Polymorphisms of Fatty Acid Elongase 2 Gene Afftects Risk of Pulmonary Tuberculosis in China Han Population

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Abstract

Background: As an infectious disease closely related to *Mycobacterium tuberculosis*, autoimmunity, inflammation, environment and heredity, the relationship between the single nucleotide polymorphism of elongase 2 gene and the susceptibility to tuberculosis is still unknown.

Methods: Between January 2016 and November 2018, a hospital-based case-control study was conducted. This epidemiological survey was conducted in both hospitals every three months. *rs3798719*, *rs1570069*, and *rs2236212* in *ELOVL2* gene were detected by Sanger sequencing.

Results: Stratified by gender, the genotypes and allele frequencies of *rs3798719*, *rs1570069* and *rs2236212* showed significant differences between the two groups ($\chi2 = 6.987$, $P = 0.030$), Genetic modeling showed that *rs3798719* was statistically different in the overdominance model ($\chi2 = 4.784$, OR = 1.414, 95% CI: 1.036-1.929, $P < 0.05$). The polymorphism of *rs2236212* between male TB patients and healthy controls was statistically different in the dominance model. ($\chi2 = 4.192$, OR = 0.507; 95% CI: 0.262-0.981, $P < 0.05$).

Conclusion: The *rs3798719* of *ELOVL2* gene may be associated with susceptibility to TB in female population and the *rs2236212* of *ELOVL2* gene may be associated with TB incidence in male patients.

Keywords: Tuberculosis; *ELOVL2* gene; Single nucleotide polymorphism; China

Introduction

Tuberculosis (TB) is a predominantly airborne infectious disease caused by *Mycobacterium tuberculosis* and is one of the top ten causes of death worldwide. The 2018 WHO report showed that 10 million people had tuberculosis and 1.6 million died from the disease (including 300,000 people living with HIV) in 2017 (1). Interestingly,

only 5% to 15% of population who have tuberculosis develop the disease (2), suggesting that the development of *M. tuberculosis* infection to TB is determined by multifactors, for example, the host's genes and immuo response (3).

The n-3 and n-6 polyunsaturated fatty acids (PUFAs) have important biological significance

and are closely related to human health (4-6). Adding n-3 and n-6 PUFAs to the diet may reduce the risk of active TB in the Chinese population (7). The biosynthesis of n-6/n-3 PUFAs originates from a series of precursor fatty acids derived from continuous carbon chain extension and dehydrogenation of fatty acids (8). Fatty acid elongase 2 (*ELOVL2*) is located on chromosome 6 (6p24.2) and encodes the long-chain fatty acid lengthening enzyme; as such, *ELOVL2* gates an important rate-limiting step in the PUFA metabolic pathway and its activity directly affects the synthesis of PUFAs (9-10).

A single nucleotide polymorphism (SNP) of the *ELOVL* gene is correlated with fatty acid composition in a number of tissues of the human body. Fatty acid composition varies among people with different *EVOVL* genotypes (11-13). The incidence of infectious disease involves autoimmunity, inflammation, environment and genetic fingerprint. The relationship between TB and SNPs of *ELOVL2* gene is still unclear.

In this study, we explored 3 SNPs of the *ELOVL2* gene (*rs3798719*, *rs1570069*, *rs2236212*) in a Han Chinese patient population, aimed to provide further insight into the association between the gene polymorphism of PUFA and TB.

Materials and Methods

Between January 2016 and November 2018, a hospital-based case-control study was conducted in Huainan Oriental Tumor Hospital, China and physical examination center of Huainan First People's Hospital. During the study period, an epidemiological survey was conducted in both hospitals every three months. Of these cases, the TB patients were diagnosed by sputum smear, culture and chest radiography. According to the "Diagnostic criteria for pulmonary tuberculosis" issued by the Ministry of Health of China, TB was diagnosed based on *M. tuberculosis* positive sputum smear, positive sputum culture and pulmonary lesions (15). The control group was excluding the history of tuberculosis, chronic ob-

structive pulmonary disease, pneumoconiosis, asthma, lung cancer, hypertension, tumor, diabetes, and other infectious diseases.

Informed consent was obtained from all participants and the study was approved by the Ethics Committee of School of Medicine, Anhui University of Science and Technology, Anhui, China (NO.2016012).

In total, 656 qualified subjects (326 cases and 330 control subjects) were enrolled. There were 232 males (71.2%) and 94 females (28.8%) in the case group, with an average age of 55.3±14.7 years. Totally, there were 225 males (68.2%) and 105 females (31.8%) in the control group, with an average age of 57.6±13.9 years.

