo group. In comparison of treatment with calcipotriene has more adverse effects. Tazarotene is teratogenic.

**Topical Immunosuppressants**

Tacrolimus and pimecrolimus are commonly used topical immunosuppressants and they act as immune modulators, and they reduces lesion and has symptomatic relief and changes course of disease.

**METHYLENETETRAHYDROFOLATE REDUCTASE AND ITS ROLE IN PSORIASIS**

Most important vitamin in plasma homocysteine level determination is folate. Elevated homocysteine levels are frequent in patients with chronic immune mediated disorders including chronic plaque psoriasis and psoriatic arthritis.[42-45] A crucial enzyme in homocysteine/methionine metabolism is methylenetetrahydrofolate reductase. The most studied of all the polymorphism is the C677T polymorphism in the methylenetetrahydrofolate reductase gene results in a thermolabile variant (T) with reduced activity of enzyme. Carriers of the 677TT genotype with low levels of folacin (folate or Vitamin B9) has been observed to have a diminished level of Deoxyribonucleic acid methylation compared with those of 677CC wild type genotype. Deoxyribonucleic acid (Deoxyribonucleic acid) methylation plays an important role in regulation of gene expression and cellular differentiation. So the methylation state of the genes can be speculated to modify phenotypes of psoriasis as well as the responsiveness to therapy.[42-45]

In the recent times it is gained importance that the Deoxyribonucleic acid methylation disorders might play a role in etiopathogenesis of psoriasis. Moreover the lower frequency of p16 gene methylation and SHP-1 (tyrosine phosphatase gene) gene methylation is found in psoriatic skin lesions. Methylenetetrahydrofolate reductase enzyme which catalyses the formation of 5-methyltetrahydrofolatin (folate or Vitamin B9) which is the methyl donor for synthesis of methionine from homocysteine. This cycle is important for maintaining the methyl donor for Deoxyribonucleic acid methylation and hence gene regulation and cellular differentiation. Polymorphism of the methylenetetrahydrofolate reductase gene involves the substitution of the nucleotide C with T at position 677. This gene polymorphism leads to a reduced enzyme activity and interferes with homocysteine levels. Folate and vitamin B12 are determinants of homocysteine levels and methylenetetrahydrofolate reductase gene had strict interaction with those molecules in metabolic cycle.[42-45]

Plasma homocysteine concentrations are found to be significantly higher in psoriatic patients. Decrease in utilisation of folacin (folate or Vitamin B9) due to methylenetetrahydrofolate reductase polymorphism may affect production rate of keratinocytes of which had faster reproduction rates with a continuous Deoxyribonucleic acid turnover. Consequently this may affect the severity of psoriasis which is characterised by keratinocyte proliferation. Decrease in folacin (folate or Vitamin B9) usage due to methylenetetrahydrofolate reductase polymorphism may effect the reproduction rates of keratinocytes which has faster turnover and the cells which continues Deoxyribonucleic acid synthesis and consequently may lead milder forms of psoriasis. Deoxyribonucleic acid methylation also decrease psoriasis severity by modifying or changing transcription of tumor suppression genes in occurrence of the disease. The promoter demethylation may play an important role in skin pathogenesis by enhancing SHP-1 isoform II transcription in psoriatic skin lesions. Folate and vitamin B12 levels are major determinants of homocysteine levels and methylenetetrahydrofolate reductase genes had strict interactions with those molecules in metabolic cycle. Notably low plasma folacin (folate or Vitamin B9) concentrations have previously been reported among psoriatic patients. Malabsorption and increased utilization of folacin (folate or Vitamin B9) for Deoxyribonucleic acid synthesis in skin cells have been proposed to be the cause.

In those cases of psoriasis vulgaris with methylenetetrahydrofolate reductase gene polymorphism are associated with hyperhomocysteinemia which has deleterious effect on cardiovascular system. Therefore, psoriasis vulgaris patients with methylenetetrahydrofolate reductase gene polymorphism may be at a greater risk of cardiovascular diseases and thromboembolic events.

Psoriatic transcriptome is widely defined as comprising molecules found on epidermal differentiation complex (1q), molecules of antimicrobial activity, hyperproliferation associated molecules, molecules involved in the regulation of proteolysis and cytokines and cytokine induced molecules associated with keratinocytes and T cells. Methylation state of the genes can modify phenotypes of the disease as well as the responsiveness to therapy. The lower frequency of p16 gene methylation and SHP-1 (tyrosine phosphatase) have been observed. Recently the application of methylation specific microarrays for simultaneous analysis of multiple genes seems to have implications in the diagnosis and research of the disorders with epigenetic imprinting involved in pathogenesis.

