ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2013, Vol 20, No 1, 131-134
www.aaem.pl

No associations between rs2030712 and rs7456421 single nucleotide polymorphisms of HIPK2 gene and prevalence of chronic kidney disease. Results of a family-based study

Joanna Żywiec[1], Katarzyna Kiliś-Pstrusińska[2], Janusz Gumprecht[1], Władysław Grzeszczak[1], Aleksandra Werner[3]

[1] *Department of Internal Medicine, Diabetology and Nephrology, Medical University of Silesia, Katowice, Poland*
[2] *Department of Paediatric Nephrology, Medical University, Wrocław, Poland*
[3] *Institute of Computer Science, Silesian University of Technology, Gliwice, Poland*

Żywiec J, Kiliś-Pstrusińska K, Gumprecht J, Grzeszczak W, Werner A. No associations between rs2030712 and rs7456421 single nucleotide polymorphisms of HIPK2 gene and prevalence of chronic kidney disease. Results of a family-based study. Ann Agric Environ Med. 2013; 20(1): 131-134.

Abstract

Introduction: Different pathological processes can deteriorate kidney function and cause ireversible degeneration of its structure; however, an optimal way to inhibit or slow down progression of renal damage is unforunately not available. In the light of promissing data concerning homeodomain-interacting protein kinase 2 (HIPK2) upregulation in damaged kidneys animal model, and increased levels of this protein in patients with various kidney diseases, the influence of rs7456421 and rs 2030712 single nucleotide polimorphisms of HIPK2 gene on chronic kidney disease incidence and progression was studied.

Material and methods: In 109 family 'trios', consisting of an affected child with CKD (48 females and 61 males, mean age 15.5 ±6.45 years) and both his/her parents, using Transmission Disequilibrium Test allele was used for the transfer of aforementioned SNPs from biological parents to their affected offspring.

Results: No statistical significance of allele transfer was found, which means that there were no associations between rs7456421 and rs 2030712 SNPs of HIPK2 gene and the incidence of renal dysfunction. Multiple stepwise regression showed a history of chronic glomerulonephritis (OR=17.3), chronic interstitial nephritis without urinary tract defect (OR=4.4), and CT genotype of rs 2030712 SNP (OR=2.6) as determinant of a more rapid progression of renal dysfunction, in contrast to the protective action of body mass index (OR=0.86).

Conclusions: On the basis of TDT results, the influence of rs7456421 and rs 2030712 SNPs of HIPK2 gene on prevalence of chronic kidney disease was not identified. Further studies are needed to ascertain the tight relationships of HIPK2 gene polymorphisms with CKD of different etiologies.

Key words

HIPK2 gene polymorphism, chronic kidney disease, family-based study

INTRODUCTION

Most chronic renal disorders, regardless of etiology, finally develop certain irreversible degenerations, among them fibrosis. In the light of epidemiological evidence for high incidence of end-stage renal failure (ESRD), it is important to find an efficient key to inhibit or slow down its progression. In March 2012, breaking-news for nephrologists was published in *Nature Medicine* when an American-Chinese team of scientists team the significant influence of homeodomain-interacting protein kinase 2 (HIPK2) on the natural history of kidney fibrosis, which promised new directions for the therapy of ESRD patients [1]. The researchers ascertained higher HIPK2 expression both in a mice model of HIV-nephropathy (Tg26) and in the kidneys of patients with focal segmental glomerulosclerosis (FSGS), diabetic nephropathy, and IgA nephropathy. They claimed that initial factors, such as DNA-damage or oxidative stress, cause HIPK2 expression increase, activating many different signaling pathways which may result in kidney fibrosis.

Objective: To estimate the influence of two selected polymorphisms of HIPK2 gene on the occurrence and progression of chronic kidney disease (CKD). For this purpose, there were detected single nucleotide polimorphisms (SNPs) rs7456421 and rs 2030712 of gene HIPK2 (maped on chromosome 7, at 7q32-q34 according to Entrez Gene) in 109 family 'trios' consisting of an affected child with CKD and both his/her parents. The study was a continuation of a previously started scientific project aimed at determining genetic factors affecting nephropathy incidence and progression according to a family-based study. The protocol of the study was approved by the Ethics Committee of the Medical University of Silesia. All measurements were performed with the conscious agreement of the patients and their legal guardians (informed consent was obtained from parents if their children were under the age of 16, and both by parents and children at the age of 16-18 years).