Dietary patterns were assessed by a modified Food Frequency Questionnaire (FFQ). The FFQ optimized a previous questionnaire by adding dietary features of the target population, including 24 food groups that Chinese people commonly eat (14). Each item represents a food group, and participants were asked how often (daily, weekly, monthly, annually, or never) they consumed each item. We assessed the reliability and validity of the FFQ (Coefficients between 0.71 and 0.89 for major food groups).

About 5 mL of peripheral venous blood was harvested from all participants with vacuum anticoagulant tubes and stored at -80 °C until use. Genomic DNA was extracted and purified from whole blood using a commercially available genomic DNA extraction kit (Cat. #DP304-03, TianGen, Beijing, China) according to manufacturer's manual. The forward and reverse primers that were used in PCR. Genotyping was performed by Sanger sequencing (Sangon Biotech, Shanghai, China), based on the Applied Biosystems (ABI) Prism BigDye Terminator v3.1 Cycle Sequencing Kit and was run on an ABI 3730XL Genetic Analyzer.

The Chinese version of the International Physical Activity Questionnaire was used to survey the physical activity of the study population. The questionnaires were used to collect information about the following features: Physical activity, which was categorized into 3 levels: strenuous exercise, medium-intensity exercise, and low-

intensity exercise (16), Passive smoking (defined as non-smokers exposed to smoke for more than 15 minutes per day), Residence, either rural (living in the rural area more than 10 years and still living in rural area) or urban (living in the urban area more than 10 years and still living in urban area), economic status, sorted into high (family per month income more than 3000 CNY), average (1500-3000 CNY), or low (lower than 1500 CNY); and alcohol intake (defined as men drinking more than 20 g and women more than 10 g per day).

Data were manual loaded into EpiData 3.0 using double data entry approach. SPSS (version 21.0, IBM) was used to identify dietary patterns by factor analysis. We used chi-square tests for categorical variables to identify significant differences in proportion between groups. Logistic regression was used to identify the contributors of TB. In the analysis of additive interaction, the variables are required to be considered as dichotomies, so in the analysis of dietary patterns. Each pattern compares the highest quartile group with the lowest quartile group. We set Diabetes = No, Age < 40, Residence = Urban, Passive smoking = No, Medium-intensity exercise > 3 times/week, BMI > 18.5, Western food = Q4, High VD and Calcium = Q4, Traditional Chinese = Q1, High animal protein = Q4 as control, and protective factors as exposure. $P < 0.05$ was considered statistically significant.

Results

There were 326 TB cases and 330 control subjects enrolled in the study. In logistic regression analyses, after adjusting for confounders, lower economic status, smoking and alcohol drinking, <3 times/week of low-intensity, lower BMI, low protein/fat and animal protein dietary pattern, high vegetarian dietary pattern were associated with risk of tuberculosis (Table 1).

Table 1: Difference of anthropometric characteristics of in cases and controls

Variable	Tuberculosis		OR(95%CI)ᵃ	OR(95%CI)ᵇ
	Yes(n=326)	No(n=330)		
Gender				
male	232 (71.2)	225 (68.2)	1.152(0.825-1.607)	0.940 (0.697-1.266)
female	94 (28.8)	105 (31.8)	1.000	1.000
Occupation				
Worker/farmer	99 (30.4)	87 (26.4)	1.422(0.823-2.458)	1.015(0.831-1.240)
individual	120 (36.8)	105 (31.8)	1.429(0.838-2.436)	
civil servants	75 (23.0)	98 (29.7)	0.957(0.550-1.664)	
students	32 (9.8)	40 (12.1)	1.000	1.000
Marital status				
unmarried	90 (27.6)	94 (28.5)	1.197(0.531-2.696)	0.894(0.582-1.372)
Married	205 (62.9)	189 (57.3)	1.356(0.619-2.971)	0.924 (0.499-1.711)
Death of a spouse	12 (3.7)	15 (4.5)	1.000	1.000
Economic status				
Wealthy	29(8.9)	59 (17.9)	0.402(0.248-0.649) [*]	0.635(0.404-0.998) [*]
General	40(12.3)	61 (18.5)	0.536(0.346-0.831) [*]	0.787(0.503-1.231)
poor	257(78.8)	210 (63.6)	1.000	1.000
Passive smoking				
Yes	259 (64.6)	140 (49.6)	5.379(3.795-7.625) [*]	4.651 (2.199-9.859) [*]
No	65 (35.4)	189 (50.4)	1.000	1.000
Alcohol drinking				
Yes	223 (68.6)	159 (48.3)	2.338(1.699-3.215) [*]	1.925 (1.299-2.852) [*]
No	102 (31.4)	170 (51.7)	1.000	1.000

