

## Distribution of TNF- $\alpha$ -308 G/A polymorphism among pediatric patients with pneumonia in Ulaanbaatar

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**Abstract:** Genetic studies indicate to the importance of individual genetic diversity on predictor of mortality. Furthermore, Single Nucleotide Polymorphisms can be used to identify disease-causing genes in humans and they can be either neutral or deleterious. Human Tumor necrosis factor- $\alpha$  is a well-known inflammation factor that is closely associated with sepsis and severe sepsis. Our objective was to evaluate the association of TNF- $\alpha$  -308 G/A promoter polymorphism with dependency to severity of pneumonia. Respiratory diseases, especially pneumonia, is the major cause of mortality and morbidity in Ulaanbaatar, Mongolia and the leading causes of pediatric hospitalization. We collected blood samples from 101 pediatric patients of the age group between "new born" and "school aged", who were treated and diagnosed with pneumonia in February 2019 and 2020, the pneumonia season in the country. Genomic DNA was extracted and performed by PCR-RFLP method to detect the presence of SNPs. The studies showed that the TNF- $\alpha$  -308 G/A polymorphism among pediatric patients, genotype G;G was 73.27%, genotype A;G was 22.77%, and genotype A;A was 3.96%. Our study demonstrated disassociation of TNF- $\alpha$  -308 G/A polymorphism with pneumonia severity in population.

**Keywords:** TNF- $\alpha$ ; population genetics; SNP; polymorphism; pediatric pneumonia;

### INTRODUCTION

In the last few decades, incidences of respiratory diseases, including pneumonia in Ulaanbaatar increased alarmingly and are now the number one of leading cause of under-five child mortality in the country. An immature immune system makes young children particularly susceptible to infection [1, 2]. In response to pneumonia, an inflammatory reaction is produced locally in the lung, which includes both pro-inflammatory and anti-inflammatory.

TNF- $\alpha$  is potently pleiotropic pro-inflammatory and immunomediator cytokine is produced by activated macrophages and lymphocytes, which are also expressed in endothelial cells and others. It is an essential component of the host immune response to infection, and is responsible for the release of other inflammatory mediators. TNF- $\alpha$  also plays a major role in the clinical manifestations of septic shock, and serum levels inversely correlate with survival from severe sepsis.

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The biological functions are varied, complex and two sided. It confers disease resistance and it also causes pathological complications. Human TNF- $\alpha$  is 17kDa cytokine and gene is located on chromosome 6 within the major histocompatibility complex (MHC) and where genetic alterations in the TNF- $\alpha$  locus are known to be involved directly in high TNF- $\alpha$  production [3, 4, 5, 9, 10, 11].

A number of TNF- $\alpha$  gene promoter polymorphisms have been described, which are believed to affect production of TNF- $\alpha$ . Among these SNP, in the promoter region at position 308 before the transcription start site, there are substitutions of adenine for guanine (-308

G/A). This SNP potentially affect TNF- $\alpha$  synthesis and adenine allele has been suggested to be associated with higher TNF- $\alpha$  level, which may alter the course of immune response.

Genetic factors are likely to contribute to variable presentation of pneumonia. An Individual's ability to respond to infection is variable and a predisposition to death from infection is clearly inheritable [6, 7, 8, 9]. However, information regarding contribution of genetic variations to pneumonia causation is limited in Mongolia. By this study, we aimed to investigate the association of TNF- $\alpha$  -308 G/A and pneumonia.

## MATERIALS AND METHODS

Pneumonia is seasonal and is usually associated with the winter season. Samples were taken from pediatric inpatients since February 2019 (50 patients) and February 2020 (51 patients) in district hospital of Songinokhairkhan. Songinokhairkhan district has one of the highest incidences of pneumonia activity in Ulaanbaatar.

