Original Article

All Gene Expression of Leukotriene A4 Hydroxylase (*LTA4H*) Gene in Extra Pulmonary Tuberculosis Patients and Healthy Control in North Indian Population

Pooja Singh Gaur[1], Surya Kant[2], Abhilasha Tripathi[3], Kanchan Srivastava[4], Rachna Chaturvedi[5]

Initial diagnosis and timely treatment of Extra Pulmonary Tuberculosis (EPTB) continues to be a challenge in all over World as well as India. First time, this analysis will discover the role of *LTA4H* gene and may be establishing another candidate that impacts the sensitivity to EPTB in the population of North India. This study will be the first report on *LTA4H* gene various diagnostic markers, expression of gene may validate as a prognostic factor in (EPTB). The diagnosis (EPTB) poses a special challenge, as it is often missed or misdiagnosed due to its atypical presentations and difficult to isolate *M tuberculosis (MTB)* due to the small number of organisms present at these sites. Subsequently the outcome of present study will reinforce possible use of *LTA4H* as biomarkers and the therapeutic utility for (EPTB). This study will be a step to decrease the analytical and therapeutic window to identify another risk factor *LTA4H* for EPTB. Leukotriene A4 hydroxylase (*LTA4H)*, an enzyme which changes LTA 4 to LTB4, controls the balance amongst the anti-inflammatory lipoxins and pro-inflammatory LTB4, with directly consequences in TB-driven inflammation. In humans and will spawn new ways to protection and enhance the wellbeing status of individuals and population groups. On RT-PCR, Extra Pulmonary Patients had lower expression of *LTA4H* compared to the controls. Correlation of biomarkers will reveal *LTA4H* level correlated with age, Gender Smoking, Clinical Parameter Serum Total Protein, BMI Height and TLC, Laboratory Parameter. On ELISA kit and follow as per manufacturer protocol. CEA562Ge 96 Tests Enzyme-linked Immunosorbent Assay Kit For Leukotriene B4 (*LTB4) LTB4* Protein level in Extra Pulmonary Patients, (EPTB) (2304.52pg/ml) had lower expression of gene *LTB4* compared to the controls (3096.142pg/mls) (P value = 0.0012).

[*J Indian Med Assoc* 2022; **120(8):** 34-9]

***Key words* : *LTA4H gene*, Real Time PCR, Extra Pulmonary Tuberculosis, ELISA.**

Tuberculosis (TB), caused by Bacillus *Mycobacterium tuberculosis*, is a few of the top 10 causes of mortality globally and the prominent cause of death from a single infectious agent (ranked higher than HIV/AIDS)[1]. *M tuberculosis* infects almost one-quarter of the worldwide inhabitants. However, among the one-quarter of the World-wide population infected by *Mycobacterium tuberculosis,* simply just about 10% growth to clinical disease [2]. Highly affected persons take the germs exclusive of communicating apparent infection for their complete life cycle, which suggests that host sensitivity is a significant probability component for the advancement of effective Tuberculosis following disease[3]. Numerous genetic components have been discovered in research of different sources to be valuable in tuberculosis[4,5]. LeukotrieneA4hydrolase (*LTA4H*) is a monomeric, cytosolic, zinc metalloenzyme which catalyses the Leukotriene B4 (*LTB4*) generation in amino acid metabolic route and standardizes the equilibrium of *LTB4* and *LXA4* generation[6]. This equilibrium can influence TNF production. TNF is an essential component which affects the kind of macrophage killing after *M tuberculosis* contamination. Macrophage caspase-mediated cell death can monitor the distribution of *M tuberculosis,* but macrophage mortification may improve the dispersion of *M*

Editor's Comment :

- These findings suggest the expression in the LTA4H gene might influence the sensitivity to Extra Pulmonary Tuberculosis and new potential genetic risk factors of Extra Pulmonary Tuberculosis. This study will be a step to reduce the diagnostics therapeutic window to other risk factors (LTA4H) to Extra Pulmonary Tuberculosis and will span to new ways to safeguard and improve the health status.