Address for correspondence: Joanna Żywiec, Department of Internal Medicine, Diabetology and Nephrology, Medical University of Silesia, Katowice, 3-Maja 13, 41-800 Zabrze, Poland
E-mail: jzywiec@sum.edu.pl

Received: 5 July 2012; accepted: 13 December 2012

MATERIAL AND METHODS

The study was performed on a group of 109 patients with chronic kidney disease (48 females and 61 males, mean age 15.5 ±6.45 years) and 109 pairs of their biological parents. Chronic interstitial nephritis (CIN) was the reason conducive to CKD for most of the patients (72.5%); in 62 (78%) of them, the urinary tract defect was a contributing or underlying condition. Another 30 patients (27.5%) suffered from chronic glomerulonephritis (CGN). Renal biopsy had been performed in the past in 22% patients for diagnostic purposes. Forty-two patients (38%) suffered additionally from hypertension.

At the time of the study, the median glomerular filtration rate measured according to the Schwartz (for children), or respectively by MDRD (Modification of Diet in Renal Disease) formulas, was 28.2 ml/min. During the study, 51 (46.8%) patients were conservatively treated, whereas the others remained on chronic renal replacement therapy – 33 patients underwent continuous ambulatory peritoneal dialysis, 17 patients – haemodialysis, and 8 patients – kidney transplantation).

Based on patients' medical records (mean observation time period 7.1 (±5.7) years) and available serum creatinine concentration data, the progression of chronic kidney disease was estimated, and the patients subsequently divided into two groups:

Those with rapid CKD progression, 54 (49.5%) patients with replacement therapy onset within a 5-year observation period from stage 2 CKD diagnosis and/or with doubled serum creatinine concentration, where the index 1/serum creatinine concentration was below 0.3.

55 (50.5%) patients were categorised with slow CKD progression course.

The mean value of the first and the last documented serum creatinine concentration were 2.25 (±2.36) and 4.66 (±3.0) mg/dl, respectively.

All study measurements were made in the Laboratory of the Department of Internal Diseases, Diabetology and Nephrology in Zabrze (Medical University of Silesia). For all study participants, genomic DNA was isolated from peripheral blood leukocytes with a DNA isolation kit (Epicentre Technologies Corporation, Madison, Wisconsin, USA) in an own laboratory modification. Fluorsecence-labelled probes of a TaqMan Pre-designed SNP Genotyping Assay (Applied Biosystems Inc., Foster City California, USA), designed for rs7456421 and rs 2030712 SNPs measurements, were used for chosen polymorphisms genotyping. PCR and allele identifications were performed in a 7300 Real Time PCR of the Applied Biosystems Company.

After initial excluding as non-informative those families in which both parents were homozygotes, the Transmission Disequilibrium Test (TDT) was performed which estimates the allele transfer from biological parents to their affected offspring.

All statistical calculations were performed with Microsoft Office Excel 2003 and the Statistica 7 software package, with $p<0.05$ as a statistically significant value.

RESULTS

rs7456421 SNP of HIPK2 gene. The disposition of rs7456421 SNP genotype in the entire study group was as follow: 61.1% GG, 8.3% CC and 30.6% GC. There were differences in genotype distribution between subgroups with different CKD etiology (Tab. 1) but TDT did not revealed statistical significances of allele transfer in any cases (Tab. 3).

rs 2030712 SNP of HIPK2 gene. Table 2 presents the distribution of genotypes in the group of all patients and regarding etiology subgroups. For entire study group: CC 31.1%, TT 17.9% and CT 51%, and respectively for CGN i CIN subpopulations: CC 46.4% vs 25.6%, TT 7.2% vs 21.8% and CT 46.4% vs 52.6% (Tab. 2). Based on the TDT test results, no correlations were found between rs 2030712 SNP allele transfer and chronic kidney disease incidence (Tab. 4).

Table 1. Distribution of rs7456421 HIPK2 SNP genotype in study groups

	All patients	CGN	CIN	CIN without UTD	CIN with UTD
GG	61.1%	55.2%	63.3%	53%	66.1%
CC	8.3%	0	11.4%	23.5%	8.1%
GC	30.6%	44.8%	25.3%	23.5%	25.8%
Allele G	76.4%	77.6%	75.9%	64.7%	79%
Allele C	23.6%	22.4%	24.1%	35.3%	21%

Abbreviations:
CGN = chronic glomerulonephritis
CIN = chronic interstitial nephritis
CIN without UTD = chronic interstitial nephritis without urinary tract defect
CIN with UTD = chronic interstitial nephritis with urinary tract defect
TDT = Transmission Disequilibrium Test

Table 2. Distribution of rs2030712 HIPK2 SNP genotype in study groups

	All patients	CGN	CIN	CIN without UTD	CIN with UTD
CC	31.1%	46.4%	25.6%	25%	25.8%
TT	17.9%	7.2%	21.8%	31.25%	19.3%
CT	51%	46.4%	52.6%	43.75%	54.8%
Allele C	56.6%	69.6%	51.9%	46.9%	53.2%
Allele T	43.4%	30.4%	48.1%	53.1%	46.8%

