MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY SCIENCE AND TECHNOLOGY

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THE STUDY OF POLYMORPHISMS OF SOME GENES ON PATIENTS WITH GOUT

Major: Biochemistry

Code: 9420116

SUMMARY OF BIOTECHNOLOGY DOCTORAL THESIS

Ha Noi - 2023

The thesis has been completed at: Graduated University of Science and Technology - Vietnam Academy of Science and Technology

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The thesis will be defended at the Board of Examiners of Graduate University of Science and Technology – Vietnam Academy of Science and Technology at on

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INTRODUCTION

1. The urgency of the thesis topic

Gout is a metabolic disorder caused by the deposition of uric acid in tissues due to increased blood uric acid levels. Clinical symptoms of the disease include gouty arthritis, tophi nodules, gouty kidney disease, and uric stones. This is a metabolic disorder, so it can be controlled if the patient is treated according to the regimen.

Studies of correlations between variants located on gene regions are of great interest in the world. Gout now has a high prevalence, especially in developing countries due to uncontrolled diet. InEurope, the disease, which accounts for about 0.02–0.2% of the population [1], occurs mainly in middle-aged men. The disease is hereditary. In Vietnam in the period 1978-1989, the prevalence of gout accounted for 1.5% of patients with musculoskeletal disease. According to another study on disease patterns at the musculoskeletal department of Bach Mai Hospital, for 10 years (1991-2000), gout accounted for 8.57% [4].

Although the disease has characteristic manifestations, early diagnosis is still difficult. Currently, correlation studies between gout and related gene variants have not been conducted much in Vietnam, so we have conducted the topic "The study of polymorphisms of some genes on patients with gout".

2. Aims of the thesis

1. Determination of allele frequency and genotype of 6 genes ABCG2, SLC22A12, *SLC2A9, TNF, TLR4*α and *SLC17A1* in gout patients compared with healthy controls.
2. Analysis of the association between polymorphisms of 6 genes *ABCG2*, SLC22A12, SLC2A9, *TNF* , *TLR4* and α*SLC17A1* with gout and some subclinical indicators.

3. Contents of the thesis

This was a disease-control study of 521 samples, conducted on two groups of normal, healthy people and people with gout. The study used molecular biology methods to identify 09 variants located on the ABCG2, SLC22A12, *SLC2A9, TNF, TLR4α* and *SLC17A1* genes.

The project comes from the practice of combining medical literature and is conducted at the Genome Research Institute, Vietnam Academy of Science and Technology. The initial results of the study found an association between certain gene polymorphisms and the risk of gout.

CHAPTER 1. OVERVIEW

1.1. Outline of gout

1.1.1. Definition

By definition, gout is a metabolic disease, characterized by episodes of acute arthritis, recurrent phenomena, and deposition of urate crystals in tissues [8]. The cause of this disease is metabolic disorders of the purine nuclei, characterized by hyperuricemia and deposition of urate crystals in tissues [9].

1.1.2. Epidemiology

Gout is common in developed countries but is now also common in developing countries and the disease tends to increase, according to studies in the UK the prevalence of gout has increased from 0.14% in 1975 to 1.4% in 2005 [12]. In Vietnam, in the period from 1978 to 1989, the prevalence of gout accounted for 1.5% of patients with musculoskeletal disease treated inpatients at the musculoskeletal department of Bach Mai Hospital and according to a study within 10 years (1991-2000) this rate increased to about 8.57% [5].

1.1.3. Pathogenesis

a) Uric acid metabolism

Uric acid is the end product of exogenous and endogenous purine metabolism pathways [18]. The total amount of exogenous purines varies significantly with diet and the amount of protein the body absorbs each day.

b) Pathogenesis

There are many risk factors for hyperuricemia, including decreased renal and gastrointestinal urate excretion or increased urate production in the body [21, 24].

1.1.4. Gout classification

Gout is usually classified based on its cause and by clinical type. Classification by etiology includes primary gout and secondary gout.

a) Primary gout

b) Secondary gout

c) Acute gout

d) Chronic gout

1.1.5. Diagnose of gout

In the current situation, although there are many criteria used to diagnose gout, among them the most commonly used standard today is the ACR/EULAR standard 2015 [23].

1.1.6. Difficulty in diagnosing early gout

Although uric acid levels are an important factor in early diagnosis of gout, elevated uric acid is not synonymous with gout. In a few cases, patients with early gout have low blood uric acid test results.

