UNIVERSITAT
ROVIRA i VIRGILI

# ANALYSIS OF THE URATE TRANSPORTERS' SNPs INVOLVED IN HYPERURICEMIA

**-FINAL DEGREE PROJECT-**

Author: Laura Segura Vilanova

Mentored by Anna Hernández Aguilera

Biochemistry and Molecular Biology

Tarragona, 2021

# INDEX

# ABSTRACT

Hyperuricemia (HU) is a metabolic disorder caused by an increased production and/or decreased excretion of uric acid. The prevalence and incidence of HU in the world have steadily increased over the past 40 years. Therefore, the knowledge of the risk factors that trigger the disease is essential to reduce and prevent its incidence. The study focuses on genetic alterations as one of these risk factors, especially the urate transporter variants.

The aim of this project was to observe the single nucleotide polymorphisms (SNPs) present in the Czech Republic population, as well as the importance of genetic disorders in the prevalence of hyperuricemia. The Czech Republic study was performed by analysing the minor allele frequency (MAF) of HU SNPs in different groups of interest. The variants that had a major impact on disease development in this population were: the Q141K variant in the ABCG2 gene, the rs4292327 variant in the SLC2A9 gene and the rs8359 and rs8361 variants in the SLC22A12 gene.

On the other hand, the prevalence study was performed by analysing the relationship between the prevalence of hyperuricemia with the frequency of the SNPs involved in it in the different regions. The results showed a clear relationship between prevalence and the presence of SNPs related to elevated serum uric acid levels. In fact, the regions with a higher prevalence of these alleles were also the regions with a high prevalence of HU. Among these regions, that with the highest presence of these variants was East Asia, followed by the Americas, South Asia, Europe and Africa, respectively. However, further studies are needed to conclude that the prevalence of hyperuricemia is directly related to the presence of the risk alleles, because other variables are also involved.

# INTRODUCTION

## 1.1 URIC ACID

Uric acid (UA, C5H4N4O3) is the end-product of purine mononucleotide catabolism. Purines can be synthesized endogenously or derived from dietary sources and play many vital functions in the body. In particular, adenosine triphosphate (ATP) provides the energy to drive intracellular reactions and the purine nucleobases, adenine and guanine, are integral components of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). [1]

The majority of mammalian species have low levels of circulating UA as it is converted by the enzyme uricase (urate oxidase), which is situated in the hepatocyte peroxisomes, to a highly soluble compound called allantoin. Humans and some other higher primates lack the ability to produce uricase due to the presence of two truncating mutations in the uricase gene, which introduce premature stop codons. Humans are therefore exposed to relative high levels of UA. [2]

The urate synthesis pathway (figure 1) involves the degradation of purines into xanthine, which is degraded by xanthine oxidase (XO) to uric acid (EC 1.17.3.2). However, the enzyme that compromises the pathway is PPRP synthetase (phosphoribosyl-pyrophosphate synthetase, EC 2.7.6.1), which phosphorylates ribose-5-P to PRPP at the expense of ATP. Many people affected by hyperuricemia have been shown to have mutated PRPP synthetase so that it is overly active or not allosterically inhibited by their product. Another relevant enzyme is HGPRT (hypoxanthine-guanine phosphoribosyl-transferase, EC 2.4.2.8) which uses PRPP to regenerate nucleotides from purine nitrogenous bases (scavenging pathway). A deficiency of this enzyme means that PRPP is not expended in the scavenging pathway and that more nucleotides have to be produced to compensate for the deficiency by the de novo pathway, leading to the final accumulation of uric acid. [2]

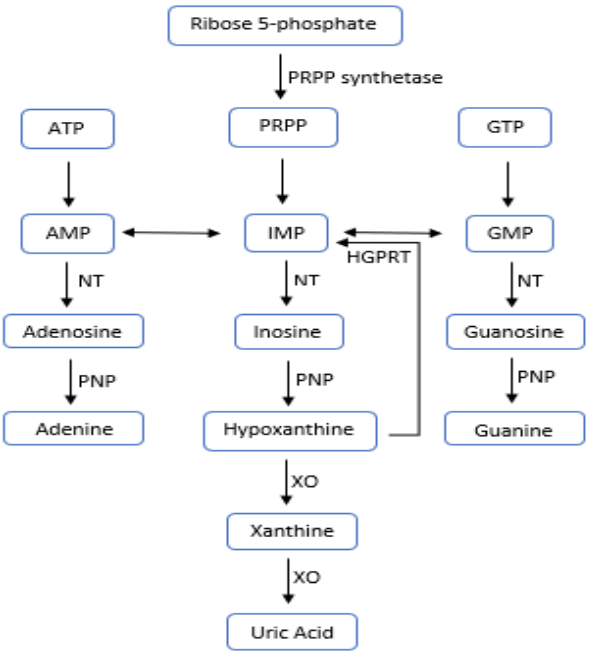


Figure 1. Uric acid pathway. [3]

UA homeostasis is governed by the balance between the rate of UA generation and UA elimination, determined by purine catabolism, renal excretion and intestinal secretion. UA is derived mainly from the breakdown of purines in the liver and intestine, as well as the kidneys, muscle and vascular endothelium. Exogenous supply of UA is obtained from dietary sources of purines including meat, seafood and alcohol. Diets with purine-free formulas have been associated with approximately a 40% reduction in the urinary UA excretion suggesting that the diet provides a significant source of urate precursors. [4]

Tissue metabolism of UA is minimal and most of its elimination occurs via the kidneys and intestine. Intestinal excretion of UA is carried out by gut bacteria in a process called intestinal uricolysis. This accounts for 25–35% of UA elimination, and renal excretion accounts for the remaining 65–75%. Most of circulating urate is free with less than 5% albumin-bound. Therefore, most urate is readily filtered by the glomeruli, although up to 90% may then be reabsorbed. [4]

Models of the urate transport are found in the proximal tubule and in the distal tubule. The process of initial uptake of uric acid is performed in the proximal tubule by URAT 1 and GLUT 9. The latter has two different isoforms: GLUT9-L and GLUT9-S. Urate outflow from the basement membrane of the proximal tubules is regulated by GLUT9-L, whereas urate inflow and outflow from the apical membrane is controlled by GLUT9-S. In the distal tubule, some transporters such as ABCG2, NPT1 and NPT4 mediate uric acid secretion. [5]

In the extracellular environment, UA is one of the strongest antioxidant compounds. Indeed, UA plays a role in preventing lipid peroxidation. However, in the intracellular environment, urate is known to act as a pro-oxidant and pro-inflammatory mediator. The upregulation of pro-inflammatory transcription factors, vasoconstrictive factors and chemokines, which may induce proximal tubular dysfunction as well as vascular smooth muscle proliferation are many of these effects. Urate induces both mitochondrial and endothelial dysfunction. UA infusion in humans has been associated with an increase in interleukin 6 (IL-6) levels in response to an oral lipid challenge, which may help explain the increased cardiovascular risk in those people with subclinical hyperuricemia. [2]

## 1.2 URATE TRANSPORTERS

Multiple urate transporters have been identified that play a role in the renal tubular reabsorption and secretion of urate, thus helping to regulate homeostasis and maintain plasma levels within a range. The several urate transporters identified (figure 2) fall into two categories: urate reabsorption transporters and urate excretion transporters. [5]

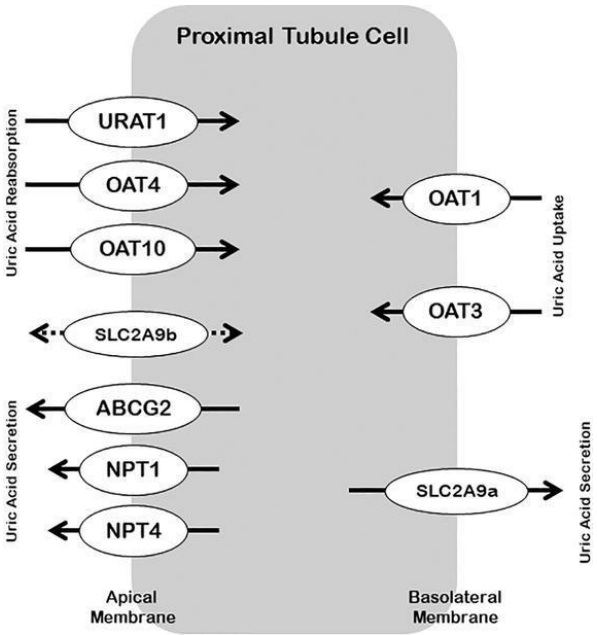


Figure 2. Urate transporters. [1]

### 1.2.1 URATE REABSORPTION TRANSPORTERS

The urate reabsorption transporters have three members, urate anion transporter 1 (URAT1), organic anion transporter (OAT4 and OAT10) and glucose transporter 9 (GLUT9). According to several researches, the URAT1 and GLUT 9 transporters are the main proteins involved in urate reabsorption. Different SNPs in the *SLC22A12* and *SLC2A9* gene, respectively, have been found to be related with a high uric acid reabsorption and consequently, hyperuricemia. [5]

