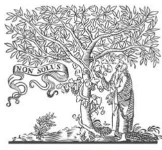
ARTICLE IN PRESS

Mitochondrion xxx (xxxx) xxx–xxx



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Mitochondrion

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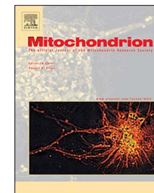

Short communication

The mitochondrial uncoupling protein 2 gene is causal for the spontaneous polycystic liver diseases in mice

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ARTICLE INFO

Keywords:
Mouse model
Mitochondria
Uncoupling protein 2 (UCP2)
Polycystic liver disease (PCLD)

ABSTRACT

Polycystic liver diseases (PCLDs) are autosomal dominant disorders. To date, 3 genes are known to be associated with the disease, *SEC63* and *PRKCSH* and *LRP5*. Here, we report that mice deficient in the mitochondrial uncoupling protein 2 gene (*Ucp2*$^{-/-}$) spontaneously developed PCLDs when they were over 12 months old. Macroscopical observation, blood chemistry as well as histopathological analysis demonstrated the PCLDs found in *Ucp2*$^{-/-}$ mice were very similar to the findings in human PCLDs. This is the first report describing the gene encoding mitochondrial protein is causative for PCLDs. *UCP2* may be a biomarker of the PCLDs in humans.

1. Introduction

Polycystic liver diseases (PCLDs) are inherited diseases of the biliary epithelium, caused by genetic defects in proteins that are associated with intracellular organelles, mainly the endoplasmic reticulum and the cilium (Strazzabosco and Somlo, 2011). PCLDs are inherited in a dominant or recessive form and can develop alone or in association with similar polycystic kidney diseases, including autosomal dominant polycystic kidney disease (ADPKD)(Perugorria et al., 2014). It has been reported that mutations in the *SEC63 gene* (Davila et al., 2004), the *PRKCSH* gene (Reynolds et al., 2000; Drenth et al., 2003) and the *LRP5* gene (Cnossen et al., 2014) are associated with PCLD in humans. The gene product of *SEC63*, SEC63p, plays a role in translocation of peptides across the endoplasmic reticulum membrane, while *PRKCSH* encodes the non-catalytic β-subunit of glucosidase II (GIIβ), which activity is crucial for protein folding. Two causative genes, *PKD1* (Hughes et al., 1995) and *PKD2* (Mochizuki et al., 1996) have been identified as causative genes for ADPKD. Tissue-specific deletion of these 4 genes as well as *Pkhd1*, the recessive polycystic kidney disease gene, resulted in the induction of liver and kidney cysts in mice (Fedeles et al., 2011). Here, we report on PCLDs observed in the mice deficient of the mitochondrial uncoupling protein 2 gene (*Ucp2*$^{-/-}$ mice).

2. Materials and methods

2.1. Mice and husbandry

Ucp2$^{-/-}$ (B6.129S4-Ucp2^{tm1Lowl}/J, stock number; 005934) and C57BL/6J wild-type (stock number; 000664) mice were purchased from Jackson laboratory (Bar Harbor, ME, USA), and *Ucp2*$^{-/-}$ mice were kept on C57BL/6 J background. Mice were provided ad libitum access to filtered water and autoclaved pellet diet (Altromin, Eastern-Westphalia/Lippe, Germany). The animal facility was maintained at 21 °C on a 12 h light-12 h dark cycle. All mice did not have breeding experience.

Animal use was approved by local authorities of the Animal Care and Use Committee (V242-7224.122-5, Kiel, Germany) and performed by certified personnel.

Abbreviations: UCP2, uncoupling protein 2; PCLD, polycystic liver disease; PCKD, polycystic kidney disease

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https://doi.org/10.1016/j.mito.2017.10.011
Received 11 July 2017; Received in revised form 12 October 2017; Accepted 26 October 2017
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Please cite this article as: Hirose, M., Mitochondrion (2017), https://doi.org/10.1016/j.mito.2017.10.011

2.2. Macroscopic observation

Mice were daily inspected, and when moribund signs, e.g. low body temperature, reduced activity, no food consumption, were observed, those mice were sacrificed and macroscopically investigated by a veterinarian. The observation was recorded, as well as organs were collected for histopathological analysis.

2.3. Histopathology

Liver samples were fixed in 4% buffered formalin and processed into paraffin blocks. Six µm sections were prepared and stained with haematoxylin and eosin according to standard protocol.

2.4. Blood chemistry

Plasma samples from *Ucp2*$^{-/-}$ mice demonstrated polycystic liver and non-affected *Ucp2*$^{-/-}$ mice as well as healthy wild-type mice were obtained. Alanine transaminase (ALT), aspartate aminotransferase (AST), urea and creatinine (Cre) were measured by commercial animal diagnostic company (Laboklin, Bad Kisseling, Germany).

2.5. Real time-PCR

Liver and kidney samples were stored in RNAlater (Thermo Fisher Scientific, Pinneberg, Germany) at − 20 °C until used in experiments. Total RNA was isolated from tissues stored by using innuPREP RNA Mini Kit (Analytik Jena, Jena, Germany) including DNase I (Thermo Scientific) digestion. After reverse transcription using First Strand cDNA Synthesis Kit (Thermo Scientific) the cDNA was added to the Maxima SYBR Green qPCR Master Mix (Thermo Scientific) and amplified using RealPlex thermal cycler (Eppendorf, Hamburg, Germany). The amplification program consisted of initial denaturation at 95 °C for 10 min, 30 cycles of denaturation at 95 °C for 15 s, and annealing at 60 °C for 30 s, followed by extention at 72 °C for 30 s. The beta-actin gene (*Actb*) was used as a housekeeping gene. Sequences for the primers used in this study are shown as follows.