The age of pediatric patients ranged from 1 month to 15 years (median 15 months, mode 10 months) and they were all unrelated. Subject blood came from 101 patients who were diagnosed with acute lower respiratory infections, including pneumonia [12]. Genomic DNA was isolated from blood by a standard phenol-chloroform method. Polymerase chain reaction (PCR) was performed using two specific primers the -308 promoter region of the TNF- $\alpha$  gene: forward primer: (5'-AGGCAATAGGTTTTGAGGGCCAT-3'),

and reverse primer: (5'-TCCTCCCTGCTCCGATTCCG -3'). PCR mix consisted was about 20 $\mu$ l, containing 100-200 ng genomic DNA, 2 $\times$ PCR mix (10 $\mu$ l), 10 pmol each of reverse and forward primer. The following cycling conditions were used: 94°C for 4 minutes and 35 cycles in PCR system (Thermo scientific<sup>TM</sup>, Arktik Thermal cycler), following conditions were applies: 95°C for 1 minutes, annealing at 60°C for 45 seconds, extension at 72°C for 7 minutes. The amplified product was digested with 10 U NcoI at 37°C for 12 hours and visualized by 2% agarose gel. A 50 bp marker (50 bp DNA Ladder, Generay) was used as a size standard for each gel lane. Resulting in a band on the gel, G;G allele identified fragments of 87 bp and 20 bp, A;G identified fragments of 107bp, 87 bp and 20 bp and A;A allele identified fragment of single 107 bp.

## RESULTS AND DISCUSSION

A total of 101 pediatric patients (58 males, 43 females) were analyzed without control group. We categorized pediatric patients into 5 age-groups (Figure 1) and three

genetic models (G;G vs A;G vs AA). 100 patients recovered and 1 patient diseased. Statistically, it's impossible to analyze in mortality.

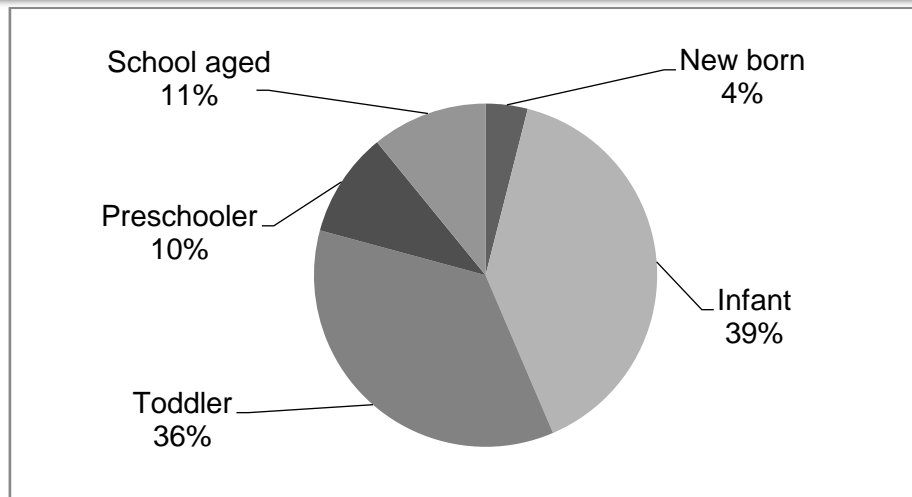


Figure 1. Age-group of pediatric patients

The studies showed TNF- $\alpha$ -308 G/A polymorphism among pediatric patients, genotype G;G was 73.27%, genotype A;G was

22.77%, and genotype A;A was 3.96% (Table 1).

