

Original Article

Gene polymorphism of leukotriene A4 hydrolase (rs17525495) among tuberculous meningitis and its associations with clinical corticosteroid treatments in Han population of Northern China

Weibin Guo¹, Xin Liu², Xiaosu Guo³, Zeyan Zhao³, Weixin Han³, Yue Zhao³, Yueli Zou^{3*}, Hui Bu^{3*}

¹Department of Neurology, The Second Hospital of Shijiazhuang, Shijiazhuang, Hebei, China; ²Department of Neurology, People's Hospital of Huanghua City, Huanghua, Hebei, China; ³Department of Neurology, The Second Affiliated Hospital of Hebei Medical University, Shijiazhuang, Hebei, China. *Equal contributors.

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Abstract: This study is to investigate the association between Leukotriene A4 hydrolase (LTA4H) single nucleotide polymorphisms and susceptibility to tuberculous meningitis (TBM) in Chinese Han population of Northern China, and its association with clinical treatment. Patients with TBM and healthy controls were enrolled. We investigated the gene polymorphism (rs17525495) of LTA4H and tuberculous meningitis by Hardy-Weinberg equilibrium, genotyping and PCR. TNF- α content in cerebrospinal fluid (CSF) and the response to corticosteroid treatment in patients with TBM were investigated. The results showed that under recessive inheritance model, the genotype frequencies of TT in TBM group, TBM grade III group were obviously higher, and higher frequency was occurred in patients with unfavorable prognosis. Patients with favorable prognosis, TBM group, TBM group with grade III, genotype with TT showed notably higher level of TNF- α . The improvement rate of patients with genotype of CC and TT showed opposite results according to corticosteroid treatment. In conclusion, polymorphism of LTA4H (rs17525495) are associated with susceptibility to TBM in Northern China. Genotype TT in rs17525495 may be more likely to be involved in TBM and tend to be with severe condition, poor prognosis, higher level of TNF- α and active response to corticosteroid treatment. While patients with genotype CC showed negative responses to corticosteroid treatment.

Keywords: Polymorphism, LTA4H, susceptibility, tuberculous meningitis, corticosteroid treatment

Introduction

Tuberculosis (TB) is caused by mycobacterium tuberculosis (MTB), leading to millions of deaths every year. As one major infectious disease, TB remains a world-wide health concern, especially in developing countries [1, 2]. Tuberculous meningitis (TBM) is the most severe form of TB causing death and neurological damage by MTB [3]. According to the data from world health organization, one third of global population has been infected with MTB, but only less than 10% would progress to TB and 1% would progress to TBM [4, 5], indicating that host genetic factor plays an important role in TBM. As the previous studies have supported the participation of host genetics as a key component in TB outcome [6-9], it would be necessary to investigate the host genetics in TBM.

Arachidonic acid metabolic pathway plays an essential role in the balance of TBM and immune system. Leukotriene A4 hydrolase (LTA4H) is a key enzyme in the pathway which catalyzes the generation of leukotriene B4 (LTB4). LTB4, as a potent inflammatory factor, regulates TNF generation which could further damage TBM by various ways like activating macrophage, regulating the generation of granuloma and the expression of helper T1 (TH1) cell [10, 11]. Different genotypes in LTA4H would determine different activities of LTB4 and the downstream factors including macrophage, leading to the changes of susceptibility to TBM. Recently Tobin *et al.* [12] showed that polymorphism of LTA4H gene had an obvious relationship with susceptibility to TB in Vietnamese population. Whilst a single nucleotide polymorphism (SNP) rs17525495 in the LTA4H promoter could regu-

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late LTA4H transcription, determine inflammatory cell recruitment. Our aim in the study was to explore the relationship between LTA4H (rs17525495) polymorphisms and susceptibility to TBM, the diverse clinical influences on different genotypes in a Chinese Han population in Northern China.