Methylenetetrahydrofolate reductase gene polymorphisms are associated with various types of
diseases including diabetes, various cancers including blood cancers like leukemia, hyperhomocysteinemia, myocardial infarction, coronary artery disease, neurological and psychiatric diseases, infertility and recurrent pregnant loss.

The methylenetetrahydrofolate reductase is located on the 1p36.3 locus and many polymorphisms have been identified in the methylenetetrahydrofolate reductase.\cite{46,48} C to T substitution in the methylenetetrahydrofolate reductase is located on the 677th nucleotide a missense mutation on the 4th exon\cite{48,49} methylenetetrahydrofolate reductase C677T polymorphism results in a valine to alanine exchange at the 222nd codon. This leads to an methylenetetrahydrofolate reductase enzyme with decreased enzymatic activity. Most common genetic cause of increased homocysteine levels is Mutation. The valine to alanine exchange at the 222nd codon frequently seen polymorphism were found to be associated with cardiovascular diseases, cancer, chronic idiopathic acrocyanosis and homocystenemia which is risk factor for developing psoriasis. A1298C (rs1801131) and C677T (rs11801133) are the most investigated methylenetetrahydrofolate reductase gene polymorphisms which results in a thermolabile variant (T) with reduced activity of the enzyme.\cite{48,49}

Sites of inflammation in psoriasis disease is controlled by a multistep adhesion cascade involving successive interactions between adhesion receptors on the surface of leucocytes and their counter receptors on vascular endothelium.\cite{49} Vascular adhesion protein-1 (VAP-1) is an adhesion molecule with an enzymatic activity that partakes in the migration process of lymphocytes into site of inflammation in rolling step and diapedesis.\cite{49} Several evidences on the other hand suggest that abnormalities in lipid metabolism, membrane lipid composition, and or features, lymphokine release, free radical generation and lipid peroxidation leads to progressive skin damage and high incidence of cardiovascular diseases in psoriatic patients. The amount of malondialdehyde (MDA) generated during lipid peroxidation of poly unsaturated fatty acids in psoriatic patients positively correlates the severity of disease. Moreover it has been demonstrated that psoriatic patients in which the higher risk of oxidative stress is supposed to contribute to the great incidence of vascular disease and other complications. Although few studies have shown increased levels of MDA, VAP-1 and lipids in psoriatic skin, erythrocytes, and serum of psoriasis patients as compare to the controls, but there are debate on association between methylenetetrahydrofolate reductase C677T (rs1801133) polymorphism and altered serum levels of MDA, VAP-1 lipids lipoproteins and apolipoproteins.

MATERIALS AND METHODS

The source of the data was those individuals who attended the department of dermatology at the Zhongnan Hospital of Wuhan University, China. In the present study we collected that the data from forty four patients who were affected with psoriasis who were willing to voluntarily participate in the study without any monetary gain. In the present study we collected that the from 1st of December 2016 to the 30th of November 2017. The patients with psoriasis were diagnosed in the department of dermatology of Zhongnan Hospital of Wuhan University. They were all of Han nationality. The patients with psoriasis and without blood relationship, There was no age limit for including the cases in the study, There was no exclusion criteria, Only those cases who agreed to participate in the study were chosen for the study. Before initiation of the study the approval of the Ethics Committee of Zhongnan Hospital of Wuhan University was taken. The study was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University, and abided by the Helsinki Declaration in the implementation process. All the subjects who were willing to participate in the study signed the Informed Consent Form prior to enrolling in the study.

The blood sample of the subjects were collected in purple capped tube that is an anticoagulant tube that contains the Ethylenediaminetetraacetic acid. To the anticoagulant tube approximately 5ml of fasting blood in the early morning and preserved at 4 degree centigrade. The genomic Deoxyribonucleic acid of peripheral blood was extracted by the phenol–protease K method, and the content and concentration of Deoxyribonucleic acid were determined by means of the ultraviolet spectrophotometer, A 260 /A 280 >18, and then it was kept at – 20 degree. After selecting the target gene, the primers were then synthesized by the Wuhan Optimus Family Innovational Biotechnology. The amplification conditions were as follows: 3 minutes at 94 degree centigrade, 30 seconds at 94 degree centigrade, 30 s at 56degree centigrade, 30 seconds at 72 degree centigrade, 30 seconds at 35 cycles, and 5 minutes at 72 degree centigrade.