- URAT1 (urate transporter 1) – It belongs to the organic acid transporter (OAT) family and is encoded by the *SLC22A12* (solute carrier family 22 member 12) gene on chromosome 11. It reabsorbs UA in exchange for monovalent anions such as acetoacetate, hydroxybutyrate, lactate and nicotinoate. It was the first urate transporter described in detail and plays a role in urate reabsorption at the apical surface of the proximal tubular epithelium. Certain drugs, such as benzbromarone, probenecid and losartan, are known to exert a uricosuric effect through inhibition of URAT1. [5]

  In a meta-analysis, the rs475688 and rs3825016 polymorphisms in *SLC22A12* were associated with susceptibility to gout and hyperuricemia. [13] Although *SLC22A12* polymorphisms have been associated with elevated serum urate levels and decreased fractional urate excretion in Caucasians, the functional mechanisms underlying these associations remain unclear. Overall, a recent meta-analysis of studies in European descent populations suggested that SLC22A12 contributed only 0.13% to the variance in serum uric acid (SUA). [6]

- OAT4 (organic acid transporter 4) – It belongs to the organic acid transporter (OAT) family and is encoded by the *SLC22A11* gene with strong expression in the placenta and some expression in the kidney. Potential substrates of OAT4 include sulphated steroids, NSAIDs (Nonsteroidal anti-inflammatory drug), antihypertensives, prostaglandins and uric acid. It is expressed in the apical membrane of renal proximal tubular cells, where it is believed to contribute to reabsorption of organic anions, including uric acid, from the urine into the proximal tubular cells. Genome-wide association studies (GWAS) have associated SNPs in OAT4 with elevated levels of serum uric acid in at least one case. [5]

- OAT10 (organic acid transporter 10) - It belongs to the organic acid transporter (OAT) family and is encoded by the *SLC22A13 gene.* The encoded protein is a transmembrane protein involved in the transport of small molecules. This protein can mediate urate uptake and is a high affinity nicotinate exchanger in the kidneys and the intestine. As OAT4, some SNPs have been associated with hyperuricemia due to an excessive uric acid reabsorption. [5]

- GLUT9 (glucose transporter 9) – It belongs to the glucose transporter family (GLUT) and is encoded by the *SLC2A9* gene on chromosome 4. GLUT9 reabsorbs urate at the basolateral membrane of the proximal tubule and is the single most important genetic determinant of SUA levels and hyperuricemia. SLC2A9 variants have shown a significant association with clinical gout cohorts in Caucasian, Chinese and Pacific Island populations. The effect of variation in *SLC2A9* is more pronounced in females, in whom it accounts for approximately 6% of the variance of serum urate, compared with 2% in males. [6] The rs7442295 single nucleotide polymorphism in the *SLC2A9* gene is robustly associated with hyperuricemia, increasing plasma uric acid levels and urate reabsorption. [7]

### 1.2.2 URATE SECRETION TRANSPORTERS

The urate secretion transporters consist of three families, nicotinate phosphoribosyltransferase (NPT1 and NPT4), organic anion transporter (OAT1 and OAT3) and ATP-binding cassette subfamily G member 2 (ABCG2). According to several researches, the ABCG2 transporter is the main protein implicated in the urate secretion. For this reason, different SNPs in the ABCG2 gene have been found to be related with a low urate secretion and consequently, hyperuricemia. [8]

- ABCG2 (ATP-binding cassette subfamily G member 2) - It belongs to the ABC family and is encoded by the *ABCG2* gene. It is known to have effects on both intestinal and renal excretion of urate. It acts on the apical surface of the proximal tubular epithelium as an urate extrusion pump. *ABCG2* polymorphisms may contribute to a pseudo-overproduction phenotype due to reduced UA intestinal excretion. This suggests that SUA homeostasis may be maintained in the context of renal failure by means of upregulated intestinal excretion through ABCG2. [2] Several functional variants of BCRP (breast cancer resistance protein) have been identified that enhance the risk of hyperuricemia and gout. In hereditary hemochromatosis, iron/heme overload enhances the activity of xanthine oxidase and accelerates the degradation of p53, causing the reduction of ABCG2 expression. Consequently, intestinal excretion of uric acid through ABCG2 is reduced and its production enhanced, leading to the accumulation of uric acid in tissues and serum, promoting the progression of hereditary hemochromatosis-associated arthritis. [7]

- OAT1 (organic acid transporter 1) - It belongs to the organic acid transporter (OAT) family and is encoded by the SLC22A6 gene. It is highly expressed in the kidney proximal tubule. It transports many metabolites and signaling molecules, including tricarboxylic acid (TCA) cycle intermediates, gut microbiome products, bile acids, dietary phytochemicals, short chain fatty acids, uremic toxins and odorants. It is considered to be one of the major transporters involved in the uptake of uric acid from the blood into the proximal tubule cell. [8]

- OAT3 (organic acid transporter 3) - It belongs to the organic acid transporter (OAT) family and is encoded by the SLC22A8 gene. As OAT1, it is highly expressed in the kidney proximal tubule and transports many metabolites and signalling molecules. Together with OAT1, it is the other main transporter involved in the uptake of uric acid from the blood into the proximal tubule cell. However, in GWAS studies, both OATs (and others, such as OAT4 and OAT10), although implicated, do not nearly achieve the levels of significance seen in genes like SLC2A9 and ABCG2. [5]

- NPT1 and NPT4 (nicotinate phosphoribosyltransferase) - They belong to the SLC17 family and is encoded by the NPT1 gene and NPT4, respectively. They transport urate and a number of organic anions, a fact unappreciated in the organic anion transporter field. SNPs in NPT1 and NPT4 correlate weakly to moderate with altered uric acid levels. [8]

## 1.3 HYPERURICEMIA

Hyperuricemia is a metabolic disorder, unique to humans, characterized by an excess of uric acid (UA) in the blood. It can be caused by an increased production or/and decreased excretion of uric acid through the kidney and gastrointestinal tract. Levels of uric acid up to 7mg/dL in men and 6mg/dL in women are considered hyperuricemia, according to established reference values. [9]

The prevalence and incidence of hyperuricemia in the world population have steadily increased over the past 40 years. With rapid economic development and prolonged life expectancy, the study of the UA in the developing countries has become even more important. Although the risk factors for hyperuricemia have not been fully determined, recent studies have shown that genetic variations, several lifestyle or dietary factors are the main associated risk factors. The prevalence of hyperuricemia ranges from 8.9% to 24.4% in the different developed populations (table 1), although the real number is much higher. However, due to the impossibility of detecting all asymptomatic cases, it is very difficult to estimate the real percentage of the population affected. [10]

Table 1. Prevalence of hyperuricemia in the different countries. [11]

| REGION | HYPERURICEMIA PREVALENCE'S (%) |
|---|---|
| **ALL** | 7 |
| **AFRICA** | 5 |
| **AMERICA** | 20-22 |
| **EAST ASIA** | 23-25 |
| **EUROPE** | 9-12 |
| **SOUTH ASIA** | 18-22 |

The countries with a higher prevalence of hyperuricemia are those who are developed or in developing ways. As shown in the table 1, East Asia is the region with the highest prevalence followed by America and South Asia. On the other hand, Africa is the region with the lowest prevalence. However, there is not data on the prevalence of hyperuricemia in all regions. [11]

### 1.3.1 SYMPTOMATOLOGY

Depending on the clinical scenarios, hyperuricemia can be divided into asymptomatic or symptomatic. Asymptomatic patients accompany an elevated serum urate level, but never experience signs or symptoms of monosodium urate crystal deposition disease, such as nephrolithiasis, gout or uric acid renal disease. Therefore, the majority of people with asymptomatic hyperuricemia go undetected, resulting in an unreal number for the worldwide prevalence of this disease. There is no need to carry medical therapy for these multitude of asymptomatic patients. Lifestyle modifications such as exercise, dietary changes and alcohol consumption are sufficient to lower the levels of uric acid. Clinical symptoms of symptomatic hyperuricemia may include gout, nephrolithiasis and uric acid kidney disease. In these cases, a treatment would be required to lower the uric acid levels and consequently, decrease its effects. [7]

#### 1.3.1.1 THE GOUT

Gout is one of the most common forms of inflammatory arthritis and affects approximately 1 to 6% of the population in several countries. It is characterized by acute arthritis, joint deformity and severe pain caused by deposition of monosodium urate crystals in and around synovial tissue promoted by an elevated serum uric acid concentration (hyperuricemia). In acute gouty arthritis, abrupt changes in uricemia and trauma are two recognized precipitating factors. Free urate crystals in joint space promote the synthesis and release of various inflammatory mediators (chemotactic factors), phagocytes, synovial cells and other cells. [12]

Hyperuricemia is a key risk factor in the pathogenesis of gout, but only a quarter of people with hyperuricemia develop gout, suggesting that an elevated serum uric acid concentration is necessary but not sufficient for the pathogenesis of gout. [13]

Inflammation in this pathology begins when macrophages phagocyte monosodic urate crystals and they cause the formation of protein complexes in the cytosol of these cells (NLRP2 inflammasomes). These complexes unite dampase-1, which activates IL-1beta, which promotes vasodilation and monocytes recruitment, as well as amplifying the inflammatory cascade. A prolonged release of IL-1beta cytokine causes irreversible damage to bone and cartilage. Other cytokines such as TNF-1, IL-6, CXCL8, and COX-2 are also found in the inflammatory response. [12]

#### 1.3.1.2 NEPHROLITHIASIS

Nephrolithiasis is a systemic metabolic disorder associated with the formation and deposition of kidney stones. It consists of small hard deposits of calcium oxalate or calcium phosphate, or occasionally, of other salts. They are a common cause of blood in the urine or pain in the abdomen, side or groin. It increases the risk of cardiovascular and kidney diseases in adults and fractures in children and adults. [14]

#### 1.3.1.3 URIC ACID RENAL DISEASE

There are two different types of renal disease induced by uric acid or urate crystal deposition: acute uric acid nephropathy and chronic urate nephropathy.