1.1.7. Gout treatment

a) Nonsteroidal anti-inflammatory drugs (NSAIDs)

b) Colchicine

c) Glucocorticoids

d) Treatment of bacterial infections

e) Drugs that lower blood uric acid

1.1.8. Gout prophylaxis

Prevention of gout requires a combination of factors [29]. Follow a proper diet such as avoiding seafood foods, eating less saturated fat and low-fat products, and discontinuing or reducing the use of alcoholic beverages, especially alcohol.

1.2. Overview of single nucleotide polymorphisms (SNPs)

1.2.1. Characteristics of single nucleotide polymorphism (SNP)

Single nucleotide polymorphisms are defined as DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered relative to other individuals in the same biological species or to the other chromosome in a pair of homologous chromosomes [31].

1.2.2. Methods for determining single nucleotide polymorphism (SNP)

a) Enzyme-based methods

b) Hybridization-based methods

c) Sequencing solutions

1.2.3. The importance and use of single nucleotide polymorphisms (SNPs)

a) SNP Map

b) SNP development and medicine

1.3. Study of the role of genes in world gout and the world

1.3.1. The general state of research on gout in the world

Currently in the world there are many studies related to gout such as the pathogenesis of gout are carried out all over the world. The prevalence of

gout patients in countries of different regions varies widely, ranging from 0.1% to 10% [11].

1.3.2. Studies that correlate whole chromosomes with gout

In recent years, genome-wide studies (GWAS), replication studies, and analysis of large samples have found many new genes associated with increased levels of uric acid in the blood and gout. In 2015, the GWAS study of 140,000 Europeans identified polymorphisms on 28 genes that are associated with uric acid levels in the blood and gout.

1.3.3. The role of the ABCG2 gene in gout

Polymorphism studies on *the ABCG2* gene are widely performed in gout patients across a number of different populations such as Japanese, Han and Tibetan populations in China, Caucasian populations, and black populations.

1.3.4. Correlation of SLC22A12 gene and gout

A study in Han Chinese populations showed that two polymorphisms on the *SLC22A12* gene were strongly associated with elevated uric acid [88]. Polymorphism rs7932775 has also been shown to be associated with elevated uric acid in Czechs.

1.3.5. The importance of the SLC2A9 gene and gout

Among the polymorphisms of the SLC2A9 gene, in addition to the rs12510549 *SLC2A9* polymorphism which has been shown to reduce the risk of gout in Caucasian and Asian people [98], the rs16890979 polymorphism has also been associated with gout [*99*].

*1.3.6. Correlation of TNF gene and gout*α

Research conducted with rs1800629 *TNF* in α 10 European populations, 3 Latin American populations and 1 Asian population has been associated with an associated risk of gout in Latin Americans [105].

1.3.7. The association of the TLR4 gene and gout

Several studies on the rs2149356 *TLR4* polymorphism suggest that this gene has been shown to be associated with disease risk and may be involved in immunomodulation, processes such as inflammation, and lipid exchange [107].

1.3.8. SLC17A1 gene and gout

Many studies have demonstrated an association between polymorphisms on SLC17A1 and gout risk [100]. This polymorphism has been shown to be associated with gout risk in Japanese and New Zealand populations.

1.3.9. Studies of gout in water

Up to now, there have been many studies on gout in Vietnam, but the studies mainly investigate issues related to clinical features, subclinical, diagnosis and treatment directions. Recently, studies on the influence of genetic factors on gout have just begun to be interested and gradually more studies are involved.

CHAPTER 2. OBJECTS AND METHODS OF RESEARCH

1.4. Sample collection location and study time

1.4.1. Sample collection locations

Five hundred and twenty-one gout patients and controls were not related by blood, aged 18 years or older. All were sampled at Nguyen Trai Hospital and Dai Phuoc General Clinic.

1.4.2. Duration of study

Project duration: from 01/01/2016 – 31/12/2018.

1.5. Study design

The study was designed according to the disease-control description.

1.6. Study subjects and sample sizes

1.6.1. Subjects of study

The disease group includes patients with acute gout or chronic gout being treated at Nguyen Trai Hospital and Dai Phuoc General Clinic selected to meet the selection and exclusion criteria.