Materials and methods

Study subjects

The study was approved by the ethics committee of the Second Affiliated Hospital of Hebei Medical University and informed consent was obtained from each subject. All subjects were Chinese Han from Northern China. Patients with TBM from the Second Affiliated Hospital of Hebei Medical University from January, 2014 to December, 2015 were selected into TBM group.

We divided TBM group into subgroups of definite TBM group and probable TBM group. Definite TBM was confirmed if one or more of the following criteria were met [13]: (1) acid-fast bacilli seen in the cerebrospinal fluid (CSF); (2) MTB cultured from the CSF; (3) a CSF positive commercial nucleic acid amplification test; (4) a CSF positive commercial nucleic acid amplification test. Probable TBM was confirmed if one or more of the following criteria were met: (1) a total diagnostic score of 10 or more points according to clinical criteria without cerebral imaging; (2) 12 or more points with cerebral imaging. At least 2 points should either come from CSF or cerebral imaging criteria in the diagnosis of probable TBM. The clinical criteria included the symptom of meningitis plus one or more of signs including headache, irritability, emesis, fever, nuchal rigidity, convulsion, partial neurologic impairment, altered mental status. Patients with autoimmune disease, HIV infection, malignancies, hormones or immunosuppressors for long-term usage would be excluded.

TBM was evaluated with 3 grades according to British Medical Research Council (BMRC) scale [14] as follows. Grade I: clear consciousness without neurological symptoms. Grade II: meningeal irritation sign; slight impaired neurological function; dysfunction of movement function. Grade III: convulsions or coma; severe impaired neurological function.

According to the retrospective analysis of corticosteroid treatment for TBM, patients with TBM

were divided into corticosteroid treatment group and no corticosteroid treatment group. Basing on the antituberculosis therapy in two groups, patients received dexamethasone (≥ 10 mg/day) by intravenous drip more than 5 days were included in corticosteroid treatment group. The prognosis on discharge was evaluated with Modified Rankin Scale (MRS). MRS runs from 0-6 as follows: 0-No symptoms; 1-No significant disability. Able to carry out all usual activities, despite some symptoms. 2-Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities. 3-Moderate disability. Requires some help, but able to walk unassisted. 4-Moderately severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted. 5-Severe disability. Requires constant nursing care and attention, bedridden, incontinent. 6-Dead. Favorable prognosis (≤ 2) and unfavorable prognosis (≥ 3) were applied in the study.

Healthy controls included in this study must be met with all the following criteria: healthy cases without genetic relationship; no history of tuberculosis or autoimmune disease; no long-term usage of hormone or immunosuppressor; no cancer or HIV infected diseases.

DNA extraction and detection

DNA from blood samples was extracted by a DNA extraction kit (Genomic DNA Isolation Kit; SBS Company, Beijing, China) under the manufacturer's instructions. DNA purity was detected by ultraviolet-visible spectrophotometer at the optical density (OD) 260/OD280. DNA concentration was calculated by $OD_{260} \times \text{dilution ratio} \times 50/1000$. DNA quality was checked by 1% agarose gel electrophoresis.

Genotyping and PCR of LTA4H gene (rs17525495)

We got the target primer sequences according to LTA4H gene (rs17525495) information from GenBank. The design and synthesis of the PCR primers was accomplished by Sangon Biological Company, China (rs17525495-F 5' AC-TTGTAGTTCCTTCA CCCATC 3'; rs17525495-R 5' CCAACGAACAGGTATC CACTAT 3'). The PCR protocol for amplification of LTA4H gene SNP in rs17525495 was proceeded of 30 cycles included pre-desaturating at 95°C for 3 min, desaturating at 95°C for 30 s, and annealing at