The Polymerase chain reaction products were then analyzed by 2 agarose gel (containing bromoaceticdine). After the amplification results were verified, the Polymerase chain reaction products were sequenced by SnAmp shot method by the Wuhan Optimus Innovation Biotechnology Co., Ltd.

The Instrument and Reagents used for the data collection

Instrument and Reagents 5424 Desktop Centrifuge (Ependorf) SI000 Thermal Cycler PCR BIO (BIO-AD), DYY-6C electrophoresis instrument (Beijing Liuyi Instrument Factory), Tanon1600 Gel Imaging System (Shanghai Tianeng Technology Co., Ltd.); 2Xtsingke Master Mix (Wuhan Optimus Innovation Biotechnology Co., Ltd.), Blood Genomic Deoxyribonucleic acid Extraction Kit (DP318, Tiangen Biochemical Technology Co., Ltd.).
THE AIMS AND OBJECTIVES OF THE STUDY

The objectives of the study was as follows:

(i) After gene detection, synthesis of primers by Wuhan Optimus Family Innovational Biotechnology.

(ii) Polymerase chain reaction Amplification of target genes. Pre denaturation at 90degC for 3 minutes, then denaturation at 94degC for 30 seconds, followed by annealing for 30 sec at 56 degree Centigrade, extension for 30 seconds at 72 degree Centigrade, 35 cycles, last extension at 72 degree Centigrade for a period of 5 minutes. Agarose gel electrophoresis.

(iii) Analysis of Polymerase chain reaction products. The amplified results were then verified.

(iv) Graph 1:

Graph 2.
### CHAPTER THREE: RESULTS AND OBSERVATIONS

Table 2: Genotype and allele frequencies of MR-C677T (rs 1801133) in psoriasis and healthy controls.

<table>
<thead>
<tr>
<th>Gene and single nucleotide protein</th>
<th>Genotype/allele</th>
<th>Psoriasis (n=44)</th>
<th>Controls (n=50)</th>
<th>Chi - square</th>
<th>P – value</th>
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<tbody>
<tr>
<td>MR - C677T Gene (rs 1801133)</td>
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<tr>
<td></td>
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<td>TT</td>
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<td>T</td>
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<td>Dominant model</td>
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<td></td>
<td>TT</td>
<td>35</td>
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<td>Homozygotic</td>
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<td>NOT APPLICABLE</td>
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<td></td>
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</tbody>
</table>

Graph: Genotype and allele frequencies of MR-C677T (rs 1801133) in psoriasis and healthy controls.
Table 4: Genotype and allele frequencies of MR-C677T (rs 1801133) in psoriasis.

<table>
<thead>
<tr>
<th>Gene and single nucleotide protein</th>
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In the present study we had evaluated the Gene and single nucleotide protein MR - C677T Gene (rs 1801133) and we were of the conclusion that TC type (20.5), CC type, TT C type, T type were seen in 9 cases, 00 cases, 35 cases, cases, 9 cases and 79 cases respectively. the absence of the c allele was seen in 35 Cases of psoriasis. all the control displayed the TT allele of MR - C677T Gene (rs 1801133).

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On the 44 cases evaluated, on evaluation of the dominant model we found that 9 cases, accounting for 20.5 percent had the TC+CC allele variation the rest had the TT allele.

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On the 44 cases evaluated in the Recessive model we found that none had the CC allele all cases had the TT+CC allele.

<table>
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</table>

On the 44 cases evaluated in the Co – Dominant, Homozygotic model we found that none had the CC allele 35 cases had the TT+CC allele, that 9 cases, accounting for 20.5 percent had the only TT allele.

In the present study we had about 44 patients, on evaluation of the gender we had 40 males and 4 females. the age of the individuals in the study ranged between 9 to 86 years. On evaluation of the type of psoriasis in the 44 patients evaluated. 30 patients had plaque psoriasis, 7 patients had guttate psoriasis, 3 patients had pustular psoriasis, 2 patients had psoriatic arthritis, 1 patient with erythrodermic psoriasis and 1 plaque and pustular psoriasis. This study could not find a possible association with risk of psoriasis and methylenetetrahydrofolate reductace MTHFR C677T gene polymorphism in Chinese Han population.