- Acute uric acid nephropathy (UAN) is characterized by a reduction in the urine excretion due to uric acid precipitation within the distal tubules and collecting ducts. In most cases, it is due to overproduction and overexcretion of uric acid in patients with rapid malignant cell turnover, such as in lymphoma, leukemia or a myeloproliferative disease (eg, polycythemia vera). It usually occurs after chemotherapy or radiation has induced rapid cell lysis, as nucleic acids released during cell lysis are converted to uric acid. [15]

- Chronic tubulointerstitial nephritis is caused by the deposition of sodium urate crystals in the medullary interstitium in patients with chronic hyperuricemia. The effects are chronic inflammation and fibrosis with subsequent chronic renal failure and renal insufficiency. [15]

### 1.3.2 TREATMENT

The several treatments to effectively manage hyperuricemia are based on inhibiting uric acid synthesis and reabsorption, as well as facilitating its excretion. As shown in the figure 3, urate-lowering medications can be roughly divided into three main categories: reducing the synthesis of uric acid (xanthine oxidase inhibitors), enhancing the excretion of uric acid (URAT1 inhibitors) and regulating the metabolic hydrolysis of uric acid (uricase inhibitors). Xanthine oxidase inhibitors are classified into purine analogues (including allopurinol) and non-purine analogue agents (including febuxostat and topiroxostat), which can decrease endogenous uric acid production and further reduce the levels of uric acid. [7]

Allopurinol is one of the most effective drugs in reducing uric acid concentrations. It is a purine-based competitive xanthine oxidase inhibitor. It can be metabolized to alloxanthine, an inhibitor of xanthine oxidase enzyme. Allopurinol promotes the secondary utilization of hypoxanthine and xanthine for the synthesis of nucleic acid and nucleotide by a metabolic reaction associated with hypoxanthine-guanine phosphoribosyltransferase (HGPRTase). This metabolic reaction accounts for an elevated level of nucleotide, leading to feedback suppression of de novo synthesis of purine. Eventually, the reduced levels of urine and serum uric acid are responsible for the reduction in the incidence of hyperuricemia. [7] [3]

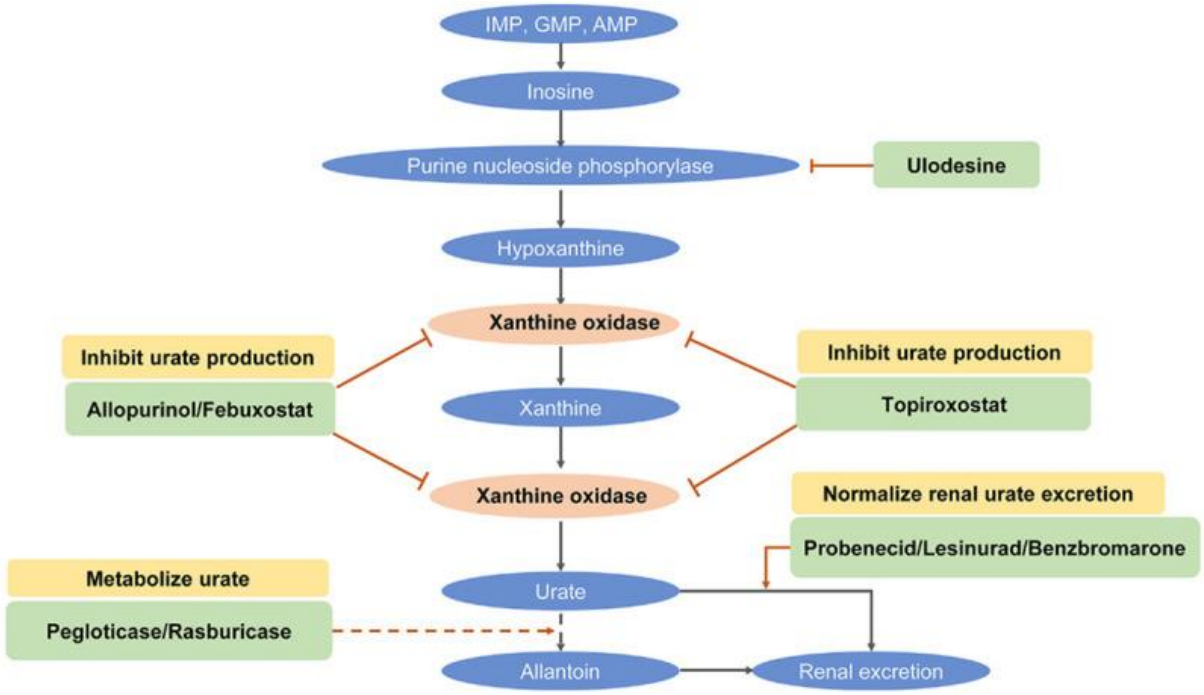


Figure 3. Compounds inhibited by the hyperuricemia treatment. [7]

## 1.4 RISK FACTORS

Hyperuricemia has been associated with several risk factors that increase the likelihood and the age of onset of the first effects. These can be broadly classified into factors associated with the increased purine synthesis and/or urate production (~10% of cases), versus factors that inhibit urate excretion and clearance (~90% of cases). Environmental factors such as overweight/obesity, gout-prone diet, alcohol consumption and diuretic use could individually account for a substantial proportion of hyperuricemia cases. However, there is also a genetic risk that benefit the development of the disease. [17]

### 1.4.1 AGE AND GENDER

Age and gender are two of the main factors related with an increased possibility of hyperuricemia. According to several researches, men suffer more from hyperuricemia than women. The risk of hyperuricemia in male is 1.772 times higher than in females. [10]

Regarding the age, it has been seen that, in women, the older the person, the higher the risk of triggering hyperuricemia. The average uric acid levels gradually increased after the age of 45 years old in women. As for men, it has been concluded that the risk does not increase after 55 years of age. [10]

### 1.4.2 GENETIC DISORDERS

A minor percentage of hyperuricemia cases have been related with genetic disorders. This is because hyperuricemia is not a monogenic disease, so more than one genetic variation has to occur to develop the disease. The main disorders associated with hyperuricemia are the Lesch-Nyhan syndrome (Hypoxanthine-guanine phosphoribosyltransferase deficiency), uromodulin pathogenic variants secondary to autosomal dominant tubulointerstitial kidney disease and genetic polymorphisms in genes encoding urate transporters: SLC2A9 (GLUT9), SLC22A12 (URAT1) and ABCG2. [2]

Genetic polymorphisms in urate transporters have been considered one of the principal causes of hyperuricemia due to genetic disorders. Although the precise role of many of the common variants remains to be established, the majority appears to be involved in the control of renal excretion of uric acid. This is because reduced FEUA (fractional excretion of uric acid) is the primary mechanism of hyperuricemia in more than 90% of patients with gout. [7]

Moreover, a significant correlation has been observed between the genetic risk score and both serum urate concentrations and the prevalence of hyperuricemia. This can be interpreted as an indication that certain SNP markedly determine the kidneys' handling of urate. This suggests that investigation of patients' genotypes could help identify individuals at risk of hyperuricemia, such as those with metabolic syndrome or cardiovascular diseases, well before the onset of clinical features and may help in clinical decisions. [16]

GWAS have identified single nucleotide polymorphisms (SNPs) at 29 genomic loci involved in UA regulation (table 2), accounting for an estimated 6.0–7.7% of serum uric acid variability. A strong association was found in all study subjects between the presence of rs11231825 (URAT1), rs16890979 (GLUT9) and rs2231142 (ABCG2) and serum urate concentrations. [2]