[1]MSc, Research Scholar, Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Lucknow 226028 and Department of Respiratory Medicine, King George Medical, University, Lucknow 226003

[2]MBBS, MD, Professor and Head, Department of Respiratory Medicine, King George Medical University, Lucknow 226003

[3]MSc, Research Scholar, Department of Neurology, Sanjay Gandhi Postgraduate Institute, Lucknow 226014

[4]MSc, PhD, Scientist Department of Respiratory Medicine, King George Medical University, Lucknow 226003

[5]MSc, PhD, Assistant Professor, Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Lucknow 226028 and Corresponding Author

Received on : 27/04/2022

Accepted on : 05/08/2022

tuberculosis. Therefore, here is an assumption that the activity of *LTA4H* can influence the development of Tuberculosis subsequently M *tuberculosis* contagion. Newly, as a contender gene participating in TB, the polymorphisms in *LTA4H* gene were investigated in a case management group study in a Vietnamese inhabitant[7]. Of the six individual nucleotide polymorphisms (SNPs) findings, two have being discovered to be substantially correlated with Tuberculosis. The identical 6 SNPs were also explored in a Russian inhabitant but nothing of them demonstrated considerable correlation with pulmonary tuberculosis[8]. Polymorphisms in the gene *LTA4H* might influence sensitivity to Extra Pulmonary Tuberculosis and shift the possibility of developing the infection in the Han population in the East China[9].

Materials nd Methods

Study Design : It is a case control study. The current study was performed on patients suffering from Extra Pulmonary Disease, fulfilling the inclusion & exclusion criteria. Patients were enrolled from the Respiratory Medicine Department, King George's Medical University (KGMU), Lucknow attending OPD. Authored notified authorization was carried from each subject before inclusion in the research. The research was endorsed by the official Ethics Committee (Registration no: ECR/262/Inst/up/2013/RR-16)-Ref.code:94th CMIIB-PhD/PI

EPTB Patients :

The investigational work was accomplished in the Departments of Biochemistry and Respiratory Medicine -KGMU. Lucknow.

Study Participants : Selection Criteria

A clinically EPTB subject was kept in case group and healthy subjects for control group. Inclusion and exclusion standards of both groups was as follows-

We enrolled a total of 274 EPTB patients effecting the inclusion and exclusion criteria from Outpatient and Indoor patient Department of Respiratory Medicine, Lucknow. Data were collected on a standardized questionnaire for each subject. This included demographic characteristics age and Gender, Clinical history (Case definitions), Past and family history, Anthropometric data. Notified agreement was taken from 274 patients or guardian.

Healthy Control : Age and Gender -matched had been selected. An only a healthy person was enrolled with not any history of Respiratory disease.

Collection of Blood Samples : Transferred Blood in Triazole vial (1:3 ratio; 1 ml blood and 3 ml of Triazole) for gene expression was stored at -80^{0}C till further use.

Diagnostic Category : The patients were confirmed as EPTB by difference diagnostic tools as X-ray, Microscopy, ultrasound, CT scan and others (Table 1) and also diagnosed and confirmed by the PPD and blood investigation (Table 2)The patients were believed distinct EPTB if along with essential criteria there were Acid Fast Bacilli (AFB) in CSF smear or culture, Gene-Xpert or PCR positive for AFB nucleic acid or demonstration of AFB in extra CNS Tuberculosis. Presence of essential and two supportive criteria was considered highly probable EPTB Post-PCR dissociation curves were utilized to validate the specificity of single target amplification. Expression of all genes was normalized to the expression of Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), a housekeeping gene. The Cp value of gene is used for calculation of fold expression of gene. The 10 µl of sybergreen (Takkara), 1 micromolar forward and 1micromolar reverse primer, 1µl cDNA and 7 µl of water were used for amplification of 20 µl one well reaction. RT-PCR was performed using a Roche system (Light cycler 480;).