1.6.2. Sample size:

Calculated according to the sample size calculation formula for the disease-control study

1.7. Sampling and research process

1.7.1. Disease group

The study collected data that, after reviewing the exclusion criteria, the study obtained 170 samples with the disease that matched the study.

1.7.2. Control group

After screening and sampling, the study obtained 351 samples that matched the study's criteria

1.8. Research instruments, equipment and chemicals

1.9. Research methodology

1.9.1. Research Implementation Process

1.9.2. Data collection methods

1.10. Methods and techniques of use

1.10.1. Biochemical and hematological methods

1.10.2. Primer design methods for PCR

1.10.3. Total DNA extraction

1.10.4. DNA quantification method by spectrophotometer

1.10.5. DNA fragment multiplication by PCR

1.10.6. PCR product purification

1.10.7. Sanger Sequencing

1.10.8. Polymorphism/gene mutation analysis

1.11. Variables in the study

1.11.1. Group of variables: general characteristics of the object of study

1.11.2. Group of subclinical variables

1.11.3. Group of gene variables

1.12. Data processing and analysis methods

1.12.1. Raw Data Processing

1.12.2. Data analysis

1.13. Ethical issues in biomedical research

This research has been approved by the Ethics Council in Biomedical Research of the Genome Research Institute under Decision No. 01-2017/NCHG-HDDD of the Genome Research Institute.

CHAPTER 3. RESULTS AND DISCUSSION

1.14. Result

1.14.1. Clinical and subclinical features in the study sample

a) Clinical factors

Table 0. 1. Results of analysis of clinical features in disease groups and controls

Character	Amount (n=521)	Control group (n=351)	Disease group (n=170)	P-value
Sample	505	351	154	**<0,001**
Age*	52.82±11.33	53.40±10.03	51.62±13.58	**0,0469**
Height (cm)	164.99±5.76	165.06±5.52	164.84±6.24	0,697
Weight (kg)	66.3±9.52	65.75±9.72	67.47±9.00	**0,026**
BMI ($kg/^{m2}$)	24.57±3.35	24.45±3.54	24.82±2.91	**0,038**
SBP (mmHg)	120.06±11.3	119.83±10.8	120.53±12.32	0,84
DBP (mmHg)	74.74 ±7.77	74.59 ±7.77	75.06 ±7.79	0,533

The value of the continuous variable is expressed as the mean ± standard deviation;

b) Subclinical

Table 0. 2. Results of analysis of relevant subclinical characteristics in disease groups and controls

Character	Amount (n=521)	Control group (n=351)	Disease group (n=170)	P-value
ALT (U/L)	32.77±23.88	32.03±22.74	34.31±26.08	0,1563
AST (U/L)	28.21±21.70	27.82±22.46	28.99±20.07	0,2842
BUN (mg/dL)	25.87 ± 12.30	26.29±9.71	25.00±16.41	0,1342
CREA (mg/dL)	1.09±0.23	1.08±0.17	1.11±0.31	0,9537
Uric acid (mg/dL)	**7.72±1.94**	**6.29±1.51**	**9.29±1.81**	**<0,001**
CRP (mg/dL)	**3.92±4,507**	**3.42±4.37**	**4.96±4.61**	**0.048**
GLU (mg/dL)	103.61±15.45	102.27±10.33	105.37±21.81	0,145
HDL-C (mg/dL)	47.2 ± 20.72	47.9 ± 22.64	45.76 ± 16.03	0,204
LDL-C (mg/dL)	**104.5 ± 27.8**	**102.76 ± 26.9**	**108.12 ± 29.4**	**0,04**
TG (mg/dL)	**171.1±60.01**	**165.9±58.8**	**184.2±61.24**	**0,001**
WBC (above μL)	7681±2306	7655±2222	7735±2478	0,778

1.14.2. Total DNA extraction

1.14.3. Evaluation of correlation between ABCG2 rs72552713 and gout

a) Determination of polymorphic genotype rs72552713 on ABCG2

b) Determination of polymorphic genotype composition ABCG2

rs72552713

Table 0. 3. Table of genotype and allele frequency statistics of polymorphism ABCG2 rs72552713

	Genotype			Allele		P-value	HWE
	GG	**GA**	**AA**	**G**	**A**		
Control group	350 (0,997)	1 (0,003)	0 (0,0)	0,999	0,001	1,000	+
Disease group	160 (0,94)	10 (0,06)	0 (0,0)	0,971	0,029	0,925	+
Amount	510 (0,98)	11 (0,02)	0 (0,0)	0,98	0,02	0,977	+