Table 2. Acid uric transporters' SNPs involved in HU. [2]

| SNPs | | | | |
|---|---|---|---|---|
| **GLUT 9** | | **ABCG2** | **URAT 1** | |
| rs16890979 | rs13129697 | rs2231142 | rs11602903 | rs1529909 |
| rs7442295 | rs2241480 | rs2725220 | rs7929627 | rs475688 |
| rs737267 | rs7663032 | rs2231137 | rs3825017 | rs7932775 |
| rs6855911 | rs3775948 | rs72552713 | rs11231825 | rs75786299 |
| rs16890979 | rs1014290 | rs2199936 | rs3825016 | |
| rs717615 | rs6449213 | | | |
| rs6856396 | rs734553 | | | |
| rs10489070 | | | | |

### 1.4.3 PREVIOUS DISEASES

The relationship between hyperuricemia and disease is evident with gout and hyperuricemia. Epidemiologic associations with some conditions such as cardiovascular and chronic kidney diseases have been reported, despite the inability to establish direct causality. [2]

Major cardiovascular risk factors such as hypertension, insulin resistance, dyslipidemia and obesity are known to be associated with an elevation of the SUA level. Individuals with hyperuricemia have significantly higher body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), uric acid (UA), fasting blood glucose (FBG), triglyceride (TG) and total cholesterol (TC), which are the main factors observed in cardiovascular diseases. [17]

Moreover, the most important risk factor between them is obesity. Prevalence ratios increase for individuals with a BMI (body mass index) >25.0 kg/m2 compared with those of BMI <25.0 kg/m2. In several researches, 44% of hyperuricemia cases were attributed to overweight or obesity (BMI ≥25 kg/m2). Obesity increases the risk of hyperuricemia by raising the serum urate level, through decreased renal urate excretion and increased urate production. [17]

Another risk factor that increases the risk of hyperuricemia is the chronic kidney diseases. Some of them affect the level of urate excretion, causing an increase in the uric acid levels and therefore, hyperuricemia or gout. [18]

### 1.4.4 ENVIRONMENTAL FACTORS

With the development of the economy and changing lifestyle, the dietary pattern has also changed. In developed countries, the consumption of high-purine foods (≥1000 mg/kg) has increased, as well as alcoholic beverages and sedentary lifestyle. [17]

Purine content is mostly present in seafood, red meat, poultry, legumes, some vegetables and fungi. Based on previous research studies, it appears that the purine-rich foods of meat origin are related with the risk of hyperuricemia, whereas purine-rich foods of plant origin are not as effective as those of meat origin. The DASH (Dietary Approaches to Stop Hypertension) diet lowers serum uric acid levels, particularly among people with hyperuricemia. [17]

Alcohol contributes to the production of uric acid precursors and its excretion due to its content of purine. Previous studies have found a direct relation between the prevalence of hyperuricemia and a high alcohol consumption. [18]

# HYPOTHESIS AND OBJECTIVES

The hypothesis of the study was that the variants of the urate transporters genes (*ABCG2, SLC2A9* and *SLC22A12*), which are involved in the uric acid (UA) metabolism, might be related to the hyperuricemia (HU) prevalence.

On the one hand, the objective of the present project was to find out which were the most relevant urate transporters' single nucleotide polymorphisms (SNPs) related with HU in the Czech Republic.

On the other hand, we tested if there was a direct relationship between the prevalence of hyperuricemia and genetics, in particular, with the SNPs of the urate transporters. To carry out this research, the minor allele frequencies (MAF) of the different world regions were compared.

# METHODOLOGY

## 3.1 CLINICAL ASPECTS OF THE URATE TRANSPORTERS´ SNPs IN THE CZECH REPUBLIC

The Stiburkova, B. *et al* study had three groups of participants: patients with hyperuricemia, patients with gout and control group. [19] The criteria followed to diagnose hyperuricemia was:

- Men: > 420 µmol/L of serum uric acid (SUA) on two repeated measurements taken at least 4 weeks apart.

- Women and children under 15 years: > 360 µmol/L of SUA on two repeated measurements taken at least 4 weeks apart.

The diagnosis of gout was determined using the criteria developed by the American College of Rheumatology (ACR) Board of Directors and the European League Against Rheumatism (EULAR) Executive Committee. Gout was defined as the presence of sodium urate crystals in the synovial fluid using a polarized microscope or at least six of their 12 clinical criteria being met. Patients with secondary gout and other purine metabolic disorders associated with pathological concentrations of SUA were excluded. [19]

Data obtained from the three groups were used to analyse the single nucleotide polymorphisms (SNPs) (table 3) involved in the hyperuricemia and gout. The urate transporters studied were ABCG2, GLUT9 and URAT1. [19]

Table 3. Urate transporters' SNP. [19] [20]

| **ABCG2's SNPs** | | **GLUT9'S SNPs** | **URAT1's SNPs** |
|---|---|---|---|
| rs2231137 | rs199854112 | rs18678 | rs7932775 |
| rs2231142 | rs769734146 | rs21155 | rs8359 |
| rs372192400 | S476P | rs2240722 | rs8361 |
| rs753759474 | rs200894058 | rs2240720 | |
| rs752626614 | rs34783571 | rs4292327 | |
| pK360del | | | |

All testing was performed in accordance with the standards established by the institutional ethics committees. Prior to data collection, all participants signed informed consents. Ethical approval for this study was obtained from the Ethics Committee of the Institute of Rheumatology (reference number 6181/2015). [20]

### 3.1.1 ANALYSIS OF THE ABCG2's SNPs

CLINICAL SUBJECTS

The analysed set had three groups of participants: a hyperuricemic group consisting of 68 subjects, a gout group consisting of 182 subjects and a control group with no pathologies consisting of 132 subjects. The unhealthy cohort (HU + gout) was selected among patients of the Institute of Rheumatology, Prague, Czech Republic. The control group was selected from the staff of the Institute of Rheumatology. [19]

METHODS

**PCR amplification and sequence analysis**

Genomic DNA was extracted from EDTA whole blood using a QIAmp DNA Mini Kit (Qiagen, GmbH., Hilden, Germany). All protein-coding exons were amplified using PCR and purified using a PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan). DNA sequencing was performed with a DNA sequencer (Applied Biosystems 3130 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA). The genotypes of allelic variants in the control cohort were determined by PCR with allele-specific primers. [19]

### 3.1.2 ANALYSIS OF SLC2A9 and SLC22A12's SNPs

CLINICAL SUBJECTS

The analysed set had three groups of participants: a hyperuricemic group consisting of 64 subjects, a gout group consisting of 46 subjects and a control group with no pathologies consisting of 150 subjects. The unhealthy cohort (HU+ gout) was selected among patients of the Institute of Rheumatology, Prague, Czech Republic. The control group was selected among the staff of the Institute of Rheumatology. [20]

METHODS

**PCR amplification and sequence analysis**

The genomic DNA for PCR analysis was isolated from subjects' blood samples (Qiagen columns). PCR and direct sequencing were used to amplify exons of SLC2A9 and exons of SLC22A12. Fifty nanograms of genomic DNA was amplified in 50 µl containing 2.5 U Taq-Purple DNA polymerase, 200 µM dNTPs and 0.15 µM primers. Amplification products were gel purified using 1% agarose gel in 1xTAE buffer and Wizard SV gel and Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). DNA sequencing was performed with an automated DNA sequencer (Applied Biosystems 3100-Avant Genetic Analyzer; Applied Biosystems, USA). [20]

## 3.2 DATA COLLECTION OF THE MAF OF THE URATE TRANSPORTERS' SNPs IN THE DIFFERENT REGIONS OF THE WORLD

The 1000 Genomes Project database from the Ensambl website was used to conduct cross-population allele and genotype frequencies for alleles associated with hyperuricemia in major racial populations. The variants were obtained from the genome-wide association studies (GWAS), which have identified single nucleotide polymorphisms (SNPs) in 29 genomic loci implicated in uric acid regulation. The regions analysed in the study were: America, Europe, Africa, East Asia and South Asia. [21]

Data obtained from the different populations were used to analyse the urate transporters' SNPs (table 4) involved in hyperuricemia and gout. The urate transporters studied were ABCG2, GLUT9 and URAT1. [2]

Table 4. Urate transporters' SNPs. [2]

| ABCG2's SNPs | GLUT9'S SNPs | | | URAT1's SNPs | |
|---|---|---|---|---|---|
| rs2231142 | rs16890979 | rs13129697 | rs717615 | rs11602903 | rs1529909 |
| rs2725220 | rs7442295 | rs2241480 | rs6449213 | rs7929627 | rs475688 |
| rs2231137 | rs737267 | rs7663032 | rs734553 | rs3825017 | rs7932775 |
| rs72552713 | rs6855911 | rs3775948 | rs6856396 | rs11231825 | rs75786299 |
| rs2199936 | rs16890979 | rs1014290 | rs10489070 | rs3825016 | |

# REVIEW

## 4.1 ANALYSIS OF THE URATE TRANSPORTERS' SNPs IN THE CZECH REPUBLIC

The urate transporters' SNPs are an important risk factor for developing hyperuricemia. The aim of this analysis was to find out which were the main SNPs associated with hyperuricemia and their prevalence in the Czech Republic.