Strenuous exercise				
< 3 times/week	156 (48.1)	142 (43.2)	1.223(0.898-1.665)	1.014(0.605-1.699)
> 3 times/week	168 (51.9)	187 (56.8)	1.000	1.000
Medium-intensity exercise				
< 3 times/week	102 (31.4)	123 (39.7)	0.766(0.554-1.059)	0.923(0.522-1.633)
> 3 times/week	223 (68.6)	206 (60.3)	1.000	1.000
Low-intensity exercise				
< 3 times/week	78 (24.0)	106 (32.2)	1.847(1.299-2.625) *	1.608 (1.414-1.829) *
> 3 times/week	247 (76.0)	223 (67.8)	1.000	1.000
BMI				
<16.0	48 (14.8)	10 (3.0)	8.000(3.482-18.379) *	7.617(4.508-12.867) *
16.1-17.5	52 (16.0)	30 (9.1)	2.889(1.500-5.564) *	2.763 (1.240-6.156) *
17.6-18.5	49 (15.1)	36 (10.9)	2.269(1.193-4.313) *	1.867(1.293-2.696) *
18.6-23.9	148 (45.7)	208 (63.2)	1.186(0.704-1.998) *	0.971(0.325-2.901)
>24	27 (8.3)	45 (13.7)	1.000	1.000
Dietary patterns				
High protein/fat				
Q1	90(27.8)	79 (24.6)	0.177(0.046-0.677) *	0.585(0.369-0.928) *
Q4	19(5.8)	118 (36.2)	1.00	1.000
VD and Calcium				
Q1	103(31.7)	106 (32.5)	0.812(0.412-0.994) *	0.998(0.565-4.413)
Q4	16(4.9)	34 (10.7)	1.00	1.000
Vegetarian				
Q1	23(6.9)	105 (32.1)	3.617(1.508-6.867) *	3.269(1.170-8.135) *
Q4	116(35.8)	40 (12.2)	1.00	1.000
High animal protein				
Q1	105(32.4)	46 (14.6)	0.370(0.381-2.810) *	0.721(0.386-0.931) *
Q4	18(5.6)	153 (46.3)	1.00	1.000

[a] Unadjusted; [b]Adjusting for gender, age, residence, education, economic status, passive smoking, drinking, married status, physical activity * $P < 0.05$

Stratified by gender, the genotypes and allele frequencies of *rs3798719*, *rs1570069* and *rs2236212* showed significant differences between the two groups ($\chi^2 = 6.987$, $P = 0.030$), and the rest variables were not statistically different as shown in Table 2.

Genetic modeling showed that *rs3798719* between the case and control group was statistically different in the overdominance model ($\chi^2 = 4.784$, OR = 1.414, 95% CI: 1.036-1.929, $P < 0.05$)(Table 3). The polymorphism of *rs2236212* between male TB patients and healthy controls was statistically different in the dominance model. ($\chi^2 = 4.192$, OR = 0.507; 95% CI: 0.262-0.981, $P < 0.05$) (Table 4).

Table 2: Associations between the SNP genotypes of ELOVL 2 and the risk of PTB

Variable	Genotype	PTB	HC	OR(95%CI)	χ^2	P
All subjects						
rs3798719	C/C	161	183	0.682(0.323,1.439)	5.797	0.055
	C/T	153	127			
	T/T	12	20			

				OR(95%CI)	χ²	P
rs1570069	A/A	1	5	5.240(0.608,45.140)	3.020	0.221
	A/G	61	54			
	G/G	264	271			
rs2236212	C/C	142	150	0.896(0.515,1.559)	0.872	0.647
	C/G	156	147			
Males						
rs3798719	C/C	115	125	1.004(0.426,2.363)	2.024	0.364
	C/T	106	88	1.314(0.553,3.123)		
	T/T	11	12	1.00		
rs1570069	A/A	1	4	0.239(0.026,2.162)	1.915	0.384
	A/G	44	42	1.003(0.627,1.604)		
	G/G	187	179	1.00		
rs2236212	C/C	99	96	1.856(0.930,3.704)	4.532	0.104
	C/G	118	102	2.082(1.050,4.129) *		
Females						
rs3798719	C/C	46	46	6.345(0.766,52.573)	6.987	0.030
	C/T	47	47	9.641(1.155,80.462) *		
	T/T	1	1	1.00		
rs1570069	A/A	0	0	1.195(0.074,19.419)	2.583	0.273
	A/G	17	17	1.693(0.762,3.762)		
	G/G	77	77	1.00		
rs2236212	C/C	43	43	0.368(0.129,1.047)	3.820	0.148
	C/G	38	38	0.390(0.135,1.124)		