HWE: Hardy-Weinberg equilibrium; "+": Obeys the HWE law of equilibrium.

c) Analysis of correlation between ABCG2 polymorphism rs72552713 with gout

Table 0. 4. Univariate correlation between polymorphism rs72552713 and gout

Genotype	Control group (n, %)	Disease group (n, %)	OR	95% CI	P-value
Dominant Model					**0.0000025**
GG	350 (99,7%)	160 (94,1%)	1,00		
GA	1 (0.3%)	10 (5,9%)	21,875	2.77 - 172.34	
Allele					**0.0000038**
G	701 (99,86%)	330 (97,06%)	1,00		
A	01 (0,14%)	10 (2,94%)	21,19	3.00 - 918.96	

d) Evaluation of correlation between rs72552713 polymorphism with

other factors

Table 0. 5. Evaluation of polymorphic correlation rs72552713 with other factors

ABCG2 **rs72552713**	**Genotype**		**P-value**
	GA (n=11)	**GG (n=510)**	
Uric acid (mg/dL)	**9.37 ± 1.80**	**7.66 ± 1.92**	**0,0001**
Weight (kg)	**71.16± 8.20**	**66.15± 9.51**	**0,012**
Height (cm)	164.93±5.79	166.47±4.82	0,1261
BMI	24.54±0.15	25.69±0.67	0,0723
CRP (mg/dL)	5.77±1.76	5.40±1.33	0,4838
HDL-C (mg/dL)	47.23±21.13	45.58±8.68	0,3669
LDL-C (mg/dL)	122.47±39.26	126.63±41.28	0,3255
Triglyceride	231.00±7.19	256.46±47.53	0,2504
Glucose	112.83±1.69	108.47±3.50	0,3074
AST (U/L)	28.35±0.99	24.57±1.15	0,2285
ALT (U/L)	32.90±1.08	29.48±4.58	0,2703
BUN (mg/dL)	**26.05±0.56**	**21.18±2.57**	**0,0454**
Creatinine	1.09±0.01	1.10±0.05	0,3877
WBC (above µL)	7646±105	8002±402	0,2546

1.14.4. Correlation evaluation of rs12505410 ABCG2 gene and gout

1.14.5. Correlation evaluation of rs11231825 SLC22A12 gene and gout

a) Determination of polymorphic genotype rs11231825 gene SLC22A12

b) Determination of polymorphic genotype composition SLC22A12 rs11231825

Table 0. 6. Table of genotype and frequency statistics polymorphic allele rs11231825

	Genotype			Allele		P-value	HWE
	TT	TC	CC	T	C		
Control group	183 (0,52)	134 (0,38)	34 (0,1)	0,712	0,288	0,438	+
Disease group	99 (0,58)	66 (0,39)	5 (0,03)	0,776	0,224	0,304	+
Amount	282 (0,54)	200 (0,38)	39 (0,08)	0,733	0,267	0,914	+

c) Analysis of correlation between rs11231825 polymorphism with gout

Table 0. 7. Results of the correlation assessment of rs11231825 polymorphism with gout

Genotype	Control group (n,%)	Disease group (n,%)	OR	95%CI	P-value
Aggregation model					**0,0104**
TT	183 (52,1%)	99 (58,2%)	1,00		
TC	134 (38,2%)	66 (38,8%)	0,910	0.621 - 1.335	0,631
CC	34 (9,7%)	5 (2,9%)	0,272	0.103 - 0.717	**0,005**
Dominant model					0.190
TT	183 (52,1%)	99 (58,2%)	1,00		
TC+CC	168 (47,9%)	71 (41,8%)	0,781	0.540 - 1.131	0,578
Diving Model					**0.006**
TT+TC	317 (90,3%)	165 (97,1%)	1,00		
CC	34 (9,7%)	5 (2,9%)	0,283	0.108 - 0.736	0,233
Co-dominant model					0,697
TT+CC	217	104	1.00		

	(61,8%)	(61,2%)			
TC	134 (38,2%)	66 (38,8%)	0,720	0.521 - 1.035	0,521
Allele					**0.0302**
T	500 (71,2%)	264 (77,6%)	1,00		
C	202 (28,8%)	76 (22,4%)	0,712	0.526 - 0.964	0,602

d) Evaluation of correlation of rs11231825 polymorphism with other factors

Table 0. 8. Evaluation of correlation of rs11231825 polymorphism with clinical indicators