The present study focused on the three most important urate transporters: ABCG2, GLUT 9 and URAT1. They are involved in the UA variability by 0.57%, 3.53% and 0.13%, respectively. [6]

The characteristics and biochemical variables of the different study groups (gout, hyperuricemic, unhealthy patients (HU + gout) and normouricemic) are shown in the following tables (tables 5 and 6).

Table 5. Characteristics and biochemical variables of the different groups in the ABCG2 study. [19]

| *ABCG2* gene | Gout group N=182 | Hyperuricemic group N=68 | Unhealthy patients N=250 | Normouricemic group N= 132 |
|---|---|---|---|---|
| Number of subjects (M/W) | 166/16 | 48/20 | 214/36 | 54/78 |
| Age (years) | 54 | 36 | 51 | 41 |
| Serum uric acid (µmol/l) | 372 | 424 | 375 | 337 |
| Creatinine (µmol/l) | 82 | 79 | 81.18 | 75.5 |
| Allopurinol use (N) | 137 | 39 | 176 | Not Applicable |

N=number of subjects. M=men. W=women.

In the ABCG2 gene study, as expected, higher SUA and creatinine levels were observed in the gout and hyperuricemic groups compared to the control group. However, only the hyperuricemic group had SUA levels above the normal established range (200-390 µmol/l). Regarding creatinine, all groups were within the normal range (55-100 µmol/l).

Table 6. Characteristics and biochemical variables of the different groups in the *SLC2A9* and *SLC22A12* study. [20]

| *SLC2A9* and *SLC22A12* genes | Gout group N=46 | Hyperuricemic group N=54 | All unhealthy patients N=100 | Normouricemic group N= 150 |
|---|---|---|---|---|
| Number of subjects (M/W) | 43/3 | 43/11 | 86/14 | 75/75 |
| Age (years) | 59.5 | 47.46 | 53 | 49 |
| Serum uric acid (µmol/l) | 395.71 | 450.21 | 425.34 | 264 |
| Creatinine (µmol/l) | 89 | 76.03 | 82 | 81 |
| Allopurinol use (N) | 22 | 10 | 32 | Not Applicable |

N=number of subjects. M=men. W=women

In the SLC2A9 and SLC22A12 genes study, higher levels of SUA were observed in the gout and hyperuricemic groups compared to the control group. In this case, both the hyperuricemic and gout groups had SUA levels above the normal established range (200-390 µmol/l). Regarding creatinine, all the groups were within the normal range (55-100 µmol/l).

### 4.1.1 ANALYSIS OF THE ABCG2'S SNPs

The analysis of the ABCG2's SNPs was performed to determinate the SNPs related to hyperuricemia and their prevalence in the Czech Republic. To conduct this research, data from the Stiburkova, B. et al study was used. [19]

Determination of the several hyperuricemia SNPs was done by performing a targeted exon sequencing of the ABCG2 gene. Only the non-synonymous allelic variants related with the risk of gout or hyperuricemia were shown in the table below (table 7). [19]

In the following table the allele distribution and the MAF of every variant divided into different groups can be observed: gout, hyperuricemia, unhealthy patients (gout + HU) and normouricemia (control group).

Table 7. Allele distribution and MAF of the several ABCG2's SNPs presents in the Czech population according to gout, hyperuricemic, unhealthy and healthy patients. [19]

| | GOUT N=182 | | | | HYPERURICEMIA N=68 | | | | UNHEALTHY PATIENTS N=250 | | | | NORMOURICEMIA N=132 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF |
| V12M (rs2231137) | 177 | 2 | 3 | 2.2 | 67 | 1 | 0 | 0.7 | 244 | 3 | 3 | 1.8 | 129 | 1 | 2 | 1.9 |
| Q141K (rs2231142) | 108 | 58 | 16 | 24.7 | 44 | 19 | 5 | 21.3 | 152 | 77 | 21 | 23.8 | 113 | 16 | 3 | 8.3 |
| R147W(rs372192400) | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 249 | 1 | 0 | 0.2 | 131 | 1 | 0 | 0.4 |
| T153M (rs753759474) | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 249 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |
| F373C (rs752626614) | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 249 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |
| T421A (rs199854112) | 182 | 0 | 0 | 0 | 67 | 1 | 0 | 0.7 | 249 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |
| T434M (rs769734146) | 181 | 1 | 0 | 0.3 | 67 | 1 | 0 | 0.7 | 248 | 2 | 0 | 0.4 | 131 | 1 | 0 | 0.4 |
| S476P | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 249 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |
| S572R (rs200894058) | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 249 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |
| D620N (rs34783571) | 180 | 2 | 0 | 0.5 | 68 | 0 | 0 | 0 | 248 | 2 | 0 | 0.4 | 132 | 0 | 0 | 0 |
| pK360del (rs750972998) | 181 | 1 | 0 | 0.3 | 68 | 0 | 0 | 0 | 248 | 1 | 0 | 0.2 | 132 | 0 | 0 | 0 |

W=wild type. M= mutant type. MAF (%) = minor allele frequency.

Eleven exonic non-synonymous variants were identified: V12M (rs2231137), Q141K (rs2231142), R147W (rs372192400), T153M (rs753759474), F373C (rs752626614), T421A (rs199854112), T434M (rs769734146), S476P (not annotated), S572R (rs200894058), D620N (rs34783571) and a three-base deletion pK360del (rs750972998). [19]

All variants were rare and not well-characterized except the V12M (rs2231137) and Q141K (rs2231142), which were related with a decrease in the ABCG2 function. [19] Among the rare variants, only two of them (T153M and F373C) had been related with uric acid, in particular, with a reduction in the secretion of UA according to Stiburkova, B. and Hoque, KM. et al studies. [22] [29]

The MAF analysis obtained in the study revealed a clear difference in the prevalence of the Q141K variant in the unhealthy group compared to the healthy one. This could indicate a relationship between having this mutation and developing the condition.

Moreover, there was a higher prevalence of the Q141K variant in the gout group compared to the hyperuricemia one, showing a possible relation with symptomatic hyperuricemia. The MAF of the other variants did not show a clear difference to be able to establish a conclusion in the present study.

### 4.1.2 ANALYSIS OF THE SLC2A9'S SNPs

The analysis of the SLC2A9's SNPs was done to determine the SNPs related with hyperuricemia and its prevalence in the Czech Republic. To conduct this research, data from the Stiburkova, B. et al study was used. [20]

Determination of the several SLC2A9's SNPs related with hyperuricemia was done by performing a targeted sequencing of the gene. Only the non-synonymous allelic variants related with the risk of gout or hyperuricemia were shown in the table below (table 8). [20]

In the following table, the allele distribution and the MAF of every variant divided into different groups can be observed: gout, hyperuricemia, unhealthy patients (gout + hyperuricemia) and normouricemia (control group).

Table 8. Allele distribution and MAF of the several SLC2A9's SNPs presents in the Czech population according to gout, hyperuricemic, unhealthy and healthy patients. [20]

| SLC2A9's SNPs | GOUT N=46 | | | | HYPERURICEMIA N=54 | | | | UNHEALTHY PATIENTS N=100 | | | | NORMO URICEMIA N=150 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF |
| rs18678 | 46 | 0 | 0 | 0 | 54 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 74 | 76 | 0 | 25.0 |
| rs2240722 | 11 | 16 | 19 | 58.7 | 11 | 26 | 17 | 55.5 | 22 | 42 | 36 | 57.0 | 52 | 26 | 72 | 56.6 |
| rs21155 | 43 | 3 | 0 | 3.2 | 54 | 0 | 0 | 0 | 97 | 3 | 0 | 1.5 | 104 | 46 | 0 | 15.3 |
| rs2240720 | 12 | 9 | 25 | 64.1 | 11 | 25 | 18 | 56.4 | 23 | 34 | 43 | 60 | 31 | 13 | 106 | 75.0 |
| rs4292327 | 21 | 24 | 1 | 28.2 | 28 | 20 | 6 | 29.6 | 49 | 44 | 7 | 29 | 150 | 0 | 0 | 0 |

W=wild type. M= mutant type. MAF (%) = minor allele frequency.

Five intronic variations that could be related with hyperuricemia were identified: rs18678, rs2240722, rs21155, rs2240720 and rs4292327. Among the variants, there were three which were related with hyperuricemia (rs2240722, rs2240720 and rs4292327) and two of them (rs18678 and rs21155) with no clear implication in the conditions according to Jeanin, G. and Stiburkova, B. studies. [23][26] The variants related with an increase in the risk to develop hyperuricemia shown an increase in the reabsorption of UA, decreasing its excretion and increasing the levels of SUA. [25]

MAF analysis obtained in the research revealed a clear difference in the prevalence of the variant rs4292327 in the unhealthy group compared to the healthy one, which could indicate a relationship between this mutation and the development of these conditions. The rs2240720 presented a difference between the unhealthy and the healthy group. However, the difference was not as significant as the previous one. On the other hand, the rs2240722 did not present a difference between the unhealthy and healthy group in the study. The other not well-characterized variants had a higher MAF in the normouricemic group, indicating a possible hypouricemic effect or no implication in the diseases.