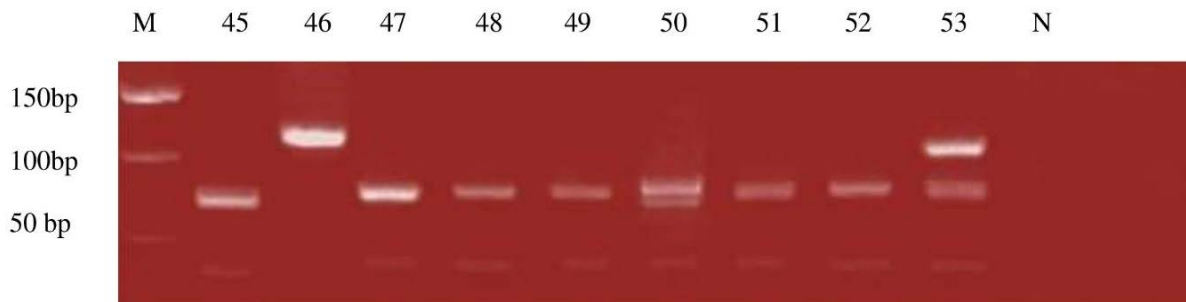


Figure 2. PCR product of the TNF- $\alpha$  308 G/A polymorphisms among patients. M-Marker; 45, 47, 48, 49, 50, 51, 52, 11, 12 G;G alleles (87 bp, 20 bp); 53 A;G alleles (107 bp, 87 bp, 20bp); 46 A;A allele (107 bp)

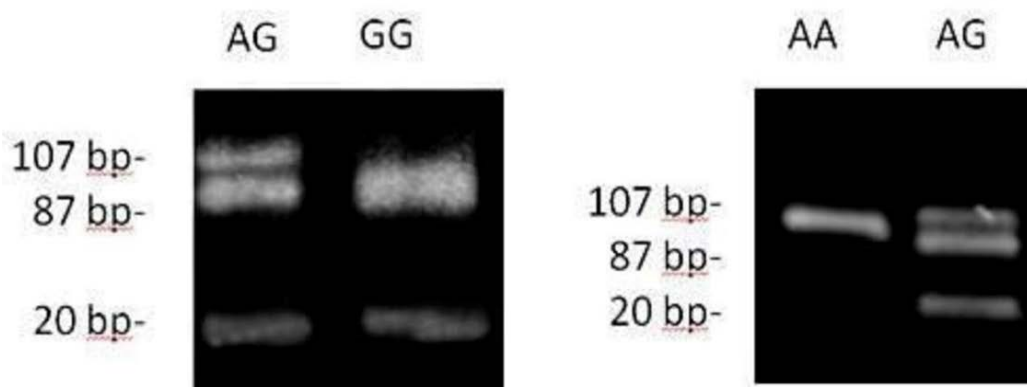


Figure 3. PCR product of TNF- $\alpha$  308 G/A polymorphisms

We considered genotype and severity. These results suggest that G;G allele group predominated 41% non-severe and A;G allele group predominated 27.1% severe diagnoses in severity. Results are shown in Figure 4. We analyzed these differences using a chi-square

test. According to the test results, there is no reason to reject the null hypothesis ( $\chi^2= 1.011$ , p-value= 0.603), which means statistically TNF- $\alpha$  -308 G/A polymorphism does not any relation to pneumonia severity (Table 2).

Table 1. Crosstab of severity and genotypes of patients

			Genotype			Total
			A;A	A;G	G;G	
Diagnosis	Non-severe	Count	2	10	41	53
		%	3.8%	18.9%	77.4%	100.00%
	Severe	Count	2	13	33	48
		%	4.2%	27.1%	68.8%	100.00%
Total		Count	4	23	74	101
		%	4.00%	22.80%	73.30%	100.00%

For the dominant model (GG, AG+AA) and severity was ( $\chi^2= 0.953$ , p-value= 0.373)

and for recessive model (GG+AG, AA) the severity was ( $\chi^2= 0.919$ , p-value= 0.653).