SLC22A12 rs11231825	**Genotype**			**P-value**
	TT (n=282)	**TC (n=200)**	**CC (n=39)**	
Uric acid (mg/dL)	7.82±1.92	7.61±1.99	7.56±1.85	0,4515
Weight (kg)	66.13±9.42	66.85±9.39	65.18±10.69	0,5311
Height (cm)	165.03±6.25	165.00±4.97	164.59±5.92	0,9030
BMI	24.55±3.35	24.62±3.14	24.67±4.45	0,9588
CRP (mg/dL)	7.00±51.94	4.20±5.88	4.74±5.78	0,7266
HDL-c (mg/dL)	46.82±24.77	47.76±14.58	46.74±16.34	0,8836
LDL-c (mg/dL)	**124.56±41.07**	**117.56±36.59**	**134.33±36.73**	**0,0245**
Triglyceride	222.11±135.30	240.74±196.01	257.62±137.54	0,2737
Glucose (mg/dL)	**108.83±25.46**	**115.09±38.27**	**127.79±76.27**	**0,0055**
AST (U/L)	29.15±23.01	27.71±21.63	23.96±7.56	0,3510

ALT (U/L)	33.21±25.29	32.31±23.47	32.01±13.84	0,9024
BUN (mg/dL)	25.71±13.12	25.98±11.40	26.45±10.85	0,9290
Creatinine	1.07±0.24	1.11±0.22	1.04±0.10	0,0824
WBC (above µL)	7701±2380	7579±2177	7773±2396	0,8104

1.14.6. Correlation evaluation of rs7932775 SLC22A12 gene and gout

1.14.7. Correlation evaluation of rs12510549 SLC2A9 gene and gout

a) Determination of polymorphic genotype rs12510549 SLC2A9 gene

b) Determination of polymorphic genotype component SLC2A9 rs12510549

Table 0. 9. Table of genotype and allele frequency statistics SLC2A9 rs12510549

	Genotype			**Allele**		**P-value**	**HWE**
	TT	**TC**	**CC**	**T**	**C**		
Control group	253 (0,72)	91 (0,26)	7 (0,02)	0,850	0,150	0,491	+
Disease group	126 (0,74)	39 (0,23)	5 (0,03)	0,854	0,146	0,372	+
Amount	379 (0,73)	130 (0,25)	12 (0,02)	0,852	0,148	0,813	+

c) Correlation analysis of SLC2A9 rs12510549 with gout

Table 0. 10. Results of the correlation assessment of polymorphism rs12510549 with gout

Genotype	**Control group n (%)**	**Disease group n (%)**	**OR**	**95%CI**	**P-value**
Aggregation model					0,785

TT	253 (72.1%)	126 (7 4.1%)	1,00		
TC	91 (25.9%)	39 (2 2.9%);	0,888	0,576 – 1,368	0,590
CC	0.7 (2%)	0.5 (3.0%)	1,463	0,455 – 4,703	0,521
Dominant model					0,606
TT	253 (72.08%)	126 (7 4.1%)	1,00		
TC+CC	98 (27.92%)	44 (2 5.9%);	0,888	0,576 – 1,368	0,729
Diving models					0,541
TT+TC	344 (98.01%)	165 (97%)	1,00		
CC	7 (1.99%)	5 (3.0%)	1,648	0,493 – 5,514	0,486
Co-dominant model					0,426
TT+CC	260 (74.07%)	131 (7 7.1%)	1,00		
TC	91 (25.93%)	39 (2 2.9%);	0,734	0,367 – 1,469	0,549
Allele					0,513
T	597 (8.5%);	291 (85,6%)	1,00		
C	105 (1.5%);	49 (14,4%)	0,827	0,461 – 1,482	0,679

n (%): Number of individuals (percent); OR: Odds ratio;95% CI: 95% confidence interval; the p-value is calculated by the Mann Whitney U test.

d) Evaluation of correlation of rs12510549 polymorphism with some clinical indicators

Table 0. 11. Correlation between polymorphism rs12510549 with some clinical indicators