Table 3. Frequency of rs7456421 HIPK2 SNP allele transmission in study groups (TDT results)

	Allele C transmitted observed/ expected		Allele G transmitted observed/ expected		chi^2	p
Groups ↓	Yes	No	Yes	No		
All patients	40/42	44/42	44/42	40/42	0.1905	0.6625
CGN	11/10.5	10/10.5	10/10.5	11/10.5	0.0476	0.8272
CIN	29/31.5	34/31.5	34/31.5	29/31.5	0.3968	0.5287
CIN without UTD	8/6	4/6	4/6	8/6	1.3333	0.2482
CIN with UTD	21/25.5	30/25.5	30/25.5	21/25.5	1.5882	0.2076

Table 4. Frequency of rs 2030712 HIPK2 SNP allele transmission in study groups (TDT results)

	Allele C transmitted observed/ expected		Allele T transmitted observed/ expected		chi^2	p
Groups ↓	Yes	No	Yes	No		
All patients	45/44.5	44/44.5	44/44.5	45/44.5	0.0112	0.9156
CGN	14/11	8/11	8/11	14/11	1.6364	0.2008
CIN	31/33.5	36/33.5	36/33.5	31/33.5	0.3731	0.5413
CIN without UTD	8/6.5	5/6.5	5/6.5	8/6.5	0.6923	0.4054
CIN with UTD	23/27	31/27	31/27	23/27	1.1852	0.2763

The results of multiple stepwise regression demonstrated the influence of CKD etiology, body mass index and CT genotype of rs 2030712 SNP on rapid progression of renal dysfunction. Although a history of chronic glomerulonephritis (odds ratio OR=17.3, p=0.000027) or chronic interstitial nephritis without urinary tract defect (OR=4.4, p=0.019) and CT genotype (OR=2.6, p=0.05) causes more rapid worsening of renal function, the body mass index was a protective factor (OR=0.86, p=0.033).

DISCUSSION

The nuclear serine/threonine kinases, which belong to the homeobox-interacting protein kinase (homeodomain-interacting protein kinase, HIPK) family, was indentified by Kim et al. in 1998 as a highly evolutionary conserved protein structure in vertebrates (more than 90% homology in the kinase domain and about 70% in homeobox-interacting domain) that influence on homebox proteins and other types of transcriptor factors as coactivatores or corepressors [2, 3]. It regulates by phosphorylation gene transcription during two fundamental biological processes: either embryonic differentation and development, or cellular response to DNA-damaging agents [3, 4, 5, 6, 7, 8]. During the last fifteen years, from among four HPIK family members (HIPK1, HIPK2, HIPK3 and HIPK4) the functional role of homeodomain-interacting protein kinase 2 (HIPK2) has been defined for the best. HIPK2, called in literature as a 'multi-talent partner for transcripton factors in DNA damage and development'[3], is involved in different pathophysiological pathways by modulating activity of several proteins, among them: tumour supressor protein p53 and its family members, p53 inhibitor MDM2, CtBP transcriptional co-repressor, bone morphogenetic protein (BMP), TGF-β and beta-catenin regulator axin [3, 4, 5, 9, 10, 11]. It also helps to control the hypoxic response, influences hematopoesis, vasculogenesis, transformation in the axial skeleton and differentiation of neuronal system [12, 13, 14, 15, 16, 17, 18, 19, 20]. There is strong evidence that HIPK2 expression presents a barier against oncogenic transformation and tumour development [21, 22, 23, 24, 25, 26].

The multipotential role of HIPK2 and direction for future research has been appropriately expressed by Calzado et al. in *Cell Cycle* in 2007 with the words:

How can a single kinase fulfil so many different functions? (…) The combined use of experimental approaches from cell biology, biochemistry and genetics will help to unravel further secrets of this ancient protein kinase [4].