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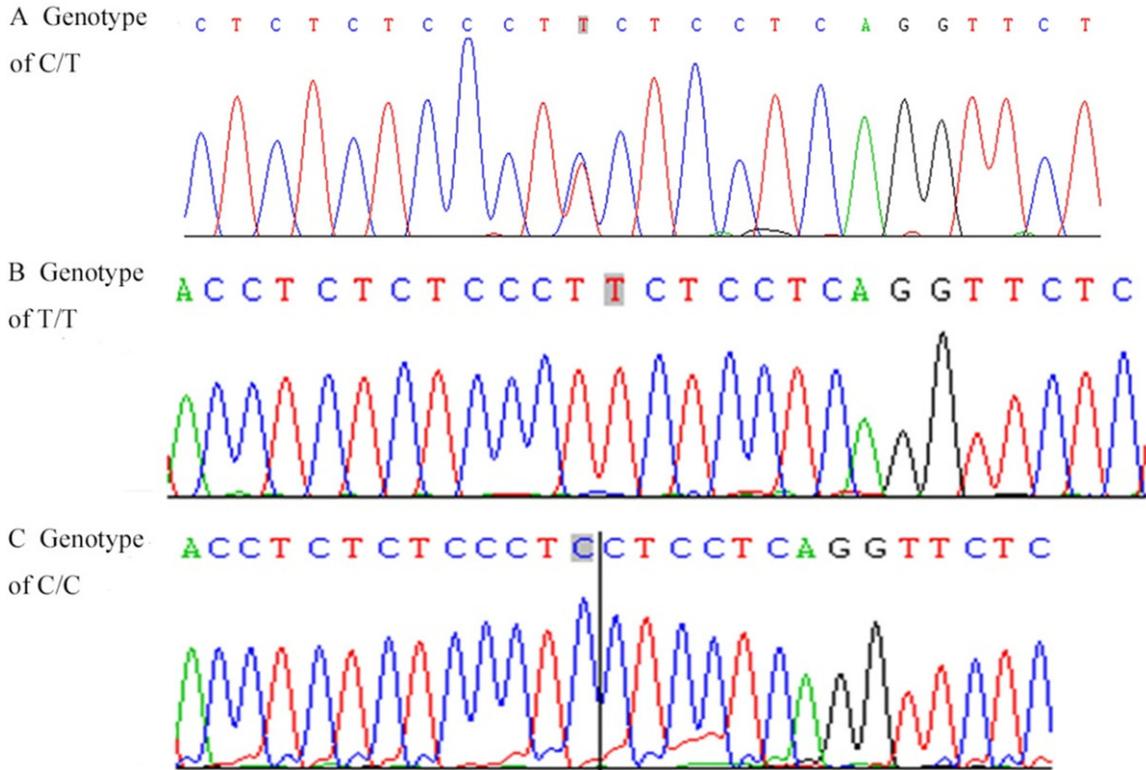


Figure 1. Sequencing results of rs17425495 SNP of LTA4H gene.

Table 1. The expected and observed genotype distribution in TBM group and control group

Groups	rs17525495			X ²	P
	C/C	C/T	T/T		
Expected number in TBM group (n)	56	45	9		
Observed number in TBM group (n)	59	39	12	1.92	0.16
Expected number in control group (n)	66	35	5		
Observed number in control group (n)	65	36	4	0.13	0.72

Data are expressed as number. TBM: tuberculous meningitis.

variable temperatures of 55°C to 60°C for 30 s, followed by extension at 72°C for 40 s to 50 s, repairing extension at 72°C for 10 min. The gene sequencing was accomplished by Sangon biological company, Shanghai (**Figure 1**).

The measurement of TNF-α level

TNF-α level in CSF was measured by a TNF-α Elisa kit (Qiaoyi Biological Company, China) under the manufacturer's instructions.

Statistical analysis

Data were analyzed by SPSS version 21.0 (SPSS Inc, Chicago, IL). Hardy-Weinberg equilibrium (HWE) was used to assess the association of the SNPs with diseases. Allele and genotype frequencies were calculated based on the genotyping results of the subjects. Differences of genotype distribution and allele frequency, patients with response to corticosteroid treatment were assessed using chi-square analysis. TNF-α content was expressed as mean±standard deviation. Student's t-test was used in the comparison of two groups and one-way analysis of variance was used in the comparison of multiply groups. Fisher's exact test was used for small sample numbers. P<0.05 was considered as a statistical significance.