The common variants associated with a protective effect in hyperuricemia in the *SLC2A9* gene (rs16890979 and rs13113918), which carry out a reduction in the UA reabsorption, were not present in this study. [25]

### 4.1.3 ANALYSIS OF THE SLC22A12'S SNPs

The analysis of the *SLC22A12*'s SNPs was done to determine the SNPs relation with hyperuricemia and its prevalence in the Czech Republic. To conduct this research, data from the Stiburkova, B. *et al* study was used. [20]

Determination of the several *SLC2A9's* SNPs related with hyperuricemia was done performing a targeted sequencing of the gene. Only the non-synonymous allelic variants related with the risk of gout or hyperuricemia were shown in the table below (table 9). [20]

In the following table, the allele distribution and the MAF of every divided into different groups can be observed: gout, hyperuricemia, unhealthy patients (gout + hyperuricemia) and normouricemia (control group).

Table 9. SLC22A12´s SNPs presents in the Czech population and the MAF of them according to gout, hyperuricemic and normouricemic patients. [20]

| SLC22A12's SNPs | GOUT N=46 | | | | HYPERURICEMIA N=54 | | | | ALL UNHEALTHY PATIENTS N=100 | | | | NORMO URICEMIA N=150 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF | WW | WM | MM | MAF |
| rs8359 | 9 | 37 | 0 | 40.2 | 2 | 52 | 0 | 48.1 | 11 | 89 | 0 | 44.5 | 150 | 0 | 0 | 0 |
| rs8361 | 9 | 37 | 0 | 40.2 | 2 | 52 | 0 | 48.1 | 11 | 89 | 0 | 44.5 | 150 | 0 | 0 | 0 |
| rs7932775 | 31 | 12 | 3 | 19.5 | 48 | 0 | 6 | 11.1 | 79 | 12 | 9 | 15 | 149 | 1 | 0 | 0.3 |

W= wild type. M= mutant type. MAF (%) = minor allele frequency.

Three variants that could be related with hyperuricemia were identified: rs8359, rs8361 and rs7932775. Among the variants, there were two SNPs (rs8359 and rs8361) which were associated with an increase in the reabsorption of UA. On the other hand, the rs7932775 was associated with a decrease in the reabsorption of UA. [21] Therefore, it is considered a protective SNP in front of hyperuricemia, according to Vidanapathirana, DM. *et al* study. [26]

The MAF analysis obtained in the research revealed a clear difference in the prevalence of the rs8359 and rs8361 variants in the unhealthy group compared to the healthy controls, which could indicate a relationship between having this mutation and developing these conditions. On the other hand, the rs7932775 presented a difference between the unhealthy and healthy group in the study, although the difference was not as large as in the other SNPs.

The common variants associated with hyperuricemia in the *SLC22A12* gene (rs11602903 and rs11231825), which carry out an increase in the UA reabsorption, were not present in this study. [21]

## 4.2 ANALYSIS OF THE HYPERURICEMIA'S SNPs MAF IN THE DIFFERENT REGIONS OF THE WORLD

Genetic polymorphisms in urate transporters have been considered one of the principal causes of hyperuricemia due to genetic disorders. A significant correlation has been observed between the genetic risk score and both serum urate concentrations and the prevalence of hyperuricemia. [2]

The aim of the analysis of the MAF of the urate transporters' single nucleotide polymorphisms (SNPs) in the different regions was to assess whether there was an important relation between genetic polymorphisms and the hyperuricemia prevalence. The analysis has been performed by comparing the minor allele frequencies (MAF) of the SNPs identified in Genome wide association studies (GWAS) between the different regions using the Ensambl database. [21] GWAS identified SNPs in 29 genomic loci involved in uric acid regulation were estimated to be responsible for 6–7.7% of serum uric acid variability. [8]

The prevalence of hyperuricemia in the different regions is shown in table 10.

Table 10. Prevalence of hyperuricemia in the different regions. [2]

| REGION | PREVALENCE (%) |
|---|---|
| **ALL** | 7 |
| **AFRICA** | 5 |
| **AMERICA** | 20-22 |
| **EAST ASIA** | 23-25 |
| **EUROPE** | 9-12 |
| **SOUTH ASIA** | 18-22 |

### 4.2.1 ANALYSIS OF THE ABCG2'S SNPs MAF

The *ABCG2* gene is strongly associated with SUA levels, early-onset gout and the progression from HU to gout. Therefore, individuals with polymorphisms in this gene have an increased risk of developing HU and gout. [5]

According to the GWAS study [2], the ABCG2 SNPs involved in the UA variation are shown in the table 11. Besides, the different MAF were compared among the worldwide regions.

Table 11. Worldwide allele frequencies of the different risk alleles in the *ABCG2* gene. [26]

| | POPULATION MAF | | | | | |
|---|---|---|---|---|---|---|
| SNPs | ALL | AFRICA | AMERICA | EAST ASIA | EUROPE | SOUTH ASIA |
| rs72552713 | 0.1 | 0 | 0 | 0.6 | 0 | 0 |
| rs2199936 | 15.0 | 12.7 | 14.4 | 29.1 | 9.4 | 9.6 |
| rs2231137 | 15.8 | 6.3 | 23.8 | 32.6 | 6.1 | 15.4 |
| rs2231142 | 11.9 | 1.3 | 14.1 | 29.1 | 9.4 | 9.7 |
| rs2725220* | 49.1 | 80.1 | 39.5 | 24.1 | 48.6 | 40.5 |

The variants related with a protective effect in front of hyperuricemia were marked with an asterisk (*).
MAF= minor allele frequencies

The allele frequencies shown in the table 11 indicated a lower frequency of the risk alleles and a higher frequency of the protective allele in the African region compared to the other regions. On the other hand, the East Asia presented the highest frequency of the risk alleles in all the cases and the lowest frequency in the protective variant. Regarding the other places, Europe was the second region with the lowest frequencies in the risk variants, followed by South Asia, America and East Asia, respectively.

### 4.2.2 ANALYSIS OF THE SLC2A9'S SNPs MAF

Polymorphisms in the *SLC2A9* gene are associated with an increased susceptibility to develop HU, gout and diabetes due to altered transporter affinity in some cases. [7]

According to the GWAS study [2], the SLC2A9 SNPs involved in the UA variation are shown in the table 12. Besides, the different MAF were compared among the worldwide regions.

Table 12. Worldwide allele frequencies of the different risk alleles in the SLC2A9 gene. [26]

| | POPULATION MAF | | | | | |
|---|---|---|---|---|---|---|
| *SNPs* | ALL | AFRICA | AMERICA | EAST ASIA | EUROPE | SOUTH ASIA |
| rs744229* | 25.5 | 43.9 | 37.6 | 1.4 | 20.9 | 21.7 |
| rs13129697* | 47.8 | 62.1 | 54.9 | 48.4 | 28.3 | 42.8 |
| rs7663032 | 37 | 33.8 | 43.5 | 49.5 | 24.9 | 36.2 |
| rs3775948 | 34.3 | 33.7 | 42.5 | 41.9 | 24.6 | 31.6 |
| rs16890979* | 26.4 | 48 | 38.2 | 1.3 | 21.4 | 19.7 |
| rs6855911* | 30.3 | 55.6 | 41.4 | 1.8 | 25.3 | 22.7 |
| rs1014290 | 32.5 | 29.1 | 40.5 | 40.9 | 24.5 | 31.2 |
| rs6856396 | 13.8 | 18.9 | 21.2 | 2.2 | 12.4 | 14.9 |
| rs734553* | 30.5 | 57.3 | 41.4 | 1.7 | 24.5 | 22.7 |
| rs6449213 | 14.2 | 13.1 | 26.4 | 2.5 | 17.7 | 15.7 |
| rs737267* | 30.3 | 55.6 | 41.4 | 1.8 | 25.1 | 22.7 |
| rs16890979* | 26.4 | 48 | 38.2 | 1.3 | 21.4 | 19.7 |
| rs717615 | 43.1 | 29.9 | 56.6 | 50.4 | 52.4 | 39.1 |
| rs10489070 | 16.2 | 13.2 | 27.4 | 14.6 | 18.4 | 12 |

The variants related with a protective effect in front of hyperuricemia were marked with an asterisk (*).
MAF= minor allele frequencies

The allele frequencies shown in the tables indicated a lower frequency of the risk alleles in the African region compared to the other regions. Moreover, in this region there was a major presence of the variants that had a protective effect. On the other hand, the East Asia and America had the highest frequency of the risk alleles followed by South Asia and Europe, respectively. Besides, East Asia was the place with the lowest frequency of the variants with a protective effect on the condition.

### 4.2.3 ANALYSIS OF THE SLC2A22'S SNPs MAF

SLC22A12 is responsible for the majority of urate reabsorption in the kidneys and the primary targets for urate-lowering therapies. Genetic polymorphisms in *SLC22A12* could significantly modulate SUA levels. [27]

According to the GWAS study [8], the SLC2A22 SNPs involved in the UA variation are shown in the table 13. Besides, the allele frequency of the several SNPs in the different worldwide regions were compared.