RNA Extraction and CDNA Synthesis :

Complete RNA was separated from EDTA blood samples using Triazole reagent (Invitrogen, Rockville, MD, USA). The dilution and reliability of the RNA have been determined by determining the absorbance at 260 and 280 nm by Spectro-photometer. Total RNA was having reverse transcription to c-DNA using a cDNA reverse transcription kit (Applied Bio system, USA) and amplified by Real-time PCR (RT- PCR) and the melting temperature (Tm) of primer was standardized by simple RT-PCR. RT-PCR was accomplished using

Table 1 — *Confirmation of EPTB patients*

Clinical diagnosis	Frequency	Percent
X-ray	40	29.2
Microscopy	32	23.4
Ultrasound	15	10.9
CT scan	13	9.5
Other	37	27.0
Total	137	100.0

Table 2 — *Clinical data of case (EPTB) case groups*

	Groups						p-value
	Cases		Controls		Total		
	N	%	N	%	N	%	
PPD test :							
Present	67	48.9%	0	0%	67	24.5%	<0.001
Absent	70	51.1%	137	100%	207	75.5%	
Blood investigation :							
Present	127	92.7%	0	0%	127	92.7%	NA
Absent	10	7.3%	0	0%	10	7.3%	

SYBR Green Super mix (MAKE) and Single-Color RT-PCR Recognition System. The quantity of template utilized in the PCR responses was cDNA corresponding to 200 ng reverse-transcribed total RNA. DNA polymerase was first triggered at 95°C for 3 minutes, denatured at 95°C for 30 seconds and annealed/ extended at 61°C for 30 seconds, for n cycles according to the manufacturer's protocol (REF). Manifestation of the maintenance gene GAPDH functioned as an inside positive control in each assay performed.

Quantification of Nucleic Acids :

Extracted RNA was evaluated and assessed for purity using the Nano Drop 2000C UV-Vis Spectrometer. The Nano Drop loading surface was cleaned and 1 µl of dH2O applied to initialize the Nano Drop 2000C Software (Nano Drop Technologies, Wilmington, USA). A second application of 1 µl dH2O was used to blank the Nano Drop 2000C software to register a zero value. 1 µl of RNA was utilized to the Nano Drop and the concentration was determined by the instrument in n g/µL units at 260nm wavelength and the veracity was evaluated by determining the ratio of absorbance at 260nm to the absorbance at 280nm wavelength. Ratios of absorbance at 260/280 nm and 260/230 nm were determined by the Nano Drop 2000C Software. A ratio of 1.8 – 2.0 is of pure RNA.

RNA Gel Electrophoresis :

Above eluted RNA samples of each BLOOD sample were equally diluted to make equal concentration of each RNA samples and were run on the 0.8% agarose gel along Ladder

Polymerase Chain Reaction (PCR) :

Specific sequences can be easily amplified using conventional PCR (using templates)[10]

Statistical Analysis :

Constant data were surmised as mean ±SD while categorical data in number and percentage. One way to analyse covariance (ANOVA) was done for the analysis the correlation between Demographic and *LTA4H* and *LTB4* Gene. Statistical Package for the Social Sciences (SPSS) software version 20.0 was used to analyze the data. Statistical significance was set as $p<0.05$.

Results

The demographic data were documented and calculated the deformities as represented in (EPTB) Case group. (Table 3). The demographic data of the participants: 137 cases representing out of 274 representing the case and 137 case representing as control group. Respondents <18 years male were 3(1.09%), female were 5 (1,82 %). Total no 8 (2.9%)

Table 3 — *The demographic data were recorded and calculated the deformities as represented in (EPTB) Case groups*