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Results

Patients' characteristics

One hundred and ten TBM cases were composed by 77 males and 33 females with the mean age of 38.6±16.8 years. The control group (n=105) included 65 males and 40 females with the mean age of 37.5±13.7 years.

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Table 2. LTA4H rs17525495 allele and genotype frequencies in TBM and control groups

Groups	Allele		Genotype			Allele comparison		Allele comparison	
	C	T	CC	CT	TT	OR (95% CI)	P value	X ²	P value
TBM	157 (71.4)	63 (28.6)	59 (53.6)	39 (35.5)	12 (10.9)	1.514 (0.972, 2.357)	0.065	4.296	0.117
Control	166 (79.0)	44 (21.0)	65 (61.9)	36 (34.3)	4 (3.8)				

Data are expressed as number (percentage). TBM: tuberculous meningitis, OR: odds ratio, CI: confidence interval.

Table 3. LTA4H rs17525495 genotype frequencies in TBM and control groups in dominant inheritance model

Groups	Dominant inheritance model analysis			
	CT+TT	CC	OR (95% CI)	P value
Control	40 (0.381)	65 (0.619)	1	
TBM	51 (0.464)	59 (0.536)	1.405 (0.816, 2.419)	0.221
Grade I	25 (0.403)	37 (0.597)	1.098 (0.578-2.087)	0.775
Grade II	6 (0.429)	8 (0.571)	1.219 (0.394-3.771)	0.731
Grade III	20 (0.588)	14 (0.412)	2.321 (1.055-5.107)	0.034
Clinical prognosis				
Favorable prognosis	36 (0.429)	48 (0.571)	1.219 (0.679-2.187)	0.507
Unfavorable prognosis	15 (0.577)	11 (0.423)	2.216 (0.926-5.300)	0.070

Data are expressed as number (percentage). Each group was compared with the control group. TBM: tuberculous meningitis. OR: odds ratio. CI: confidence interval.

Table 4. LTA4H rs17525495 genotype frequencies in TBM and control groups in recessive inheritance model

Groups	Dominant inheritance model analysis			
	TT	CC+CT	OR (95% CI)	P value
Control	4 (0.038)	101 (0.962)	1	
TBM	12 (10.9)	98 (89.1)	3.092 (0.964-9.914)	0.047
Grade I	3 (0.048)	59 (0.592)	1.284 (0.278-5.935)	0.711 ^a
Grade II	2 (0.143)	12 (0.857)	4.208 (0.696-25.454)	0.147 ^a
Grade III	7 (0.206)	27 (0.794)	6.546 (1.784-24.015)	0.005 ^a
Clinical prognosis				
Favorable prognosis	7 (0.083)	77 (0.917)	2.295 (0.649-8.123)	0.221 ^a
Unfavorable prognosis	5 (0.192)	21 (0.808)	6.012 (1.488-24.289)	0.015 ^a

Data are expressed as number (percentage). Each group was compared with the control group. TBM: tuberculous meningitis. OR: odds ratio. CI: confidence interval. ^aFishers exact test was used for small sample numbers.

There were no significant differences between TBM group and control group in sex ratio and age.

HWE analysis

As shown in **Table 1**, no significant difference between the expected and observed genotype distributions of LTA4H gene (rs17525495) in TBM group ($P>0.05$) meant the samples of TBM group had demographic representation. The similar result could also be seen from no obviously difference between the expected and

observed genotype distributions of LTA4H gene (rs17525495) in control group ($P>0.05$).