Table 13. Worldwide allele frequencies of the different risk alleles in the SLC22A12 gene. [25]

| | POPULATION MAF | | | | | |
|---|---|---|---|---|---|---|
| *SNP* | ALL | AFRICA | AMERICA | EAST ASIA | EUROPE | SOUTH ASIA |
| *rs75786299* | 0.2 | 0 | 0 | 1.1 | 0 | 0.1 |
| *rs7929627* | 39.8 | 67.4 | 25.4 | 51.3 | 20.2 | 21.2 |
| *rs11231825* | 38.6 | 9.5 | 43.2 | 22.1 | 70.6 | 58.7 |
| *rs3825017* | 7.1 | 0.4 | 6.3 | 29.6 | 0.4 | 0.3 |
| *rs3825016* | 38.7 | 10.1 | 43.2 | 21.9 | 70.6 | 58.7 |
| *rs1529909* | 38.9 | 9 | 43.7 | 22.1 | 71.5 | 59.9 |
| *rs475688* | 29.5 | 16.3 | 39.8 | 40.1 | 24.6 | 34 |
| *rs7932775** | 39.7 | 67.4 | 25.5 | 51.3 | 20.2 | 20.7 |
| *rs11602903* | 38.6 | 9.5 | 43.2 | 22.1 | 70.6 | 58.6 |

The variants that have a protective effect in front of the hyperuricemia are marked with an asterisk (*).
MAF= minor allele frequencies.

The allele frequencies shown in the tables indicated a lower frequency of the risk alleles in the African region compared to the other regions. On the other hand, the East Asia and Europe had the highest frequency of the risk alleles, followed by South Asia and America, respectively. Regarding the protective allele, Africa had the highest frequency followed by East Asia, America, South Asia and Europe.

# DISCUSSION

Hyperuricemia (HU) is an increasing worldwide disease due to the rapid economic development and prolonged life expectancy. The study of the HU in the developing countries has become even more important nowadays. There are some factors that have been established as risk factors for the hyperuricemia such as sedentary lifestyle or genetic disorders. The last one, although not the most relevant, plays an important role in the hyperuricemia prevalence. The knowledge of patients' genotypes could help in identifying individuals at risk of hyperuricemia, such as those with metabolic syndrome or cardiovascular diseases, long before the onset of clinical features, thus helping in clinical decisions. [9][10]

On the one hand, single nucleotide polymorphisms (SNPs) involved in HU were analysed to find out which were the most relevant variants in the Czech Republic population.

The ABCG2 gene is involved in the secretion of UA. Therefore, individuals with polymorphisms in this gene that produce a reduction in its functionality, are strongly associated with high SUA levels and consequently, hyperuricemia. [5] In this gene, the Q141K variant was the SNP that was mostly present in the population and showed a significant difference in the minor allele frequency (MAF) between the control group and the unhealthy group (HU+ gout), indicating a relationship with the diseases. According to Hoque, KM. and Stiburkova, B. studies [29] [22], this variant carried out an important reduction in the function of the urate transporter producing an important reduction in the excretion of uric acid (UA) and therefore, a high serum uric acid (SUA) level. Furthermore, this variant was involved in the development of gout, which explains the difference in the MAF observed between the HU and gout groups in the study.

The SLC2A9 gene is involved in the reabsorption of UA. Therefore, individuals with polymorphisms in this gene that produce an increase in its functionality, are strongly associated with high SUA levels and consequently, hyperuricemia. [6] In this gene, the rs4292327 variant was the SNP that presented the most important difference in the MAF between the control group and the unhealthy group (HU + gout). According to Jeanin, G. and Stiburkova, B. studies [28] [26], this variant was related with an increase in the reabsorption of UA, increasing its concentration in serum. Besides, this variant was not related to symptomatic HU (gout), which could explain why no differences in the MAF were observed between HU and gout groups.

The SLC22A12 gene is involved in the reabsorption of UA. Therefore, individuals with polymorphisms in this gene, which produces an increase in its functionality, are strongly associated with high SUA levels and consequently, hyperuricemia. [13] In this gene, the rs8359 and rs8361 variants were the SNPs that presented the most important difference in the MAF between the control group and the unhealthy group (HU + gout). The MAF of these variants were almost 50% in the unhealthy group in front of 0% in the healthy group, indicating a significant involvement in the development of the diseases. According to Vidanapathirana, DM. *et al* study [26], these variants were involved in UA variability with an increase in the reabsorption of it, increasing SUA concentration.

In conclusion, these results showed that the most relevant SNPs in the Czech Republic were: the Q141K variant in the *ABCG2* gene, the rs4292327 variant in the *SLC2A9* gene and the rs8359 and rs8361 variants in the *SLC22A12* gene. However, further studies would be needed to establish the relevant SNPs in the Czech Republic, as these studies did not have a significant number of subjects to establish a solid conclusion.

On the other hand, our hypothesis was that genetic disorders are directly related to the disease's prevalence and therefore, play an important role in the probability of developing the condition. To prove this hypothesis, the MAF of the risk variants were compared between different world regions.

In the *ABCG2* gene, the allele frequencies showed a lower frequency of risk alleles and a higher frequency of protective allele in the African region compared to the other regions. On the other hand, East Asia presented the highest frequency of the risk alleles in all the cases and the lowest frequency in the protective variant. Regarding the other places, Europe was the second region with the lowest frequencies in the risk variants, followed by South Asia, America and East Asia, respectively. These results correlate with a genomic meta-analysis done by over 28.000 individuals worldwide [23], which showed a significant increase in the SUA levels due to polymorphisms in the *ABCG2* gene. The risk alleles were present in 9.4% of Europeans, 25% of Americans and 32% of Asians. These results suggested a possible genetic basis of the documented higher prevalence of HU and gout due to mutations in the *ABCG2* gene in Asian populations compared to others.

In the *SLC2A9* gene, the allele frequencies indicated a lower frequency of risk allele in the African region compared to the other regions. Moreover, in this region there was a higher presence of the variants with a protective effect. On the other hand, East Asia and America had the highest frequency of the risk alleles, followed by South Asia and Europe, respectively. Besides, East Asia had the lowest frequency of the variants with a protective effect on the condition. These results were related to genome Wide Association Studies (GWAS) performed in different populations [23], which showed that polymorphisms in this gene were involved in hyperuricemia. The risk alleles were present in 36.2% of Americans, 22.7% of Europeans and 21.5% of Asians. However, East Asians had less than 2% of protective alleles, increasing the probability to develop HU. The data suggested that Asian populations have a higher risk of developing HU or gout due to mutations in the *SLC2A9* gene compared to other populations, especially East Asia. [23]

In the *SLC22A12* gene, the allele frequencies indicated a lower frequency of risk allele in the African region compared to other regions. On the other hand, East Asia and Europe had the highest frequency of the risk alleles, followed by South Asia and America, respectively. Regarding the protective allele, Africa had the highest frequency followed by East Asia, America, South Asia and Europe. These results were related to the Ivonne, T. *et al* study performed in individuals worldwide, which showed that polymorphisms in this gene were involved in hyperuricemia, increasing the risk of its development in most cases. The risk alleles were present in 53.3% of Americans, 75.5% of Europeans and 95.5% of Asians. With such a marked differential prevalence and large effect size on SUA levels, the data suggested that Asian populations had a higher risk of developing HU or gout due to mutations in the *SLC22A12* gene compared to other populations. [25]

In conclusion, the results showed a clear relationship between the hyperuricemia prevalence and the presence of the genetic variants. However, further studies would be needed to conclude that the prevalence is directly related to the presence of the risk alleles, since other variables are involved.

# CONCLUSIONS

The most relevant urate transporters' single nucleotide polymorphisms (SNPs) associated with hyperuricemia (HU) in the Czech Republic were:

- the Q141K variant in the *ABCG2* gene.
- the rs4292327 variant in the *SLC2A9* gene.
- the rs8359 and rs8361 variants in the *SLC22A12* gene.

On the other hand, a direct relationship was observed between HU prevalence and genetics, in particular, with the SNPs of the urate transporters. Therefore, the regions with a high presence of the HU variants were also the regions with a high prevalence of hyperuricemia. Accordingly, the region with the highest presence of these variants, as well as HU prevalence, was East Asia, followed by the Americas, South Asia, Europe and Africa, respectively. However, there are other risk factors, such as diet, that may be involved in this relationship. Therefore, further studies would be necessary to establish this conclusion.

# REFERENCIES


1. Chen CJ, Lü JM, Yao Q. Hyperuricemia-related diseases and xanthine oxidoreductase (XOR) inhibitors: An overview. Vol. 22, Medical Science Monitor. International Scientific Literature Inc.; 2016. p. 2501–12. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4961276/> [Accessed 25 February 2021].

