RESEARCH LETTER

Potential role of plant microRNAs in the pathogenesis of autosomal dominant polycystic kidney disease: an in silico study

Mariusz Niemczyk[1], Arkadiusz Gładki[2], Agnieszka Gromadka[3], Piotr Zielenkiewicz[3,4], Leszek Pączek[1]

1 Department of Immunology, Transplant Medicine, and Internal Diseases, Medical University of Warsaw, Warsaw, Poland
2 DoBio Arkadiusz Gładki, Skierniewice, Poland
3 Institute of Biochemistry and Biophysics; Polish Academy of Sciences, Warsaw, Poland
4 Faculty of Biology, University of Warsaw, Warsaw, Poland

Introduction Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder. Its prevalence is estimated at 1:500 to 1:4000. In a substantial number of adult patients, ADPKD leads to end-stage kidney disease (ESKD).[1] Autosomal dominant polycystic kidney disease is caused by a mutation in the *PKD1* or *PKD2* genes that encode polycystin 1 (PC1) and polycystin 2 (PC2), respectively.[2] Polycystin 1 is expressed in many tissues.[3] Therefore, the clinical presentation of ADPKD is not limited to the kidneys and numerous extrarenal manifestations of the disease can be observed.[1]

Both PC1 and PC2 form a complex to play a role in intracellular calcium homeostasis. The loss of function of the PC1 / PC2 complex leads to upregulated cAMP signaling, which ultimately results in ADPKD through a myriad of signaling pathways. Autosomal dominant polycystic kidney disease is genetically dominant at the organismal level, but cystogenesis requires the inactivation or severe reduction of functional activities of alleles of both *PKD1* or *PKD2*. The critical level of decline in the functional activity of these genes, required for the initiation of cystogenesis can vary depending on different factors, such as kidney developmental stage, environmental effects, and genetic variants at modifier loci.[2] However, the mechanism of selective inhibition of activity of the normal copy of *PKD1* or *PKD2* in an individual patient with an inherited mutation in the second copy of the gene remains unclear. Due to the fact that ADPKD is observed worldwide, the mechanism of inhibition of the normal haplotype gene should be universal. That is why we speculated that it may be a dietary component that causes the inactivation of the normal copy of the gene.

MicroRNA (miRNA) are short RNA molecules that are present in eukaryotic organisms and regulate the post-transcriptional expression of genes through target mRNA translation inhibition or degradation. As a result, miRNAs control crucial biological processes such as metabolism, apoptosis, developmental timing, cell proliferation, immune responses, and differentiation.[4] It was shown that exogenous plant miRNAs acquired by ingesting food are present in sera and tissues of animals and may decrease the expression of target genes in mammals.[5] Other studies also support the opinion that plant miRNAs can effectively regulate the expression of human genes in a cross-kingdom manner.[4,6] That led to the hypothesis that vegetable miRNAs supplied with food interact with a normal copy of the *PKD1* gene leading to its inactivation and, ultimately, to the clinical manifestations of ADPKD. To test our hypothesis, we performed an in silico study in which vegetable miRNAs potentially interacting with the *PKD1* gene were sought. The term 'in silico' denotes studies performed on a computer that model natural or laboratory processes, reducing the need for laboratory work or clinical trials. In silico studies are among major experimental methods in genomics.

Methods To search for plant miRNAs interacting with human *PKD1* mRNA, we searched for potential miRNAs targeting 2 isoforms of *PKD1* mRNA. For further analyses, we considered only miRNA–mRNA pairs predicted in at least 4 out of 8 tools used. In the next step, we restricted our analyses only to the miRNAs from edible plants. The list of edible plants is shown in

Correspondence to:
Mariusz Niemczyk, MD, PhD, Department of Immunology, Transplant Medicine, and Internal Diseases, Medical University of Warsaw, ul. Nowogrodzka 59, 02-006 Warszawa, Poland, phone: +48225021641, email: mniemczyk@wum.edu.pl
Received: December 31, 2020.
Revision accepted: January 31, 2021.
Published online: February 10, 2021.
Pol Arch Intern Med. 2021; 131 (3): 306-308
doi:10.20452/pamw.15804

Supplementary material, *Table S1*. Finally, we checked which of the miRNAs found can be considered as plant-derived xenomiRs.