Ucp2 forward, 5′- GCGTTCTGGGTACCATCCTA-3′; *Ucp2* reverse, 5′-GCTCTGAGCCCTTGGTGTAG-3′ (Zhang et al., 2010).

Actb forward, 5′- CCCCATTGAACATGGCATTG-3′; Actb reverse, 5′-ACGACCAGAGGCATACAGG-3′ (Thundathil et al., 2005).

2.6. Gene network analysis

GeneMANIA (http://www.genemania.org/) was used to perform gene network analysis (Warde-Farley et al., 2010).

2.7. Statistical analysis

GraphPad Prism 6 was used for the statistical analysis. The incidence of liver and kidney cysts were analyzed using Fisher's exac *t*-test. Results for blood chemistry and RT-PCR were analyzed using *t*-test and two-way ANOVA, respectively. *P*-values < 0.05 were considered as statistically significant difference.

3. Results and discussion

The *Ucp2*$^{-/-}$ mice grew normally and were fertile, though they gained less body weight in aging (data not shown). When mice were found to be moribund, they were sacrificed and macroscopically investigated. To our surprise, of 142 *Ucp2*$^{-/-}$ mice dissected (male; $n = 64$, female; $n = 78$) the presence of cysts in the liver was identified in 63 mice (male; $n = 28$, female; $n = 35$), while only 1 mouse exhibited the phenotype in 147 wild-type mice (Table 1, $P < 0.0001$, Fisher's exact test and Fig. 1A and B).

Thus, the *Ucp2* gene is protective for polycystic liver (relative risk, 1.785, 95% CI; 1.540–2.069, odds ratio; 116.4, 95% CI; 15.84–855.9). The liver cysts observed in the *Ucp2*$^{-/-}$ mice were varied in the number and size (Fig. 1A). We also observed kidney cysts in a small number of *Ucp2*$^{-/-}$ mice that carried liver cysts (supplementary Fig. 1A). The incidence of kidney cysts was significantly higher in *Ucp2*$^{-/-}$ mice compared to wild-type ($P = 0.0059$, Fisher's exact test), suggesting that the *Ucp2* gene is protective for kidney cysts (relative risk; 1.131, 95% CI; 1.041–1.229, odds ratio; 4.544, 95% CI; 1.486–13.89, supplementary Table 1). In addition, pancreas cysts were observed in 4 male and 4 female *Ucp2*$^{-/-}$ mice, while none was in wild-type mice (supplementary Fig. 1B). All those mice carrying kidney cysts or pancreatic cysts had liver cysts concurrently except one mouse. We observed liver cysts phenotype only in *Ucp2*$^{-/-}$ mice over 11 months old (Fig. 1C), while the age of moribund *Ucp2*$^{-/-}$ mice varied between 4 and 34 months old in males and between 6 and 29 months old in females. None of the *Ucp2*$^{-/-}$ mice sacrificed at younger age, between 3 months old and 10 months old for other studies did present the polycystic liver nor kidney phenotypes ($n = 24$ in male and $n = 28$ in female *Ucp2*$^{-/-}$ mice). Furthermore, the same phenotype was also independently identified in different colony of *Ucp2*$^{-/-}$ mice kept at a different institute (18 mice with liver cysts among 30 mice aged between 12 and 24 months old). Blood chemistry analysis demonstrated that the liver enzyme (ALT; alanine transaminase and AST; aspartate transaminase) were within the normal range and did not show significant difference between mice with polycystic livers and non-diseased *Ucp2*$^{-/-}$ or wild-type mice (Fig. 1D and supplementary Table 2), which is also characteristics of human PCLDs (Chandok, 2012).

Interestingly, the polycystic disease phenotype was primarily found in the liver, an organ reportedly with less expression levels of UCP2 protein and mRNA compared with other organs (Pecqueur et al., 2001). The UCP2 protein is highly expressed in spleen, lung and stomach, while the protein is, at least in basal condition, not detected in muscle, heart, and brain mitochondria (Pecqueur et al., 2001), and there are few example in liver. In line with this, we were only able to detect UCP2 expression at the RNA level (supplementary Fig. 2A, B), but not at the protein level in the liver mitochondrial samples obtained from the wild-type mice (data not shown).

Although the specificity of UCP2 antibodies currently available is usually not high enough for immunohistochemistry (Pecqueur et al., 2001), it has been reported, the putative presence of UCP2 in biliary epithelial cells in primary biliary cirrhosis, but not in other liver diseases, including autoimmune hepatitis and chronic viral hepatitis, as well as normal liver (Taniguchi et al., 2002). As matter of fact, the

Table 1
Incidence of liver cysts in *Ucp2*$^{-/-}$ and wild-type mice.

Strain	Case (N)	Case (%)	Unaffected (N)	Unaffected (%)	Total (N)	*P*-value	Relative risk	95% CI	Odds ratio	95% CI
Wild-type	1	0.68	146	99.32	147	< 0.0001	65.22	9.164–464.1	116.4	15.84–855.9
Ucp2$^{-/-}$	63	44.37	79	55.63	142					

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