2. Stewart DJ, Langlois V, Noone D. Hyperuricemia and hypertension: Links and risks. Vol. 12, Integrated Blood Pressure Control. Dove Medical Press Ltd.; 2019. p. 43–62. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6935283/> [Accessed 25 February 2021].

3. Jin M, Yang F, Yang I, Yin Y, Luo JJ, Wang H, *et al*. Uric acid, hyperuricemia and vascular diseases. Front Biosci. 2012 Jan 1;17(2):656–69. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3247913/> [Accessed 25 February 2021].

4. Xu L, Shi Y, Zhuang S, Liu N. Recent advances on uric acid transporters. Vol. 8, Oncotarget. Impact Journals LLC; 2017. p. 100852–62. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725069/> [Accessed 27 February 2021].

5. Nigam SK, Bhatnagar V. The systems biology of uric acid transporters: The role of remote sensing and signaling. Vol. 27, Current Opinion in Nephrology and Hypertension. Lippincott Williams and Wilkins; 2018. p. 305–13. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6275126/> [Accessed 1 March 2021].

6. Riches P, Chapter 7 - Genetics of Gout. Gout & Other Crystal Arthropathies. 2012, 85-93. Available at: <https://www.sciencedirect.com/science/article/pii/B9781437728644100077O> [Accessed 2 March 2021]

7. Li L, Zhang Y, Zeng C. Update on the epidemiology, genetics, and therapeutic options of hyperuricemia. Vol. 12, American Journal of Translational Research. E-Century Publishing Corporation; 2020. p. 3167–81. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725069/> [Accessed 27 February 2021].

8. Nigam SK, Bush KT, Martovetsky G, Ahn SY, Liu HC, Richard E, *et al*. The organic anion transporter (OAT) family: A systems biology perspective. Physiol Rev. 2015 Jan 1;95(1):83–123. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4281586/> [Accessed 1 March 2021].

9. Aihemaitijiang S, Zhang Y, Zhang L, *et al*. The Association between Purine-Rich Food Intake and Hyperuricemia: A Cross-Sectional Study in Chinese Adult Residents. Nutrients. 2020;12(12):3835. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7765492/> [Accessed 20 February 2021].

10. Qiu L, Cheng XQ, Wu J, *et al*. Prevalence of hyperuricemia and its related risk factors in healthy adults from Northern and Northeastern Chinese provinces. *BMC Public Health*. 2013;13:664. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3722003/> [Accessed 20 February 2021].

11. Kuo CF, Grainge M, Zhang W, *et al.* Global epidemiology of gout: prevalence, incidence and risk factors. *Nat Rev Rheumatol*. 2015; 11, 649–662. Available at: <https://www.nature.com/articles/nrrheum.2015.91#citeas> [Accessed 20 February 2021].

12. Un paciente con hiperuricemia. Medicina Integral. Available at: <https://www.elsevier.es/es-revista-medicina-integral-63-articulo-un-paciente-con-hiperuricemia-15362> [Accessed 27 February 2021]

13. Li Z, Zhou Z, Hou X, Lu D, Yuan X, Lu J, *et al*. Replication of Gout/Urate Concentrations GWAS Susceptibility Loci Associated with Gout in a Han Chinese Population. Sci Rep. 2017 Dec 1;7(1). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725069/> [Accessed 27 February 2021].

14. Kim SY, Bang WJ, Min C, Choi HG. Association of nephrolithiasis with the risk of cardiovascular diseases: A longitudinal follow-up study using a national health screening cohort. BMJ Open. 2020 Nov 14;10(11). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725069/> [Accessed 27 February 2021].

15. Metabolic Nephropathies - Genitourinary Disorders - MSD Manual Professional Edition. Available at: <https://www.msdmanuals.com/professional/genitourinary-disorders/tubulointerstitial-diseases/metabolic-nephropathies> [Accessed 1 March 2021].

16. Chen BD, Chen XC, Pan S, Yang YN, He CH, Liu F, *et al*. TT genotype of rs2941484 in the human HNF4G gene is associated with hyperuricemia in Chinese Han men. Oncotarget. 2017;8(16):26918–26. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5432307/> [Accessed 5 May 2021]

17. Choi HK, McCormick N, Lu N, Rai SK, Yokose C, Zhang Y. Population Impact Attributable to Modifiable Risk Factors for Hyperuricemia. Arthritis Rheumatol. 2020 Jan 1;72(1):157–65. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6935419/> [Accessed 1 March 2021].

18. Li Z, Guo X, Liu Y, Chang Y, Sun Y, Zhu G, *et al*. The relation of moderate alcohol consumption to hyperuricemia in a rural general population. Int J Environ Res Public Health. 2016 Jul 20;13(7). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4962273/> [Accessed 2 March 2021]

19. Toyoda, Mančíková, Krylov, Morimoto, Pavelcová, Bohatá, *et al*. Functional Characterization of Clinically-Relevant Rare Variants in ABCG2 Identified in a Gout and Hyperuricemia Cohort. Cells. 2019 Apr 18;8(4):363. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6523779/> [Accessed 25 March 2021]

20. Hurba O, Mancikova A, Krylov V, Pavlikova M, Pavelka K, Stiburková B. Complex analysis of urate transporters SLC2A9, SLC22A12 and functional characterization of non-synonymous allelic variants of GLUT9 in the Czech population: No evidence of effect on hyperuricemia and gout. 2014 Sep 30;9(9). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4182324/> [Accessed 25 March 2021]

21. Kevin L Howe, Premanand Achuthan, James Allen, Jamie Allen, *et al*. Ensembl 2021. Nucleic Acids Research. 2021 Volume 49, Issue D1. Available at: <https://www.ensembl.org/index.html> [Accessed 20 May 2021]

22. Stiburkova B, Pavelcova K, Pavlikova M, Ješina P, Pavelka K. The impact of dysfunctional variants of ABCG2 on hyperuricemia and gout in pediatric-onset patients. Arthritis Res Ther. 2019 Mar 20;21(1). Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6425717/> [Accessed 20 May 2021]

23. Butler F, Alghubayshi A, Roman Y. The epidemiology and genetics of hyperuricemia and gout across major racial groups: A literature review and population genetics secondary database analysis. J Pers Med. 2021 Mar 1;11(3). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8005056/> [Accessed 20 May 2021]

24. Zhou Z, Wang K, Zhou J, Wang C, Li X, Cui L, *et al*. Amplicon targeted resequencing for SLC2A9 and SLC22A12 identified novel mutations in hypouricemia subjects. Mol Genet Genomic Med. 2019 Jul 1;7(7):722. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6625124/> [Accessed 31 May 2021]

25. Le Hellard S, Lee AJ, Underwood S, *et al*. Haplotype analysis and a novel allele-sharing method refines a chromosome 4p locus linked to bipolar affective disorder. Biological Psychiatry. 2007 Mar;61(6):797-805. Available at: <http://europepmc.org/article/MED/16996484/> [Accessed 20 May 2021]

26. Vidanapathirana DM, Jayasena S, Jasinge E, Stiburkova B. A heterozygous variant in the SLC22A12 gene in a Sri Lanka family associated with mild renal hypouricemia. BMC Pediatr. 2018 Jun 29;18(1). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6025733/> [Accessed 31 May 2021]

27. Cho SK, Kim S, Chung JY, Jee SH. Discovery of URAT1 SNPs and association between serum uric acid levels and URAT1. BMJ Open. 2015 Nov 24;5(11). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4663448/> [Accessed 20 May 2021]

28. Jeannin G, Chiarelli N, Gaggiotti M, Ritelli M, Maiorca P, Quinzani S, *et al*. Recurrent exercise-induced acute renal failure in a young Pakistani man with severe renal hypouricemia and SLC2A9 compound heterozygosity. BMC Med Genet. 2014 Jan 7;15(1). Available at: <https://pubmed.ncbi.nlm.nih.gov/22527535/> [Accessed 31 May 2021]

29. Hoque KM, Dixon EE, Lewis RM, Allan J, Gamble GD, Phipps-Green AJ, *et al*. The ABCG2 Q141K hyperuricemia and gout associated variant illuminates the physiology of human urate excretion. Nat Commun. 2020 Dec 1;11(1). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7265540/> [Accessed 31 May 2021]

# ABBREVIATIONS

ABCG2- ATP-binding cassette subfamily G member 2

BMI- Body mass index

BCRP- Breast cancer resistance protein

DASH- Dietary Approaches to Stop Hypertension

DBP- Diastolic blood pressure

FBG- Fasting blood glucose

FEUA- Fractional excretion uric acid

GLUT9- Glucose transporter

GWAS- Genome-wide association studies

HU- Hyperuricemia

MAF- Minor allele frequency

NPT- Nicotinate phosphoribosyltransferase

NSAID- Nonsteroidal anti-inflammatory drug

OAT- Anion transporter

SBP- Systolic blood pressure

SNP- single nucleotide pholymorphisms

SUA- Serum uric acid

TC- Total cholesterol

TG- Triglyceride

UA- Uric acid

URAT 1- Urate anion transporter 1

WC- Waist circumference