Due to the robust number of predicted miRNAs, we decided to repeat prediction using stricter parameters. The predictions were followed by the manual verification of miRNAs and the target's binding sites.

Detailed data from the bioinformatic analysis are presented in Supplementary material. No additional statistical analysis was performed in this study, as it was found to be redundant.

The study was conducted in accordance with the principles of the Declaration of Helsinki. Due to study design, the approval of the ethics committee was not necessary.

Results Using psRNATarget, we found 709 and 711 miRNAs potentially interacting with the first and second variant of the *PKD1* gene, respectively (Supplementary material, *Table S2* and *Table S3*). Among those, we found 341 unique miRNA families (ie, miRNAs with the same sequence found in the same and / or different species) potentially interacting with *PKD1* variant 1 and 343 unique miRNA families potentially interacting with *PKD1* variant 2 mRNA.

All miRNA family–*PKD1* mRNA pairs predicted in the previous step were validated with 8 additional tools. In Supplementary material, *Tables S4* (variant 1) and *S5* (variant 2), we presented the most potentially interesting miRNAs: the ones predicted to be binding to *PKD1* mRNA by more than 5 out of the 9 tools used, found in at least a single species that is considered to be edible and / or drug for humans, and supposed to be plant-derived xenomiRs in the study by Zhao et al (Supplementary material, *Reference M12*). An additional column ("strict parameters") informs whether the given finding was also confirmed by the second, stricter approach and highlights the most promising targets for future experimental validation. The results for all miRNA families can be found in Supplementary material, *Tables S6* (variant 1) and *S7* (variant 2). MicroRNAs identified in our study have origins in corn, potato, cabbage, barley, soya, Asian rice, grapes, cassava, artichoke, melon, milo (sorghum), marsh pine, reed fescue, barrel-clover, peanuts, and common radish. The most promising plant miRNAs, potentially interacting with the *PKD1* gene include zma-miR164e-5p, stu-mir408a-3p, hvu-miR6187, and hvu-miR6186, originating from corn, potato, and barley, respectively.

Discussion According to the literature data, miRNAs are involved in the pathophysiology of ADPKD.[7,8] Plant miRNAs are supplied orally, with food. They are quite stable and do not undergo degradation during boiling or at low pH in the stomach. They can be detected in serum and tissues and decrease the expression of mammalian genes.[5] The results of our in silico study suggest that it may also be the case in ADPKD. MicroRNAs present in plants that constitute a basis of diet in different parts of the world, may potentially decrease *PKD1* expression, leading to the occurrence of ADPKD symptoms. Our findings show that these miRNAs are found in such plants as corn, potato, cabbage, barley, soya, Asian rice, milo, grapes, and cassava. It corresponds with the fact that ADPKD is present worldwide, in patients of all races. It may also explain individual differences in the course of ADPKD in members of particular families who inherited the same mutation. In those cases, various dietary habits would be responsible for differences among disease phenotypes.

The dietary origin of disease manifestations would also explain the cystic rebuilding of the liver, which is the most common extrarenal manifestation of ADPKD.[9] The liver is significantly exposed to plant miRNAs, absorbed by the gut, and supplied via the portal vein. Liver cystogenesis occurs later in the course of ADPKD compared with renal cystogenesis and undergoes other regulation processes; however, as mentioned above, numerous additional factors play a role in the initiation of this process.[2]

Similarly, the presence of plant miRNAs in serum leads to the exposure of endothelial cells to them, which may explain why the endothelium is disturbed very often and early in the course of ADPKD.[10] Finally, the filtration of blood in the kidney leads to exposing the epithelial cells of renal tubules to plant miRNAs,[11] which may account for renal involvement in ADPKD.