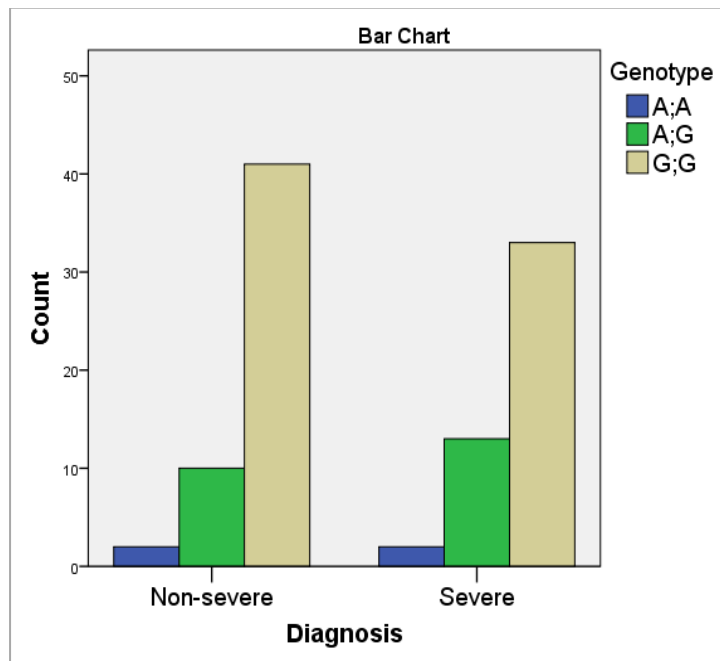


Figure 4. Bar chart of severity and genotypes of patients

Table 2. Distribution of the TNF- $\alpha$  genotypes and alleles among patients

Genotype	Severe n=48	Non-severe n=53	OR (95% CI)	p value
<b>Dominant model</b>				
GG	33 (44.6%)	41 (55.4%)	1.553 (0.64-3.77)	0.373
AG+AA	15 (55.6%)	12 (44.4%)		
<b>Recessive model</b>				
AA	2 (4.2%)	2 (3.8%)	1.109 (0.15-8.193)	0.653
AG+GG	46 (95.8%)	51 (96.2%)		

Frequencies of severity and genotypes were compared. Among dominant and recessive model, no significant difference was found in the prevalence. Given the suggestion of different age group association between severities, we analyzed age dependency. Among the pediatric patients, non-severe diagnoses (50.0%) was predominant among the new born and infant group, severe diagnoses (60.0%) predominated the toddler group and non-severe diagnoses (60.0%) predominated

pre-school (age between 3 and 5 years old) and school age (age over 12 years) group ( $\chi^2=6.67$ , p-value= 0.154).

We calculated allele frequencies of TNF- $\alpha$  -308 G/A polymorphism by Hardy-Weinberg equilibrium. Although these assumptions are rarely true in the natural world, they allow us to calculate an expected allele frequency (p= 0.8465, q= 0.1535). Table 3 shows the comparison with populations in other countries.

*Table 3. Distribution of TNF- $\alpha$  308 G/A alleles in different populations*

	Observed 2019 (Mongolia) %	Expected %	Chen et al 2011 (China) %	Ognjanovic 2009 (non-asian USA) %	Churnosov et al 2017 (Russia) %	Bin Cui et al 2015 (Kazakh, Xinjiang) %
G;G	73.27	83.5	88	50	79.9	51.8
A;G	22.77	15	11.11	39.1	38	48.2
A;A	3.96	1.5	0.8	10.8	1.8	4.2
Total	100	100	100	100	100	100

The polymorphism in the human TNF- $\alpha$  gene encoding high TNF- $\alpha$  level may be important in the susceptibility or severity of diseases and in other inflammatory conditions. Genetic studies of pneumonia are not numerous, while investigations assessing the role of host genetics in sepsis due to pneumonia have been carried out. Furthermore, to date, there are no genome-wide association (GWAS) study [5, 16]. Other studies reveal that TNF- $\alpha$  A allele is associated with more aggressive disease and this may be related to variations in

TNF- $\alpha$  levels in serum, which correlates with the severity of the disease [17, 18]. Several studies evaluated the associations of TNF- $\alpha$  polymorphism with pneumonia among different populations. However, the results were conflicting and controversial. In meta-analysis study, A alleles (A;G+A;A) was not associated with a higher risk mortality from pneumonia compared with G;G, but carriers of A increased pneumonia risk in the Asians, but not in the Caucasians. [19].

**CONCLUSIONS**

In conclusion, pneumonia is a complex inflammatory disease. In our study, we succeeded in demonstrating that TNF- $\alpha$  -308 G/A polymorphism was not associated with pneumonia severity and does not play a major

role in our case. In other words, 2.39% of individuals are categorized as risk group. Large-sample studies and other polymorphism interactions should be considered in future.

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