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PDZ domain containing protein 1 (PDZK1), a modulator of membrane proteins, is regulated by the nuclear receptor THRβ

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ABSTRACT

Genome wide association studies revealed single nucleotide polymorphisms (SNP) located within the promoter of PDZ domain containing protein 1 (PDZK1) to be associated with serum uric acid levels. Since modulation of transporters and particularly of membrane proteins involved in uric acid handling by PDZK1 has previously been reported, the aim of this study was to analyze the impact of the polymorphisms rs1967017, rs1471633, and rs12129861 on promoter activity and thereby transcription of PDZK1. Cell-based reporter gene assays showed transactivation of the PDZK1-promoter by triiodothyronine mediated by thyroid hormone receptors (THR) α and β. In silico analysis verified localization of the polymorphism rs1967017 within the most likely THR binding site whose deletion reduced THR-mediated transactivation. Furthermore, our study shows regulation of PDZK1 by thyroid hormones, thereby providing a mechanistic basis for the previously reported associations between thyroid hormone status and uric acid homeostasis.

1. Introduction

Genome wide association studies searching for genetic variants associated with uric acid levels, hyperuricemia, and/or gout in European and Asian populations identified polymorphisms localized in the 5′ untranslated region (5' UTR) of PDZK1 to be linked with the tested phenotypes (Yang et al., 2014,Kolz et al., 2009,Kottgen et al., 2013,van der Harst et al., 2010,Yang et al., 2010). Even if the polymorphisms in this region not always reached the level of genome wide significance (Karns et al., 2012,Dehghan et al., 2008), the observation that PDZK1 is genetically linked to urate homeostasis was basis for further studies finally providing a mechanistic explanation.

PDZK1, a multidomain protein containing four postsynaptic density 95/disk-large/ZO-1 (PDZ)-domains (Kato et al., 2006), functions as scaffold for membrane proteins, thereby contributing to the organization of specialized membrane domains. Indeed, PDZK1 stabilizes multiprotein complexes by direct protein-protein interaction, thus positioning interacting membrane proteins in correct spatial arrangement (Bezprozvanny and Maximov, 2001). Membrane proteins containing the classical type I PDZ-domain binding motif at the carboxy-terminus are likely to directly interact with the PDZ-domains of PDZK1, thereby being stabilized in the membrane and/or modulated in their function (Kato et al., 2006).

In terms of uric acid handling, PDZK1 is assumed to be a stabilizer of the so-called “urate transportosome” (Anzai et al., 2012). Based on our current understanding, the “urate transportosome” is a complex of multiple membrane transporters which contribute to the renal handling of urate. Importantly, PDZK1 interacts with membrane

Abbreviations: 5-HT$_2$A, Serotonin 2A receptor; ABC, ATP-binding cassette transporter; AhR, Transcription factor aryl hydrocarbon receptor; BCRP, Breast cancer resistance protein; CFTR, Cystic fibrosis transmembrane conductance regulator; DR1, Direct repeat with 1 bp spacer; ERα, Estrogen receptor α; FXRα, Farnesoid X receptor; GRα, Glucocorticoid receptor α; HDL, High-density lipoprotein receptor; MRP, Multidrug Resistance Proteins; NHE3, Sodium-hydrogen exchanger 3; OAT, Organic anion transporter; OATP, Organic anion transporting polypeptide; PDZ, Postsynaptic density 95/ disk-large/ ZO-1; PDZK1, PDZ domain containing protein in kidney 1; PPARα, Peroxisome proliferator-activated receptor α; PXR, Pregnane X receptor; RARβ, Retinoid acid receptor β; RXRα, Retinoid X receptor α; SLC, Solute carrier; SNP, Single nucleotide polymorphisms; SR-B1, Scavenger receptor class B member; THR, Thyroid hormone receptor; THRE, Thyroid response element; URAT1, Urate Anion Exchanger 1; 5′UTR, 5′ untranslated region; VDR, Vitamin D receptor.

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proteins localized at the luminal side of the renal tubule. Accordingly, transporters located in the apical membrane of the tubular cell like the solute carriers 22A12 (SLC22A12, URAT1), or 22A11 (SLC22A11, OAT4) were identified as interacting partners of PDZK1. This interaction has been shown to increase membrane localization, and thereby transport capacity (Anzai et al., 2004,Miyazaki et al., 2005). In addition to those uptake transporters, the ATP-binding cassette transporter C4 (ABCC4, MRP4), an efflux transporter, has been reported to be part of the "urate transportosome" (Van Aubel et al., 2005), and to interact with PDZK1 (Park et al., 2014). Importantly, ABCC4 is also expressed in other tissues such as liver or intestine and is known to play a role in drug metabolism (Giacomini et al., 2010). The list of drug transporters interacting with PDZK1 is further extended by ABCC2 (MRP2), a well-known efflux mechanism for products of the phase II biotransformation that also interacts with PDZK1 (Kocher et al., 1999). In addition to its renal expression, ABCC2 is expressed in the canalicular membrane of hepatocytes and the apical membrane of enterocytes (Jedlitschky et al., 1997), thereby providing evidence that PDZK1, even if highly expressed in kidney, executes its organizational role also in other tissues with polarized cells. This notion is supported by findings on ABCG2 (BCRP) and PDZK1 in murine intestine (Shimizu et al., 2011).

However, at this point it seems noteworthy that the function of PDZK1 is not limited to uric acid handling or drug metabolism, but also affects epithelial fluid transport by modulation of the positioning and gating of ABCC7 (CFTR) (Bezprozvanny and Maximov, 2001). Furthermore, PDZK1 is assumed to modulate cholesterol metabolism interacting with the HDL-receptor scavenger receptor class B member (SCARB1) (Silver, 2004), or to impact neurotransmitter signaling mediated by interaction with the serotonin 2A receptor (HTR2A) (Walther et al., 2015).