	SEX		
	Male	Female	Total
Age Intervals :			
<18 years	3 (1.9%)	5 (4.2%)	8 (2.9%)
18-35 years	82 (53.2%)	65 (54.2%)	147 (53.6%)
36-50 years	43 (27.9%)	34 (28.3%)	77 (28.1%)
51-65 years	26 (16.9%)	16 (13.3%)	42 (15.3%)
Total	154 (100.0%)	120 (100.0%)	274 (100.0%)

Applied χ^2 test for significance, χ^2 value=1.71; df (3); p-value=0.636

Respondents between 18 to 35 years male were 82 (53.2%), female were 65 (54.2%) and total no 147 (53.6%). Respondents between the 36-50 years male were 43(27.9%), female were 34(28.3%) and total number 77 (28.1%). Respondents between 51-65 years male were 26(16.9%), female were 16(13.3%) and total no. 46 (15.3). Applied χ^2 test for significance. χ^2 values=1.71; df (3); p-value=0. 636. There is gender difference to sample helping the significance of study. The distribution of the sample for Smoking type shows Current smoker 23(8.4%), Ex-smoker 18(6.6%), Passive smoker16(5.8%) and Non-smoker were 217(79.2%). The duration of smoking (yrs) was 20.72 and 11.36,no of Cigarette were 2.25 and 0.97 and pack years of the smoking were 21.94 and 0.97. Other distribution of Alcohol and Gutkkha chewer were present the Alcohol consumption present in 10 (7.3%),absent in 127(92.7%) and gutkkha chewing present in 17(13.7%),absent in 107(86.3%) (Table 4). Site and clinical diagnostic of Extra Pulmonary Tuberculosis (EPTB) in case representing that the participants belongs to the types of site in the Extra-pulmonary Tuberculosis for example in Abdominal 33 (24.1%) lymph node 21(15.3%) Pleural 32 (23.4%) Pots 7(5.1%) TBM 15(10.9%) Genitourinary 3(2.2%) Miliary 3(2.2%) Other 23(16.8%) (Table 5) Confirmation of

Table 4 — *Smoker, non-smoker and ex-smoker and Alcohol data of case and control groups*

		N	%
Smoking type :	Current smoker	23	8.4%
	Ex-smoker	18	6.6%
	Passive smoker	16	5.8%
	Non-smoker	217	79.2%
		Mean	**SD**
No of cigarette		2.25	0.97
Duration of smoking (years)		20.72	11.36
Pack years		21.94	12.27
		N	**%**
Alcohol	Present	10	7.3%
	Absent	127	92.7%
Gutkkha chewer	Present	17	13.7%
	Absent	107	86.3%

Table 5 — *Site and clinical diagnostic of Extra Pulmonary Tuberculosis (EPTB) in case*

Types of side	N	%
Abdominal	33	24.1%
Lymph node	21	15.3%
Pleural	32	23.4%
Pots	7	5.1%
TBM	15	10.9%
Genitourinary	3	2.2%
Miliary	3	2.2%
Other	23	16.8%
	137	100%

EPTB patients shows that the clinical diagnosis for the cases by the different method like X-Ray 40 (29.2) Microscopy 32(23.4) Ultrasound 15(10.9) CT scan 13(9.5) Other37(27.0) (Table 1) .