SLC2A9 rs1251054	**Genotype**			**P-value**
	TT (n=379)	**TC (n=130)**	**CC (n=12)**	
uric acid	7.70±1.89	7.80±2.06	7.49±2.30	0,821
BMI	24.70±3.41	24.91±3.77	23.79±0.68	0,532
CRP	4.26±6.03	3.54±3.39	4.13±4.96	0,588
HDL-c	47.81±22.72	45.88±12.92	42.61±24.57	0,489
LDL-c	124.07±38.74	119.32±38.82	105.89±49.39	0,162
Triglycerid	235.13±165.0	232.42±176.4	244.06±121.2	0,968
Blood	112.10±38.66	115.76±33.78	100.76±31.63	0,336
AST (U/L)	27.90±20.45	29.07±24.30	30.2±26.21	0,827
ALT (U/L)	32.83±22.32	33.79±28.83	28.18±21.84	0,726
BUN	26.01±11.82	24.89±12.07	34.28±21.47	0.137
Creatinine	1.07±0.21	1.12±0.27	1.19±0.24	0,054
WBC (/µL)	7671±2277	7690±2424	7759±2277	0,989

1.14.8. Evaluation of correlation of rs1680979 SLC2A9 gene and gout

a) Determination of polymorphic genotype rs16890979 SLC2A9 gene

b) Determination of polymorphic genotype component SLC2A9 rs16890979

Table 0. 12. Genotype and frequency table of allele SLC2A9 rs1 6890979

	Genotype			**Allele**		**P-value**	**HWE**
	CC	**CT**	**TT**	**C**	**T**		
Control group	338 (0,96)	13 (0,04)	00 (0,0)	0,982	0,018	0,978	+
Disease group	167 (0,98)	03 (0,02)	00 (0,0)	0,991	0,009	0,657	+
Amount	505	16	00	0,985	0,015	0,956	+

	(0,97)	(0,03)	(0,0)				

HWE: Hardy–Weinberg equilibrium; +: Follow the Hardy-Weinberg law of equilibrium.

c) Correlation analysis of SLC2A9 rs16890979 with gout

Table 0. 13. The results of the evaluation of the correlation between rs1 polymorphism6890979 with gout

Genotype	**Control group n (%)**	**Disease group n (%)**	**OR**	**95%CI**	**P-value**
Dominant model					0,843
CC	338 (96.4%)	167 (98.2%)	1.00		
CT	13 (3.6%)	03 (1.8%)	0.496	0.132 – 1.861	0.289
Allele					0,147
C	689 (98.2%)	337 (99.1%)	1.00		
T	13 (1.8%)	03 (0.9%)	0.5	0.087 – 2.027	0.381

d) Evaluation of correlation of rs16890979 polymorphism with some clinical indicators

Table 0. 14. Correlation of rs16890979 gene polymorphism with some clinical indicators

SLC2A9 rs16890979	**Genotype**		**P-value**
	CC (n=505)	**CT (n=16)**	
uric acid (mg/dL)	5.69±4.11	4.83±3.96	0,5217
BMI	23.72±3.41	22.61±3.77	0,6322
CRP (mg/dL)	4.99±1.03	1.47±0.37	0,2884
HDL-c (mg/dL)	42.81±18.72	43.88±13.92	0,1894
LDL-c (mg/dL)	126.17±28.74	129.32±48.82	0,3629

Triglyceride	**275.57±203.30**	**263.10±172.03**	**0,007**
Blood glucose	119.10±32.66	110.76±31.78	0,2362
AST (U/L)	26.90±19.45	27.07±20.30	0,9270
ALT (U/L)	30.83±21.32	32.79±26.83	0,4267
BUN (mg/dL)	25.01±10.82	25.89±9.07	0,324
Creatinine	**1.11±0.31**	**1.14±0.37**	**0,046**
WBC (/µL)	7372±2077	7610±2224	0,789

1.14.9. Correlation evaluation of rs1800629 TNF gene and gout

1.14.10. Correlation evaluation of rs2149356 TLR4 gene and gout

1.14.11. Evaluation of correlation of rs1165196 SCL17A1 gene and gout

a) Determination of polymorphic genotype rs1165196 gene SLC17A1

b) Determination of polymorphic genotype component SLC17A1 rs1165196

Table 0. 15. Genotype and frequency statistics table polymorphic allele SLC17A1 rs1165196