PTB: tuberculosis patients; HC: healthy controls *$P<0.05$

Table 3: Analysis results of different genetic models

SNP-ID	Genotype		PTB	HC	OR(95%CI)	χ²	P
rs3798719	dominance	CC+CT/TT	314 12	310 20	0.592(0.285-1.232)	2.001	0.157
	recessive	TT+CT/ CC	165 161	147 183	0.784(0.577-1.065)	2.421	0.120
	additive	CC/TT	161 12	183 20	0.682(0.323-1.439)	1.020	0.313
	overdominance	TT+CC/CT	173 153	203 127	1.414(1.036-1.929) *	4.784	0.029
rs1570069	dominance	AA+AG/ GG	62 264	59 271	0.927(0.625-1.376)	0.142	0.707
	recessive	AG+GG-AA	325 1	325 5	0.200(0.023-1.721)	2.642	0.104
	additive	AA/GG	1 264	5 271	4.871(0.565-41.971)	2.536	0.111
	overdominance	AA+GG/AG	265 61	276 54	1.177(0.786-1.761)	0.625	0.429
rs2236212	dominance	CC+CG/GG	298 28	297 33	0.846(0.498-1.435)	0.387	0.534
	recessive	GG+CG/CC	184 142	180 150	0.926(0.681-1.260)	0.239	0.625
	additive	CC/ GG	142 28	150 33	0.896(0.515-1.559)	0.150	0.698
	overdominance	CC+GG/CG	170 156	183 147	1.142(0.840-1.553)	0.722	0.396

Table 4: Analysis results of different genetic models in males

SNP-ID	Genotype		PTB	HC	OR(95%CI)	χ^2	P
rs3798719	dominance	CC+CT/TT	115 117	125 100	1.272(0.880-1.837)	1.642	0.200
	recessive	TT+CT/ CC	221 11	213 12	0.883(0.382-2.045)	0.084	0.772
	additive	CC/TT	115 11	125 12	0.761(0.320-1.808)	2.024	0.364
	overdominance	TT+CC/ CT	126 106	137 88	1.310(0.903-1.900)	2.023	0.155
rs1570069	dominance	AA+AG/GG	45 187	46 179	1.068(0.675,1.690)	0.079	0.779
	recessive	AG+GG/AA	1 231	4 221	4.181(0.464-37.698)	1.914	0.166
	additive	AA/GG	1 187	4 179	0.997(0.623-1.595)	1.915	0.384
	overdominance	AA+GG/AG	44 188	42 183	0.981(0.613-1.568)	0.007	0.935
rs2236212	dominance	CC+CG/GG	217 15	198 27	0.507(0.262-0.981) [*]	4.192	0.041
	recessive	GG+CG/CC	99 133	96 129	1.000(0.690-1.449)	0.000	0.999
	additive	CC/GG	99 15	96 27	0.480(0.242-0.952)	4.532	0.104
	overdominance	CC+GG/CG	114 118	123 102	1.248(0.864-1.803)	1.399	0.237

PTB: tuberculosis patients; HC: healthy controls [*]*P* < 0.05

Discussion

In this study, we found that *ELOVL2* genotype polymorphisms were significantly related to the development of tuberculosis. Specifically, we observed significant different frequencies of the *rs3798719* SNP in the overdominance model between the case and control groups. According to gender stratification analysis, we found that in the dominance model, the *rs2236212* of *ELOVL2* SNP is distributed statistically differently between male TB patients and healthy controls. In the homozygous and recessive models, female TB patient and healthy control genotypes differ in the frequency of the of *rs3798719* of *ELOVL2* SNP.

Associations of polymorphisms in *ELOVL2* and susceptibility to TB have not been reported, but n-6/n-3 PUFAs had benefits for preventing the development of TB (7, 22-24). Furthermore, *ELOVL2* encodes elongase 2, critical to n-6/n-3 PUFAs biosynthesis (17). An in vivo study demonstrated that the *ELOVL2* gene played a key role at two penultimate steps of PUFA synthesis in Atlantic salmon (17). In addition, the

levels of 22:6 n-3 and 22:5 n-6 PUFAs and cumulative level of 20:4 n-6, 20:5 n-3, 22:5 n-3 and 22:4 n-6 PUFAs were lower in an *ELOVL2* partial knockout mouse model (18).