Due to the common exposure of the whole population to plant miRNAs, supplied with food, our results are not limited to people with ADPKD, but may also explain the pathogenesis of conditions different than ADPKD, eg, simple cysts of the kidney. They are not hereditary, and their prevalence increases with age. Ultimately, 50% of people at 50 years or older have simple renal cysts. Their pathophysiology remains unclear.[12] Based on our findings, one can speculate that it may be the decreased *PKD1* expression, caused by long-time exposure to plant miRNAs provided with food, that leads to cystogenesis. However, in people with 2 normal copies of the *PKD1* gene, the severity of dysfunction is milder compared with those with an inherited mutation of a single copy of the gene, and the condition never becomes clinically significant.

The main limitation of our study was the fact that it was an exclusively in silico study, which might have been influenced by the availability and incompleteness of datasets and deficiencies in bioinformatic algorithms. Therefore, further in vitro and in vivo studies are needed to confirm our findings. They should include the detection of predicted plant miRNAs in human tissues and provide the evidence of their influence on the activity of target genes, as well as the association of clinical data on the progression of ADPKD with those on dietary habits.

Conclusions Exogenous plant miRNAs, acquired with food, may interfere with the *PKD1* gene activity, decreasing its expression and being responsible for the clinical presentation of ADPKD. Our findings require further validation in experimental studies.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

ACKNOWLEDGMENTS We acknowledge the contribution of Cezary Kuran (Electronic Devices Cezary Kuran, Skierniewice, Poland) in the bioinformatic analysis.

CONTRIBUTION STATEMENT MN conceived the study concept and design, interpreted the results, and wrote the manuscript. AGł and AGr performed bioinformatic analysis and wrote the manuscript. PZ performed bioinformatic analysis and interpreted the results. LP conceived the study concept and design and interpreted the results.

CONFLICT OF INTEREST None declared.

HOW TO CITE Niemczyk M, Gładki A, Gromadka A, et al. Potential role of plant microRNAs in the pathogenesis of autosomal dominant polycystic kidney disease: an in silico study. Pol Arch Intern Med. 2021; 131: 306-308. doi:10.20452/pamw.15804

REFERENCES

1 Rangan GK, Alexander SI, Campbell KL, et al. KHA-CARI guideline recommendations for the diagnosis and management of autosomal dominant polycystic kidney disease. Nephrology (Carlton). 2016; 21: 705-716.

2 Ferreira FM, Watanabe EH, Onuchic LF. Polycystins and molecular basis of autosomal dominant polycystic kidney disease. In: Li X, ed. Polycystic Kidney Disease [Internet]. Brisbane, Australia: Codon Publications; 2015.

3 Peters DJ, van de Wal A, Spruit L, et al. Cellular localization and tissue distribution of polycystin-1. J Pathol. 1999; 188: 439-446.

4 Lukasik A, Brzozowska I, Zielenkiewicz U, Zielenkiewicz P. Detection of plant miRNAs abundance in human breast milk. Int J Mol Sci. 2017; 19: 37.

5 Zhang L, Hou D, Chen X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res. 2012; 22: 107-126.

6 Marzano F, Caratozzolo MF, Consiglio A, et al. Plant miRNAs reduce cancer cell proliferation by targeting MALAT1 and NEAT1: a beneficial cross-kingdom interaction. Front Genet. 2020; 11: 552490.

7 Kim DY, Woo YM, Lee S, et al. Impact of miR-192 and miR-194 on cyst enlargement through EMT in autosomal dominant polycystic kidney disease. FASEB J. 2019; 33: 2870-2884.

8 Lee EC, Valencia T, Allerson C, et al. Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. Nat Commun. 2019; 10: 4148.

9 Bae KT, Zhu F, Chapman AB, et al. Magnetic resonance imaging evaluation of hepatic cysts in early autosomal-dominant polycystic kidney disease: the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease cohort. Clin J Am Soc Nephrol. 2006; 1: 64-69.

10 Theodorakopoulou M, Raptis V, Loutradis C, Sarafidis P. Hypoxia and endothelial dysfunction in autosomal-dominant polycystic kidney disease. Semin Nephrol. 2019; 39: 599-612.

11 Yang J, Farmer LM, Agyekum AA, et al. Detection of an abundant plant-based small RNA in healthy consumers. PLoS One. 2015; 10: e0137516.

12 Garfield K, Leslie SW. Simple renal cysts. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020.