Association of LTA4H (rs17525495) polymorphism with susceptibility to TBM

As shown in **Table 2**, genotype and allele frequencies of LTA4H rs17525495 in TBM group showed no significant differences compared with control group ($P>0.05$). **Table 3** showed that under dominant inheritance model, no remarkable differences in genotype frequencies of CT+TT of LTA4H rs17525495 in TBM group and TBM groups with grade I, II compared with control group respectively ($P>0.05$), but the frequency of CT+TT was obviously higher in TBM group with grade III compared with control group

($P<0.05$). With recessive inheritance model shown in **Table 4**, the genotype frequencies of TT in TBM group and in TBM grade III group were obviously higher than that in control group ($P<0.05$, $P<0.05$), and it showed distinct higher frequency in patients with unfavorable prognosis compared with control group ($P<0.05$).

TNF- α level

As shown in **Figure 2**, the TNF- α level in TBM group was apparently higher than that in control group ($P<0.05$). The TNF- α level in TBM patients with grade III was much higher than

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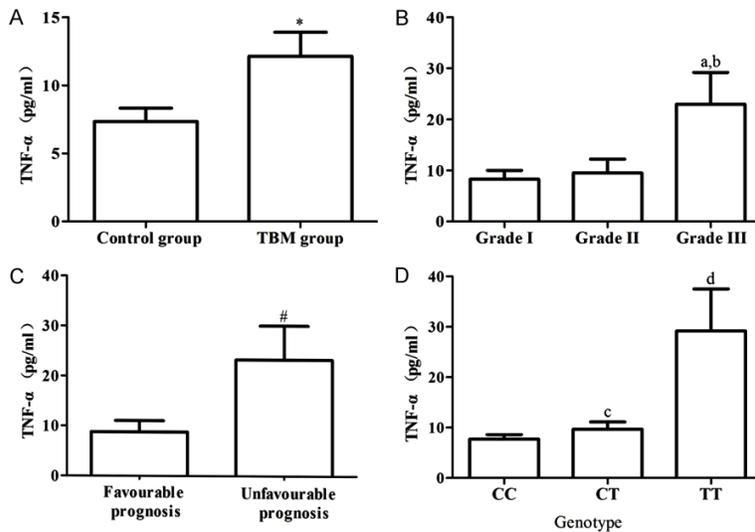


Figure 2. TNF- α levels in TBM patients with different groups, grades, outcomes and genotypes TBM: tuberculous meningitis. A: The levels of TNF- α between TBM and control groups, *vs Control groups, $P=0.01$; ^avs. Grade I group, $P=0.01$; ^bvs. Grade II group, $P=0.01$; B: The level of TNF- α in patients with different TBM grades, $P=0.01$. C: The levels of TNF- α with different clinical outcomes in TBM groups, [#]vs Favorable prognosis group, $P=0.01$. D: The levels of TNF- α with different genotypes in TBM groups, ^{c,d}vs. CC genotype group, $P=0.01$.

that in TBM patients with grade I and II ($P<0.05$, $P<0.05$). Data from patients with favorable prognosis showed notably higher level of TNF- α compared with patients with unfavorable prognosis ($P<0.05$). According to different genotypes, the TNF- α levels in the genotypes of TT and CT were obviously higher than the genotype of CC in TBM groups ($P<0.05$, $P<0.05$).

Response to corticosteroid treatment

There were 33 patients in corticosteroid treatment group and 42 patients in no corticosteroid treatment group. 35 patients were excluded due to patients' physical state. The improvement rate of patients with genotypes of TT was significant higher in corticosteroid treatment group than no hormone group and patients with CC was apparently lower in corticosteroid treatment group than no hormone group ($P<0.05$, $P<0.05$, **Table 5**).

