SIGNIFICANT ROLE OF ABCG2 TRANSPORTERS IN GOUT

PhD thesis

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Doctoral School of Molecular Medicine

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Introduction

Gout is a common crystal induced arthritis, characterised by intra- and/or extraarticular crystal deposition induced inflammation and the appearance of tophi in chronic cases. Besides erosive arthritis, it leads to severe systemic comorbidity if untreated. Crystallisation appears if the serum urate level chronically exceeds its threshold of solubility. Normal serum urate ranges vary between 180-360 μmol/l (3-6 mg/dl).

The actual level of serum urate is determined by the balance of production and excretion. Classically, hyperuricemia is categorised into primer and secunder and also overproducer and underexcretor types. The most frequent cause of primer hyperuricemia in this earlier categorisation is the underexcretion of serum urate due to urate transporter deficiencies (90 %).

ABCG2 urate transporter is a member of the ATP-binding cassette (ABC) transporter family (subfamily G2). **ABCG2 has an outstanding role in eliminating urate**, organic anions, steroids or xeno- and endobiotics. Its dysfunction is known to be strongly related to gout. Lately, it was connected with an earlier, even childhood-onset of the disease and also with intestinal urate excretion, resulting in an elevated renal load of serum urate, also known as renal-overload (ROL) type hyperuricemia.

However, despite being a key factor, **our information is still limited on how ABCG2 genetic changes relate to protein expression and that to clinical parameters and ROL-hyperuricemia in gouty patients.**

Objectives

The primary aim of this dissertation was to investigate the role of the ABCG2 urate transporter in gout, providing a comprehensive examination of the topic, ranging from ABCG2 genetic polymorphisms, to protein expression and clinical parameters in gouty patients.

Based on this, the objectives were as follows:

1. Study and further strengthen the linkage between ABCG2 urate transporter polymorphisms and gout **susceptibility**.
2. By testing and using the lately described **Ery-test**, one may gain information about ABCG2 protein expression levels through such an easily accessible human tissue as peripheral blood. This method could also make it possible to identify cases that should be put to further genetic analyses.
3. Evaluate the connection between ABCG2 **genetic changes** and **protein expression** in a clinically defined gouty population.
4. Evaluate whether ABCG2 mutations are associated with higher **disease severity,** characterised by early onset disease (*years prior to wild type group*), frequent flares (*flare/ past 12 months*) and tophi formation (*subcutaneous tophi present/absent*).
5. To put to the test how genetic changes and protein expression correlate with **clinical parameters** (se. urate level, UUE, FUE) and if the altered protein levels strengthen the concept of the new hyperuricemia classification based mostly on ABCG2 dysfunction

caused **renal-overload hyperuricemia**.

Methods

This thesis synthesises existing literature and our previous results of a prospectively recruited non-interventional study concerning 78 gouty patients and 73 healthy controls from a Hungarian population (study number 41006-1/2013/EKU). Clinical classification of gout was based on the EULAR criteria. Overproducer type patients and those taking colchicine three months prior to enrolment were not included. Healthy volunteers with no history of hyperuricemia or gout were enrolled as a control group.

All **urate and clinical parameters** were determined among **standardised circumstances.**

Genetic background was determined by **TaqMan-based qPCR** with premade assay mixes, or with custom-designed assay mixes and master mix from Thermo Fisher. TaqMan probe specificities were verified by sequencing.

The measuring of **ABCG2 protein levels** in red blood cells (RBC) were carried out according to the recently developed **flow cytometry-based Ery-test method**. EDTA-anticoagulated whole blood samples were freshly collected (2-6 hours before analysis), mildly fixed resulting in RBC membranes, also known as “ghosts”. Ghosts are incubated with ABCG2-specific primary antibody (Bxp-34), followed by a secondary, Alexa Fluor 488-labelled antibody, in 96 well plates. Cellular fluorescence was measured twice each case by a FACSCanto II flow cytometer.