**DISCUSSION**

In China; psoriasis is one of the diseases that is most often encountered in the dermatology clinic. In comparison of gender distribution with other studies in the present study we had a higher incidence of psoriasis in the male gender the male to female ratio was 10 : 1.

30 patients had plaque psoriasis, 7 patients had guttate psoriasis, 3 patients had pustular psoriasis, 2 patients had
Psoriasis is considered as a genetic disease that is very complex and it involves multiple genes, some of which interact with each other. Methyltetrahydrofolate reductase is one of the crucial enzyme in the metabolism of homocysteine/methionine. It is involved by catalysing the formation of 5-methyltetrahydrofolacin (folate or Vitamin B9) (5-methyl-THF), which is the methyl donor for synthesis of methionine from homocysteine (Hcy), thus deficiency of this leads to increase in the homocysteine levels.\cite{50-51} The folacin (folate or Vitamin B9) deficiency induces a hyper-homocysteine that is responsible for increase in the pro-inflammatory cytokine secretion leading to a constant inflammatory state in the affected individual. one of the effects of this, is, the methyltetrahydrofolate reductase polymorphism which can affect the production rate of keratinocytes. The keratinocytes begin to multiply at a faster reproduction rates with a continuous Deoxyribonucleic acid turnover and this influences the clinical outcome of psoriasis. As with other genes, many functional polymorphisms were reported in methyltetrahydrofolate reductase gene and among all of them the commonly discussed in pathologies are 2, the C677T (Ala222Val, rs1801133) polymorphism and the A1298C (Glu429Ala, rs1801131) polymorphisms. These common variations are the most studied because the alleles methyltetrahydrofolate reductase 677T and methyltetrahydrofolate reductase 1298C reduce the expression of methyltetrahydrofolate reductase enzyme.\cite{52} This mutation is a single nucleotide protein variation of the missense mutation category. The single nucleotide protein SNP was initially described by Frosst and his colleagues in the year 1995. In their research they gave the single nucleotide protein position according to the Deoxyribonucleic acid which they had cloned.\cite{53} The single nucleotide protein is in simple terms is the Deoxyribonucleic acid sequence and position given is for Deoxyribonucleic acid not the messenger ribonucleic acid, when the need to check in messenger ribonucleic acid is there the, check position for messenger ribonucleic acid in db single nucleotide protein also it is on reverse strand. Also T in messenger ribonucleic acid sequence is not found as the C allele is the wild type so, in database you will find only C.rs1801131 is a single nucleotide protein in the methyltetrahydrofolate reductase gene, representing an A > C mutation at messenger ribonucleic acid position 1298, resulting in a glu429-to-al-a (E429A) substitution (hence this single nucleotide protein is also known as A1298C or E429A). rs1801131 is a single nucleotide protein that is relatively common and has been studied for (relatively) a long time. Also known as C677T, Ala222Val, and A222V, it encodes a variant in the methyltetrahydrofolate reductase gene, which encodes an enzyme involved in folacin (folate or Vitamin B9) metabolism.\cite{54}

Many roles of these genes make a the hypothesis that their polymorphisms can be considered as a predictive factors associated with the increased susceptibility for psoriasis. The messenger ribonucleic acid gene has its location on the chromosome 1 at P arm p36.3 in human beings. There are many variants of the Deoxyribonucleic acid sequence that are associated with messenger ribonucleic acid gene. In a study that was done in the year 2012, the research team had a reported that over forty types of point mutation type of substitution take place. But among all of the point mutations that take place; the substitution of methyltetrahydrofolate reductase gene, C to T at 677th position remains the most problematic one.

Another finding of variants of mutation of the messenger ribonucleic acid gene polymorphisms is its association with various diseases. in the gene mutations coding for the methyltetrahydrofolate reductase enzyme, it has been found to be a new candidate for genetic risk factor for the following diseases in the general population:- increased cancer in the general population,\cite{54} increased risk of cardiovascular disease and venous thromboembolism,\cite{55-56} diabetic complications like peripheral neuropathy,\cite{57} increased risk of Cerbrovascular diseases,\cite{58} the increased risk of pregnancy losses,\cite{59-60} the increase in fetal disease like Down’s syndrome.\cite{61}

The other thing to be understood of this point mutation is that it is a is temperature mediated mutation, The mutation determines the loss of function of the messenger ribonucleic acid gene that is a temperature related, with the T allele having an enzyme activity of approximately 35 percent of the values observed in those individuals who carry the C allele.