Epidemiological studies support the linkage between *ELOVL2* gene polymorphisms and PUFA variation. For instance, *ELOVL2* polymorphisms were significantly related to Autism spectrum disorder risk by lowering the level of long-chain polyunsaturated acids (19). Lemaitre et al analyzed five European cohorts with a genome-wide association study (GWAS) of 8,868 individual and showed that *ELOVL2* gene polymorphism was correlated with n-3 PUFAs and that *rs2236212* carriers had significantly decreased levels of DHA (13). To elucidate the relationship between blood plasma concentration of PUFAs and genetic factors, 1,075 participants were included for GWAS of plasma levels of six types of n-3 and n-6 PUFAs. Besides FADS gene cluster, the strongest association region in this GWAS was chromosome 6 (*ELOVL2*), mapped to the region encoding long-chain fatty acid (21-23). A GWAS conducted by the Shanghai Institute of Nutrition reported that the variation of *rs2281591* by *ELOVL2* gene was positively cor-

related with erythrocyte membrane DPA levels, while the variation of *rs3734398* was negatively correlated with DHA level (26). Despite this breadth of investigation into the link between *EVOLV2* polymorphisms and PUFAs, the gene's connection with TB has been understudied.

The relationship between the n-6/n-3 series of PUFAs and risk for TB is still elusive (7, 24-29). The ability of EPA/DHA to control the growth of *M. tuberculosis* has been demonstrated in animal studies, including mouse and guinea pig models (24, 25). While omega-3 fatty acids tended to increase pathogen death in an *in vivo* study, and omega-6 increases survival of *M. tuberculosis* in mice (25). Other studies have likewise reported a limited and inconclusive relationship between n-6/n-3 PUFAs and TB (21-24). Additionally, EPA has been shown to increase mycobacterial growth by reducing TNFa secretion in macrophages (26). DHA reduces the ability of J774A.1 cells to control tuberculosis in response to activation by IFNc, by modulation of IFNc receptor signaling and function (27) In contrast to these data, a recent 15-year longitudinal cohort study of 63,257 Chinese people aged 45 to 74 in Singapore showed that n-6/n-3 PUFAs reduced the risk of pulmonary tuberculosis in a dose-dependent manner (7). Thus, n-6/n-3 PUFAs targeting to *M. tuberculosis* could be a new assist treatment strategy to alleviate the pulmonary impairment caused by *M. tuberculosis* infection. However, it should be confirmed by further epidemiological studies.

To understand better the mechanism by which n-6/n-3 PUFAs influence TB, further examination of PUFAs role in inflammatory responses should be conducted. n-6/n-3 PUFAs are beneficial to host by enhancing ability to fight tuberculosis and regulate inflammation and immune factors (24, 28-31). The n-3 PUFAs have effects on immune cells function. For example, there are three main properties (production and secretion of cytokines and chemokines, the capacity of phagocytosis and the polarization into classically activated or alternatively activated macrophages) of macrophage biology that have been identified to be altered by omega-3 fatty acids (32-33). Meanwhile,

Omega-3 fatty acids and their metabolites can modulate neutrophil function by neutrophil migration, phagocytic capacity, as well as the production of reactive oxygen species and cytokines (34-36). Moreover, DHA regulates placental inflammation by inhibiting the NLRP3 inflammasome and NF-ϰB signaling pathways (37). An *in vitro* study indicated that DHA and EPA reduced LPS-induced inflammation in HK-2 cells and PUFA promoted anti-inflammatory effect at the transcription level *in vivo* (38, 39). In a rat model of colitis, the synergistic effect of a diet rich in omega-3 PUFA and olive oil played a protective role in inflammatory bowel disease (40). Epidemiologic evidence also revealed that DHA supplements reduce serum c-reactive protein and other markers of inflammation in men with hypertriglyceridemia (41). In summary , elucidation of the mechanism between n-6/n-3 PUFAs and the risk of pulmonary tuberculosis is critical to shed lights on the supplement of n-6/n-3 series PUFAs as an intervention means to prevent tuberculosis.

Three SNPs of the *ELOVL2* gene were selected for this study, which are in line with the gene frequencies of Asian population in Hapmap database. However, we only found a difference in women, not in the whole population. The low sample size (303 cases) could be one cause. A large sample size and cross valiation by other groups should be used to study the relationship between *ELOVL2* gene polymorphisms and the risk of TB.

Conclusion

We investigated the association of three SNP (*rs3798719, rs1570069, and rs2236212*) of the *ELOVL2* gene with TB susceptibility. The *rs3798719* SNP may be associated with susceptibility to *M. tuberculosis* infection in women and the *rs2236212* SNP may be associated with TB occurring in male subjects.

Our study provide direct evidence for screening population with genetic susceptibility to tuberculosis, which alerts early prevention of TB. Our

work provides new avenue for the diagnosis and treatment of TB.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

All authors declare that they have no conflict of interest.

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