Conclusions

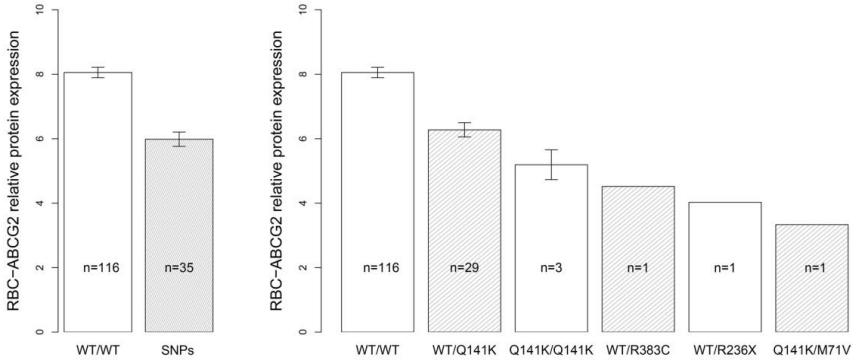
The thesis proves the significance of the ABCG2 urate transporter in presenting gout. Out of 78 gouty patients, 25 (32.1%) had at least one ABCG2 mutation, while only 10 out of 73 (13.7%) healthy controls had a mutation. These results indicate that **functional ABCG2 polymorphisms may lead to an elevated susceptibility to gout (odds ratio 3.0, 95% CI=1.2-7.5, p=0.012).**

It not only highlights the well-known Q141K and less frequent R236X and R383C polymorphisms, but also emphasises the **recently identified M71V variant** resulting in an unstable protein structure. Additionally, the thesis calls attention to the findings of collaborating partners, that the function of the M71V and also Q141K polymorphisms can be restored *in vitro* by treating with small molecule chemical compounds such as colchicine.

Previous analysis of ABCG2 urate transporter proteins has been hindered by the invasive nature of sample collection. As a result, much of the available data has come from animal studies (such as those using mice) and has lacked clinically relevant *in vivo* information. The **successful implementation of the Ery-test** enabled us to study ABCG2 transporter proteins in a well-controlled gout population, thus filling a previously existing gap in research. Our work clearly demonstrated through statistical analysis that the presence of specific **functional SNPs** led to a marked

reduction in expression levels of the ABCG2 transporter protein, as shown in Figure 10a, b of the Thesis.

Figure 10a, b of the Thesis. Correlation of functional ABCG2 mutations and protein expression levels



Mean values ± SD of erythrocyte ABCG2 relative protein expression levels in all wild type and mutant individuals (a) or wild type and individuals with specific mutations (b)

Our results demonstrate that patients with ABCG2 polymorphisms developed gout on average 8 years earlier than wild-type gout patients (mean age at diagnosis ± SD: 37.6±11.8 versus 45.7±12.3, p=0.008). Furthermore, they had significantly higher chances to experience frequent flares (presence of two or more flares during the past 12 months: 80% versus 52.8%, p=0.026) and had a higher number of tophaceous cases, although this difference did not reach statistical significance (presence of tophi: 40% versus 30.2%, p=0.445). These findings suggest that functional **ABCG2 mutations** may be a risk factor for **early onset, severe gout.**

The thesis describes that **genetic changes of ABCG2** transporter correlate with an inversely proportional shift of the studied clinical indices of fractional urate excretion (FUE) and urinary urate excretion (UUE), showing **elevated (near normal) FUE** (Mean ±SD FUE (%): 6.1 ±1.8 versus 4.6 ±1.4, p=<0.001) **and increased UUE** levels (Mean ±SD UUE (mg/24h): 663.0±190.1 versus 522.7 ±171.2, p=0.002). Additionally, these observations can also be proved in regards to altered protein levels and fractional urate excretion (FUE) and urinary urate excretion (UUE), as demonstrated in Figure 11a,b of the Thesis. These results *in vivo* confirm previous data that **impaired ABCG2** transport function acts as an extra-renal cause of urate excretion deficiency leading to a **renal-overload type hyperuricemia**.

Altogether, the thesis has made significant contributions by linking the various stages of ABCG2 genetic, protein and functional impairment, further paving the way for potential therapeutic and diagnostic advancements in the clinical field.

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