Correlation of Laboratory Parameter : lta4h gene the expression value of mean standard deviation and the median of case has mean .92, SD 1.27 and Median. 51 (Table 6) and the correlation of lta4h gene expression value in AGE, BMI Protein Haemoglobin tlc and the smoker (No of cigarette) and the correlation by the Spearman's rho is the distribution (Table 6) . lta4h gene Spearman's rho 1 and the significance p-value is nil of the 38 patients and the age Spearman's rho-0.175 significance 0.292 in 38 patients. BMI-0.241 p-value 0.145 in 38 patients. Protein 0.273 p-value 0.097 in 38 patients. Haemoglobin-0.259 p-value 0.116 in 38 patients. TLC (cumm) -0.342* p-value 0.036 in 38 patients. No. of cigarette -.678* p-value 0.022 in 11 patients. Clinical data of case (EPTB) case group shows the case and control group present Monteux test (PPD) test present case 67(48. 9%) control 0(.0%) Total no 67 (24.5%), absent 70 (51.1%) control the 137 total no 207(75.5%), significance is <0. 001. The blood investigation present in case 127(92.7%) and control 0 (0%) Total no 127 (92.7%), absent 10 (7.3%) control group 0(0%) Total no10 (7.3%) and there is no significance level (Table 2). The case and control group the applied the unpaired t test for the significance level of different factors as age, weight, height and BMI in cases mean SD age 33.09 and 12.85 control 41.83 and 11.72 total mean 37.46 and 13.03 the significance is<0.001 (Table 6) For the weight in mean 47.52 SD 6.74 and the control 161.28 SD 4.17 total mean 37.46 and significance i<0.001 (Table 6). For the height 162.68 SD 4.01 and the control. 60.88 SD 5.17 Total mean 111.78 SD 51.20 the significance level of <0.001(Table 6). For BMI mean 23.2 SD 1.60 control group 23.66 and SD 1.80 and the total mean 23.49 SD 1.71 for significance is 0.105 (Table 6).

Table 6 — *lta4h gene expression value*

	Mean	Standard Deviation	Median
lta4h gene expression value	0.92	1.27	0.51
Parameters	Spearman's rho	p-value	No of patients
lta4h gene expression value	1	-	38
AGE	-0.175	0.292	38
BMI	-0.241	0.145	38
Protein	0.273	0.097	38
Haemoglobin	-0.259	0.116	38
TLC (cumm)	-0.342*	0.036	38
No of cigarette	-0.678*	0.022	11

LTA4H Expression :

LTA4H mRNA expression evaluated by real time PCR using SYBR green method. The expression levels of LTA4H gene, were lower in patients than control (Fig 1). The above study the correlation of different parameter and *LTA4H* gene are found. *LTA4H* gene were considerably correlated with Age Sex, Medical history, Socio-economic status, Occupation and other clinical parameter We are associated with *LTA4H* mRNA expression. The expression levels of *LTA4H* gene with lower expression with case compered the control. Abdominal lymph node Pleural Genitourinary TBM, Miliary pots and other part of the tuberculosis. and other Extra Pulmonary Tuberculosis. These findings were suggested that Expression in the *LTA4H* gene might be influence the sensitivity to Extra Pulmonary Tuberculosis and a new potential genetic risk factor of Extra Pulmonary Tuberculosis. This study will be a step to reduce the diagnostic and therapeutic window to identify another risk factor (*LTA4H*) for Extra Pulmonary Tuberculosis and will spawn new ways to safeguard and improve the health status of individuals and population groups. On RT-PCR, Extra pulmonary Patients had lower expression of *LTA4H* compared to the controls. Correlation of biomarkers: *LTA4H* level correlated with Age, Sex Smoking, Protein, BMI TLC are the different level the correlation is found with significant of Extra Pulmonary Patients. Applied unpaired t test for significance.

Fig 1 — Error Bar diagram shows Lower expression of LTA4H in the patients with Extra Pulmonary (case) compared to controls