The 677TT genotype is associated with higher total plasma homocysteine significant levels than in heterozygotes or in individuals with wild-type C alleles. This is demonstrated by Frosst, and co workers in their study in the year 1995.\cite{53}

The mutation in messenger ribonucleic acid gene at the nucleotide position 677 can have two types of possibilities:

1. C (cytosine, which occurs in the wild type gene) or
2. T (thymine, which occurs in the mutated gene).

In the first type the C677T variant the nucleotide position at 677 is modified from C to T and as an effect of this the replacement of alanine by valine residue in the protein at 222 amino acid position takes place. In those Individuals who have two copies of the 677CC or the 677TT mutation will have the wild type or mutated genes respectively, in both the homologous chromosomes. Some people are heterozygous for this type mutation where one homolog carries the C residue and the other the homolog carries the T residue as showed in the study.

Psaoritic arthritis, 1 patient with erythrodermic psoriasis and 1 plaque and pustular psoriasis.
The homozygous or the TT genotype is considered as being harmful because this type of mutation is associated with a very high concentration of the total plasma Homocysteine levels and a much lower messenger ribonucleic acid enzyme activity of only 30 percent. As a result of this combination it can cause defects in Deoxyribonucleic acid repair system in the enzyme. On the other hand, the other type of mutation, that is the 677CT genotype has messenger ribonucleic acid enzyme activity of about 65 percent and hence is less harmful.

The A1298C polymorphism in the methylenetetrahydrofolate reductase gene has also been associated with the methylenetetrahydrofolate reductase activity abnormalities This A1298C polymorphism is known to have a lower effect in reducing enzyme activity, compared with the 677 mutation. This is significantly seen in the homozygous (CC) state than in the heterozygous (AC) or normal (AA) states. Heterozygote individuals for both the C677T and the A1298C mutations were found to exhibit 50 to 60 percent of control activity, a value lower than that seen in single heterozygotes for the C677T variant.

CONCLUSION
The objective of this study is to investigate the genetic polymorphism of methylenetetrahydrofolate reductase C677T in psoriasis in Chinese Han population. In the study we came to the following conclusions:-
The disease of Psoriasis can have a varied manifestion in various forms like the plaque form, inverse form, guttate form, pustular form, and the erythrodermic form. Even when localised form of psoriasis is diagnosed a careful and complete examination, including that of the nails and of the scalp is necessary to correctly know the extent of the disease. Polymorphism in the methylenetetrahydrofolate reductase gene (677C>T, rs1801133) has been associated with psoriasis in a Chinese population but due to the absence of C allele and CC genotype in this study no association could be evaluated. Those with extensive form of the disease may have an association with polymorphism in the methylenetetrahydrofolate reductase gene (677C>T, rs1801133).

Investigations of methylenetetrahydrofolate reductase gene polymorphism association with psoriasis vulgaris in different countries showed conflicting results. The contradictory findings may be due to probable differences in ethnicities. The frequency of the methylenetetrahydrofolate reductase 677T allele varies substantially on different regions of world and among ethnic groups. methylenetetrahydrofolate reductase C677T polymorphism prevalence ranges from 0.20 to 0.55 among different populations. It may be hard to find the association of psoriasis of which 2-3 percent incidence with such a gene mutation with a higher incidence in population.

LIMITATIONS
The limitation of the study was as follows: -
The treatment of the disease was not studied. The effect of treatment and association of treatment in those affected with the absence of the C allele has not been evaluated in the disease.

The short duration of the study was the most important drawback of the study.

RECOMMENDATIONS
In the study we have the following recommendations:-

Studies on a larger population to be conducted to validate the data. The long term course of the disease in those who have absence of the C allele be done. The seasonal and ethnic various of the disease is well known and is not studied in the present study.

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REFERENCES


investigative dermatology, 2000 Mar 1; 114(3): 587-90.
57. Mitraoui N, Zammiti W, Ghazouani L, Brahman NJ, Saidi S, Finan RR, Almawi WY, Mahjoub T.