Even if the number of interacting proteins and thereby physiological functions modulated by PDZK1 steadily increases, little is known about the mechanisms underlying the observed association of genetic variants located upstream of the coding region of PDZK1 with urate homeostasis. Nonetheless, the area in which the uric acid associated polymorphisms (rs12129861, rs1471633, and rs1967017) are located comprises the promoter region of the scaffolding protein (Tachibana et al., 2008), thereby potentially influencing transcription and expression. The promoter of the scaffolding protein has previously been described by Tachibana, K. et al. reporting transactivation of PDZK1 by PPARα (Tachibana et al., 2008). However, it is currently unknown whether transactivation is influenced by the above-mentioned naturally occurring polymorphisms.

Accordingly, the aim of the herein reported study was to further analyze the 5′UTR of PDZK1 including the abovementioned genetic variants in our considerations. At first, human kidney samples were assessed for expression of PDZK1, revealing a trend for lower mRNA amount in individuals harboring the minor alleles of the polymorphisms. The observed trend in this descriptive analysis was basis for a subsequent mechanistic study screening the promoter for transactivation by known ligand activated nuclear receptors including the thyroid hormone receptor (THRα, NR1A1 and THRβ, NR1A2). This study demonstrates that THRβ activated by T3 induces the expression of PDZK1 and that this transactivation is influenced by naturally occurring polymorphisms, as observed in the herein reported *in vitro* experiments.

2. Materials and methods

2.1. Kidney tissue samples

Human malignant and adjacent non-malignant kidney samples were obtained from patients undergoing surgery due to kidney tumors after their written informed consent. The study was approved by the local ethics committee of the Medical Faculty of the University of Greifswald (III UV 12/03). Once the tissue samples were prepared and pulverized as reported elsewhere (Prestin et al., 2016), the samples were stored at −80 °C until further use.

2.2. Cell culture

Cell lines were cultured at 37 °C in a humidified 5% CO_2-atmosphere. Dulbecco's Modified Eagle's Medium (D6429, 4.5 mg/L glucose and 1 mM sodium pyruvate, Sigma-Aldrich, Arlesheim, Switzerland) was used as culture medium for HepG2 (ATCC® HB-8065™) and Caco2 (ATCC® HTB-37™), while M199 (Gibco™, LuBioScience, Lucerne, Switzerland) was used for BeWo cells. The medium was supplemented with 5% FCS (AMIMED, BioConcept Ltd., Allschwil, Switzerland), 1% GlutaMAX™, (Thermo Fisher Scientific, Reinach, Switzerland). Caco2 cells were cultivated for 14 days, before starting the experiments. Human primary renal proximal tubule epithelial cells (RPTEC) derived from renal tissue of a Caucasian 55-year old male (Lot 0000324297) were obtained from RUWAG (Bettlach, Switzerland) and cultured in renal epithelial growth medium supplemented with 0.5% serum, 0.1% rhEGF, 0.1% insulin, 0.1% hydrocortisone, 0.1% epinephrine, 0.1% triiodothyronine, and 0.1% transferrin (Clonetics™ REGM™ BulletKit™, Lonza, Basel, Switzerland). Cell passaging of RPTEC was performed using the DetachKit (PromoCell, Heidelberg, Germany) containing HEPES buffered saline solution (30 mM HEPES), trypsin-EDTA solution (0.04% and 0.03%), and trypsin neutralizing solution (0.05% with 0.1% BSA). All cell lines were tested negative for contamination with mycoplasma.

2.3. Genotyping of kidney samples

Genomic DNA from the renal tissue was isolated with the NucleoSpin® Tissue Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The renal DNA was used for genotyping of rs1967017 (PDZK1 -4017 bp T > C), rs1471633 (PDZK1 -3923 bp A > C), and rs12129861 (PDZK1 -1976 bp G > A). Analysis was performed using commercially available TaqMan® genotyping assays (C___1862243_10, C___1862244_10, or C__26662675_10, LuBioScience), the ViiA™7 Real-Time PCR System, and the ViiA™7 software v1.2.2 (Thermo Fisher Scientific). However, the assay C___1862244_10 for genotyping of rs1471633 was not specific, as observed by analysis of fluorescence data after amplification according to the manufacturer's instructions. Therefore, the resulting PCR product of each sample was subsequently digested using *Hpy*CH4III (*Taa*I) (Thermo Fisher Scientific) finally identifying the genotype by RFLP. In case of exchange of A to C, the *Hpy*CH4III was not capable of cutting the PCR fragment (supplemental Fig. A1).

2.4. Western blot analysis of human kidney samples and cell lines

Protein expression of cell lines and human kidney samples was analyzed by immunoblotting. Samples were separated by SDS-PAGE and electro-transferred onto a nitrocellulose membrane using a tank blotting system (Mini Trans-Blot® Cell; Bio-Rad Laboratories, Cressier FR, Switzerland). After blocking with 5% FCS diluted in tris-buffered saline supplemented with TWEEN®20 (TBST), the membranes were incubated with the respective primary antibody at 4 °C overnight. For detection of PDZK1 and β-actin, the rabbit monoclonal anti-PDZK1 antibody ab137873 (Lucerna-Chem, Lucerne, Switzerland; diluted 1:2500 in TBST with 1% BSA) and goat polyclonal anti-β-actin antibody sc-1616 (Santa Cruz Biotechnology, Heidelberg, Germany; diluted 1:1000 in TBST 1% BSA) were

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