	Genotype			**Allele**		**P-value**	**HWE**
	AA	**AG**	**GG**	**A**	**G**		
Control group	189 (0.54)	144 (0,41)	18 (0,05)	0,744	0,256	0,1552	+
Disease group	107 (0,63)	57 (0,33)	06 (0.04)	0,797	0,203	0,6215	+
Amount	296 (0.57)	201 (0.39)	24 (0,04)	0,761	0,239	0,1619	+

HWE: Hardy-Weinberg equilibrium; "+": Obeys the HWE law of equilibrium.

c) Correlation analysis of SLC17A1 rs1165196 with gout

Table 0. 16. Results of the correlation evaluation of polymorphism rs1165196 with gout

Genotype	**Control group (n,%)**	**Disease group (n,%)**	**OR**	**95%CI**	**P-value**
Aggregation model					0,301
AA	189 (53,8%)	107 (62,9%)	1,00		
AG	144 (41,0%)	57 (33,5%)	0,706	0,479 – 1,040	0,078
GG	18 (5,2%)	06 (3.6%)	0,594	0.229 - 1.543	0,285
Dominant model					0,103
AA	189 (53,8%)	107 (62,9%)	1,00		
AG + GG	162 (46,2%)	63 (37.1%)	0,693	0.476 - 1.010	0,056
Diving models					0,608
AA+AG	333 (94,8%)	164 (96,4%)	1,00		
GG	18 (5,2%)	06 (3.6%)	0,681	0.265 - 1.748	0,422
Co-dominant model					0,703
AA+GG	207 (59.0%)	113 (66,5%)	1.00		
AG	144 (41,0%)	57 (33,5%)	0,416	0.327 - 1.140	0,358

Allele					0,557
A	522 (74,4%)	271 (79,7%)	1,00		
G	180 (25,6%)	69 (20,3%)	0,744	0.543 - 1.02	0,311

n (%): Number of individuals (%); OR: Odds score; 95%CI: 95% confidence interval.

d) Evaluation of correlation of rs1165196 polymorphism (A>G) with some clinical indicators

Table 0. 17. Evaluation of correlation between polymorphism rs1165196 with clinical indicators

SLC17A1 rs1165196	**Genotype**			**P-value**
	AA (n=296)	**AG (n=201)**	**GG (n=24)**	
uric acid (mg/dL)	7.86±1.96	7.53±1.88	7.63±2.09	0,1707
BMI	24.66±3.39	24.64±3.26	23.27±3.73	0,1457
CRP (mg/dL)	7.59±51.06	3.54±3.78	2.27±2.21	0,4686
HDL-c (mg/dL)	45.56±13.15	49.41±28.88	47.96±12.70	0,1294
LDL-c (mg/dL)	123.41±39.13	122.05±39.93	118.00±37.20	0,7831
Triglyceride	230.39±139.58	237.05±193.42	208.06±109.25	0,6877
Blood glucose	113.40±41.68	112.45±31.17	105.83±16.70	0,6268
AST (U/L)	28.18±20.53	28.30±24.17	27.71±11.98	0,9917

ALT (U/L)	33.42±23.61	32.15±24.88	30.16±18.45	0,7281
BUN (mg/dL)	25.18±12.53	27.06±12.45	24.20±6.60	0,2024
Creatinine	1.09±0.23	1.09±0.23	1.03±0.11	0,4524
WBC (above µL)	7749±2121	7538±2281	7594±4049	0,6028

Mean ± SD: Mean value ± standard deviation; n (%): Number of individuals (%);

** Oneway Anova test compares average values between 3 groups*

1.14.12. Correlation between some clinical and subclinical features with gout

a) Evaluation of correlation between biochemical laboratory indicators with gout

Table 0. 18. The results of the assessment of the correlation between subclinical indicators and gout