Discussion

The human LTA4H gene, localized in chromosome 12q22, is involved in the synthesis of LTB4 in arachidonic acid metabolic pathways. SNP rs17525495 of LTA4H could affect the activity of LTA4H, the level of mRNA, even the

response to corticosteroid treatment with different genotypes in lines of evidence [15, 16]. Dunstan *et al.* [17] showed that rs17525495 was associated with susceptibility to bacterial meningitis, and genotype with TT increased the risk of disease in recessive inheritance model. And Tobin *et al.* [17] found that rs1978331 and rs2660898 in LTA4H were related to susceptibility to TB in Vietnamese population [12]. While, the same SNPs showed no relationship with susceptibility to TB in Russian population in the finding by Curtis *et al.* [18]. The different results may due to different races, types of TB, sample sizes. In our study, we investigated the relationship between rs17525495 polymorphism and susceptibility to TBM in ethnic Han popula-

tion of northern China. The results showed no significant difference between TBM group and control group in genotype and allele frequencies. However, genotype TT in TBM group, TBM grade III group, TBM patients with unfavorable prognosis showed higher frequency compared with control group under recessive inheritance model ($P<0.05$). The results revealed that patients with genotype TT at rs17525495 may be more likely to be involved in TBM and may tend to be with severe condition and poor prognosis, which was basically consistent with the study of previous studies [15, 16].

Tobin *et al.* [12] presented that rs17525495 could affect the susceptibility to TBM on zebrafish by regulating the enzymatic activities on TNF- α . Our study with the highest level of TNF- α in CSF in genotype of TT in rs17525495 was basically consistent with the finding by Tobin *et al.* [12]. However Dunstan *et al.* [17] displayed no difference of TNF- α level in CSF of patients with bacterial meningitis among different genotypes in rs17525495 in Vietnamese population. The inconsistent results may be partly explained by different diseases. Mononuclear macrophage, which could secrete TNF- α , could be affected in migration by rs17525495 in TBM

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Table 5. Effect of corticosteroid treatment therapy on patients with TBM in different genotypes

Groups	Genotypes	Total (n)	Favorable prognosis (n)	Unfavorable prognosis (n)	Improvement rate (%)	P value
Corticosteroid treatment	CC	15	9	6	60*	0.037
No corticosteroid treatment		24	22	2	91.7	
Corticosteroid treatment	CT	12	7	5	58.3*	1.000
No corticosteroid treatment		14	9	5	64.3	
Corticosteroid treatment	TT	6	5	1	83.3*	0.048
No corticosteroid treatment		4	0	4	0	

Data are expressed as number (percentage). *vs the no hormone group respectively in each genotype.

in our study. While, bacterial meningitis displayed less TNF- α due to great number of neutrophils infiltration instead of mononuclear macrophage.

Our study demonstrated that the improvement rate of patients with CC genotype in corticosteroid treatment group was remarkably lower than that in no hormone group and contrary results were occurred in TT genotype, suggesting that different genotypes in rs17525495 will affect the response to corticosteroid treatment with the better response in TT genotype and worse response in CC genotype. In agreement with our finding, Tobin *et al.* [12] concluded that corticosteroid treatment might suppress the excessive inflammation occurred in patients with TT which could convert disease to better condition, while it could suppress the low inflammation occurred in patients with CC which could lead disease to worse condition.

There are several limitations in this study. Firstly, the patients had different physical states and varying subjective desires to hormone treatments, we had to exclude patients with varying hormone therapies to unify the regimen according to the retrospective study. Secondly, the small sample size limited the depth of the paper. Thirdly, LTB4 dehydrogenase should be involved in this study.

In conclusion, polymorphism of LTA4H (rs17525495) are associated with susceptibility to TBM in ethnic Han population of northern China. Genotype TT in rs17525495 may be more likely to be involved in TBM and may tend to be with severe condition, poor prognosis, higher level of TNF- α and active response to corticosteroid treatment. While patients with the genotype CC showed negative responses to corticosteroid treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Hui Bu and Yueli Zou, Department of Neurology, The Second Affiliated Hospital of Hebei Medical University, 215 Heping West Road, Shijiazhuang 050000, Hebei, China. Tel: +86 13831106903; E-mail: buhui881@163.com (HB); sunnygirlzyl@126.com (YLZ)

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