Discussion

An experiment was performed on animal model zebrafish and found susceptibility for *Mycobacterium marinum*, can result from either inadequate or excessive acute inflammation[7]. Researchers revealed the two distinct molecular routes to *Mycobacterial* susceptibility converging on dys regulated TNF levels: inadequate inflammation caused by excess lipoxins and hyper inflammation driven by excess *LTB4*. In humans, recognize a single nucleotide polymorphism (SNP) in the *LTA4H* promoter region that regulates its transcriptional activity. In tubercles meningitis, polymorphism was correlated together with inciting cell recruitment along with patient survival and response to adjunctive anti-inflammatory therapy. A study by[11] . and several additional findings have shown a similar effect in different diseases, single nucleotide polymorphisms in 5-lipoxygenase activating protein (ALOX5AP) and *LTA4H* genes were associated with *LTB4* over production in myocardial infarction susceptibility. A single study of India [12.] has evaluated the genetic contribution of Leukotriene variants with Coronary Artery Disease (CAD) and observed the association of these variants with plasma *LTB4* levels. Plasma LTB4 levels were measured in 150 subjects of each case and controls by *LTB4* Immunoassay. Plasma *LTB4* levels were higher in cases than in controls *LTB4* measured by ELISA and *LTA4H* mRNA expression evaluated by real time PCR using SYBR green method. The expression levels of *LTA4H* gene, TNF, IL-8 were higher in patients than. The researchers observed a promoter region SNP (rs17525495) and an intronic SNP (rs1978331) we are associated with *LTA4H* mRNA expression. We are evaluated the gene *LTA4H* mRNA expression evaluated by real time PCR using SYBR green method. The expression levels of *LTA4H* gene with lower expression with case compered the control and above study the correlation of different parameter are found. Finally, they concluded that *LTA4H* exhibit close association in subject with circulatory disease and might deliver incremental values for predicting cardiovascular risks, In the present study results of RT-PCR, revealed that Extra Pulmonary Patients had lower expression of *LTA4H* compared to the controls. Recent studies recommended extremely appropriate association of *LTA4H* gene polymorphisms along with various ethnicities and TB infection. Genomic display in zebrafish embryo and found a group of mutants hyper susceptible to *M marinum* infection[7]. Mutations in the *LTA4H* gene of zebrafish were presented heightened infectious growth and decreased TNF signalling. Mutations of the *LTA4H* gene reduced its expression and redirected

LTB4 synthesis along with production of the anti-inflammatory *LXA4.* Tobin and his co-workers were also tested whether heterozygosis at *LTA4H* protected against another mycobacterial disease in a different population in a different environment, *LTA4H* confers protection from development of severe disease among exposed persons. These outcomes recommend that conventional polymorphisms in the *LTA4HH* gene do not work any key function in sensitivity to clinical pulmonary tuberculosis. The contradictory results of these different studies are force to recognize *LTA4H* gene polymorphism in different ethnic groups as well as diseases. Due to this motive a recent study [13,9] was examined to the association of LTA4H polymorphisms with tuberculosis in a Han Chinese population of Eastern China. They were genotyped to the 5 SNPs (rs1978331, rs2247570, rs2540474, rs2660898, rs2660845) of LTA4H gene in 743 of Pulmonary Tuberculosis patients, 372 of Extra Pulmonary Tuberculosis patients and 888 of healthy controls persons. Genotyping of the SNPs was performed with the SNPlex Genotyping System (Applied Biosystems, USA). These findings were suggested that polymorphisms in the *LTA4H* gene may be influence the sensitivity to EPTB and a new potential genetic risk factor of EPTB. Polymorphisms were genotyped by real-time PCR using the TaqMan probes. They found neither rs17525495 nor rs1978331 and rs2660898 SNPs displayed significant association with Pulmonary tuberculosis. In tubercles meningitis, polymorphism was correlated with inflammatory cell recruitment all along with patient existence and response to adjunctive anti-inflammatory therapy[14,15,10]. With our best knowledge this is the primary study from India to address the *LTA4H* gene polymorphisms, its mRNA expression of present study with RT-PCR results in Extra pulmonary patients had lower expression of *LTA4H* gene to compared to the controls sample *LTA4H* level correlated with age, Gender Smoking, Serum Total Protein , SGOT, SGPT, Tubercular, Hydrocephalus. On ELISA, Extra Pulmonary Patients(2304.52pg/ml) had lower expression of LTB4 compared to the controls (3096.142pg/mls) (P value = 0.0012).