Gout	OR	95%CI	P-value
CRP (mg/dL)	**1,04**	**1.00 - 1.07**	**0,030**
BUN (mg/dL)	0,99	0,978 - 1,007	0,269
Creatinine	1,95	0.87 - 4.36	0,103
AST (U/L)	1,00	0.99 - 1.01	0,570
ALT (U/L)	1,00	0.99 - 1.01	0,316
HDL-c (mg/dL)	0,99	0.98 - 1.01	0,280
LDL-c (mg/dL)	**1,005**	**1,000 - 1,009**	**0,040**
Triglyceride	**1,003**	**1,001 - 1,004**	**<0.001**
Glucose	0,996	0,990 - 1,002	0,200

b) Analysis of correlation between each genotype and gout status

Table 0. 19. Correlation between each genotype and gout status

	Gout	**OR**	**95%CI**	**P-value**
rs72552713	GA	1,00		
	GG	0,051	0.012 - 0.225	**<0.001**
rs12505410	GG	1,00		
	TG	0,885	0.588 - 1.331	0,557
	TT	1,202	0.703 - 2.054	0,502
rs11231825	CC	1,00		
	TC	3,297	1,231 - 8,829	**0,018**
	TT	3,723	1,411 - 9,826	**0,008**
rs12510549	CC	1,00		
	TC	1,392	1,036 - 4,129	0,517
	TT	1,463	1,221 - 5,836	0,698
rs1800629	AA	1,00		
	AG	1,022	0.336 - 3.113	0,969
	GG	1,086	0.369 - 3.197	0,881
rs16890979	CC	1.00		
	CT	0.496	0.132 – 1.861	0.289
rs2149356	TT	1,00		
	GT	0,987	0.533 - 1.826	0,967
	GG	0,941	0.507 - 1.746	0,847
rs1165196	GG	1,00		
	AG	0,678	0.458 - 1.002	0,051
	AA	0,581	0.224 - 1.509	0,265

CONCLUSIONS AND SUGGESTION

In this study, we successfully identified allele frequency and genotype of 9 single nucleotide polymorphisms across 6 ABCG2, SLC22A12, SLC2A9, TNF,α, *TLR4*, and *SLC17A1* genes in healthy populations and people with gout.

Successfully investigated the correlation between SNPs and clinical and subclinical factors in the group of gout patients and healthy people. In particular, research has found an association between gene polymorphism and gout risk.

+ Afigure *ABCG2* **rs72552713** has a strong association with gout. Specifically, people with the GA genotype are nearly 19 times more likely to develop gout than people with the GG genotype.

+ Polymorphisms *SLC22A12* rs11231825 genes are associated with gout, increasing the risk of gout, specifically, people with CT and TT genotypes are 3,119 times more likely to develop gout than CC genotypes (95%CI: 1.122 - 8.669; p=0.029) and 3.195 times (95%CI: 1.439 - 10.654; p=0.008).

NEW CONTRIBUTIONS OF THE THESIS

Two polymorphisms of the ABCG2 gene, rs72552713 and SLC22A12, rs11231825, have been detected that are associated with gout in Vietnamese people. The polymorphism ABCG2 rs72552713 has a fairly strong association with gout. Specifically, people with CT genotype are nearly 19 times more likely to develop gout than people with CC genotype. SLC22A12 rs11231825 gene polymorphism, although less

related, still increases the risk of gout, specifically CT and TT genotypes are 3,119 times more likely to develop gout than CC genotypes (95%CI: 1,122 - 8,669; p=0.029) and 3.195 times (95%CI: 1.439 - 10.654; p=0.008).

PUBLICATIONS RELATED TO THE THESIS

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2. Nguyen Thuy Duong, Nguyen Thy Ngoc, **Nguyen Tran Minh Thang**, Bach Thi Hoai Phuong, Nguyen Thanh Nga, Nguyen Doan Tinh, Do Hai Quynh, Nguyen Dang Ton, Nong Van Hai, *Polymorphisms of ABCG2 and SLC22A12 Genes Associated with Gout risk in Vietnamese population*, Medicina, 2019, 55, 11.

3. **Nguyen Tran Minh Thang**, Nguyen Doan Tinh, Nong Van Hai, Nguyen Thuy Duong, *Survey on the association of SLC2A9 rs12510549 with uric acid concentration and gout in Vietnamese*, Journal of Biotechnology, 2020, 18(1), 49-57.

4. **Nguyen Tran Minh Thang**, Nguyen Thuy Duong, Nong Van Hai, *The role of risk factors for gout*, Vietnam Medical Journal, 2020, 490(5), 252-255.

5. **Nguyen Tran Minh Thang**, Nguyen Thy Ngoc, Nguyen Thanh Nga, Nong Van Hai, Nguyen Thuy Duong, *Study on the association of SLC2A9 rs16890979 with gout in 410 vietnamese individuals*, Journal of Biotechnology, 2021, 43(1), 12 9-136.