Conclusion

This study will explore the role of LTA4H gene and may be establish another candidate that influence the susceptibility to Extra Pulmonary Tuberculosis in the Northern population of India. This study will be the first

report on LTA4H gene Polymorphisms among North Indian tuberculosis patients and may suggest a new potential genetic risk factor of Extra Pulmonary Tuberculosis. Among various diagnostic markers, Expression of the plasma LTB4 protein may validate as a prognostic factor in Extra Pulmonary Tuberculosis, its mRNA expression and plasma LTB4 levels will depict that functional variant of LTA4H may modulate Tuberculosis by regulating LTB4 production. So, the level of pro-inflammatory marker LTB4 in plasma may highlighted towards a primary diagnosis of Tuberculosis. Subsequently the outcome of present study will reinforce possible use of LTA4H as biomarkers and also the therapeutic utility for Extra pulmonary Tuberculosis. This study will be a step to reduce the diagnostic and therapeutic window to identify.

References

1 Chakaya J, Khan M, Ntoumi F — *Tuberculosis (TB)* 2020.

2 Raviglione MC, Snider DE, Kochi A — Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. *JAMA* 1995; **273(3):** 220-6.

3 Schurr E — Is susceptibility to tuberculosis acquired or inherited? *Journal of Internal Medicine* 2007; **261(2):** 106-11.

4 El Baghdadi J, Grant AV, Sabri A, El Azbaoui S, Zaidi H, Cobat A, Abel L — Human genetics of tuberculosis. *Tuberculosis of the Central Nervous System:* 2017 *Pathogenesis, Imaging, and Management*.

5 Leung JM, Fowler C, Smith C, Adjemian J, Frein C, Claypool RJ, *et al* — A familial syndrome of pulmonary nontuberculous mycobacteria infections. *American journal of respiratory and critical care medicine*, 188(11), 1373-6. LTA4H polymorphisms and extra-pulmonary tuberculosis in an ethnic Han Chinese population in Eastern China. *Tuberculosis*, 2013; **94(6):** 657-63.

6 Audat SA, Al-Shar'i NA, Al-Oudat BA, Bryant-Friedrich A, Bedi MF, Zayed AL, *et al* — Identification of Human Leukotriene A4 Hydrolase Inhibitors Using Structure-Based Pharmacophore Modeling and Molecular Docking 2020; *Molecules*, **25(12):** 2871.

7 Tobin DM, Vary Jr JC, Ray JP, Walsh GS, Dunstan SJ, Bang ND, *et al* — The lta4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans 2010 *Cell*, **140(5):** 717-730.

8 Curtis J, Kopanitsa L, Stebbings E, Speirs A, Ignatyeva O, Balabanova Y — subjects. *Tuberculosis (Edinb)* 2011; **91(1):** 22-5.

9 Yang J, Chen J, Yue J, Liu L, Han M, *et al* — Relationship. 2014 Tuberculosis, 2014 – Elsevier

10 Mullis KB, Faloona FA — Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction 1987; Methods Enzymol **155:** 335-50. doi: 10.1016/0076-6879(87)55023-6.

11 Dunstan ML — Psychological Review © 2010. *American Psychological Association* 2010; **117(2):** 601-22

12 NairJ, Shanker J, Arvind P, Jambunathan S, Kakkar Vijay V — Expression analysis of Leukotriene- inflammatory Gene Interaction Network in patients with Coronary Artery Disease. *J Atheroscler Thromb* 2014; **21(4):** 329-45

13 Mabunda N1, Alvarado-Arnez LE, Vubil A, Mariamo A, Pacheco AG, Jani IV, Moraes MO — Gene polymorphisms in patients with pulmonary tuberculosis from Mozambique. *Mol Biol Rep* 2015; **42(1):** 71-6.

14 Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I — The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. *Allergy* 2008; **63(8):** 1046-53.

15 Sun H, Zhang J, Wang J, Sun T, Xiao H, Zhang JS — Association between genetic variants of the leukotriene biosynthesis pathway and the risk of stroke: a case- control study in the Chinese Han population. *Chin Med J (Engl)* 2013; **126(2):** 254-9.