The year 2012 revealed important news to renal pathophysiology when an multidisciplinary team from the USA and China identified homeodomain-interacting protein kinase 2 as a key regulator of kidney fibrosis [1]. A higher HIPK2 expression associated with tubulointerstitial injury or fibrosis and glomerulosclerosis were detected in human kidneys with HIV-associated nephropathy (HIVAN), focal segmental glomerulosclerosis (FSGS), diabetic nephropathy and severe IgA nephropathy. The HIPK2 concentration was also higher in the tubulointerstitium of other models of renal fibrosis, i.e. unilateral ureteral obstruction model and a folic acid-induced renal fibrosis model. Studies performed in a mouse model of kidney disease have identified HIPK2-mediated signaling pathways that lead to renal tubular epithelial cell (RTEC) apoptosis, which is a well known hallmark of tubulointerstinal injury. HIPK2 was also found as mediator of podocytes' de-differentiation and regulator of the NF-κB-inflammatory patchway conducive to tissue injury and fibrosis. As demonstrated by Jin et al., knocking out HIPK2 in mice with HIV-associated nephropathy model reduced proteinuria, podocyte hiperplasia and glomeruloclerosis, and led to significantly improved renal function. It was also revealed that reactive oxygen species (ROS) mediate HIV-induced HIPK2 expression and inhibition of ROS diminished kidney injury and fibrosis [1].

The relevance of all these findings gives hope for possible alternation in chronic kidney diseases' treatment, taking into account HIPK2 as a future therapeutic target. Based on this report, we expected interesting results of HIPK2 polymorphisms analysis in patients with chronic kidney disease. However, the presented family-based study performed on 109 patients with CKD secondary to chronic glomerulo- or interstitial nephritis did not demonstrate the influence of rs7456421 and rs 2030712 SNPs of HIPK2 gene on the incidence of renal dysfunction. While the findings of different genotype distribution in respective subgroups (Tab. 1-2) and results of multiple stepwise regression suggested some kind of its potential significance in chronic kidney diseases, it was supposed that the lack of relationship, apart from using TDT, may be due to the relatively small study group. When considering these suggestions, it would be more appropriate in the future to examine more patients.

CONCLUSIONS

On the basis of TDT results, no influence was identified of rs7456421 and rs 2030712 SNPs of HIPK2 gene on prevalence of chronic kidney disease.

The further studies are needed to ascertain the close connections of HIPK2 polymorphisms with CKD of different etiologies.

Acknowledgement

The study was supported by the Medical University of Silesia, Katowice, Poland (KNW-1-006/10).

REFERENCES

1. Jin Y, Ratnam K, Chuang PY, Fan Y, Hong Y, Dai Y, et al. A systems approach identifies HIPK2 as a key regulator of kidney fibrosis. Nat Med. 2012; 18(4): 580-588.
2. Kim YH, Choi CY, Lee SJ, Conti MA, Kim Y. Homeodomain-interacting protein kinases, a novel family of co-repressors for homeodomain transcription factors. J Biol Chem. 1998; 273(40): 25875-25879.
3. Rinaldo C, Prodosmo A, Siepi F, Soddu S. HIPK2: a multitalented partner for transcription factors in DNA damage response and development. Biochem Cell Biol. 2007; 85(4): 411-418.
4. Calzado MA, Renner F, Roscic A, Schmitz ML. HIPK2: a versatile switchboard regulating the transcription machinery and cell death. Cell Cycle. 2007; 6(2): 139-143.
5. Bitomsky N, Hofmann TG. Apoptosis and autophagy: Regulation of apoptosis by DNA damage signalling – roles of p53, p73 and HIPK2. FEBS J. 2009; 276(21): 6074-6083.
6. Iacovelli S, Ciuffini L, Lazzari C, Bracaglia G, Rinaldo C, Prodosmo A, et al. HIPK2 is involved in cell proliferation and its suppression promotes growth arrest independently of DNA damage. Cell Prolif. 2009; 42(3): 373-384.
7. Isono K, Nemoto K, Li Y, Takada Y, Suzuki R, Katsuki M, et al. Overlapping roles for homeodomain-interacting protein kinases

HIPK1 and HIPK2 in the mediation of cell growth in response to morphogenetic and genotoxic signals. Mol Cell Biol. 2006; 26(7): 2758-2771.
8. Pierantoni GM, Bulfone A, Pentimalli F, Fedele M, Iuliano R, Santoro M et al. The homeodomain-interacting protein kinase 2 gene is expressed late in embryogenesis and preferentially in retina, muscle, and neural tissues. Biochem Biophys Res Commun. 2002; 290(3): 942-947.
9. Hofmann TG, Möller A, Sirma H, Zentgraf H, Taya Y, Dröge W, et. al Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. Nat Cell Biol. 2002; 4(1): 1-10.
10. Sombroek D, Hofmann TG. How cells switch HIPK2 on and off. Cell Death Differ. 2009; 16(2): 187-194.
11. de la Vega L, Fröbius K, Moreno R, Calzado MA, Geng H, Schmitz ML. Control of nuclear HIPK2 localization and function by a SUMO interaction motif. Biochim Biophys Acta. 2011; 1813(2): 283-297.
12. Calzado MA, De La Vega L, Munoz E, Schmitz ML. From top to bottom: the two faces of HIPK2 for regulation of the hypoxic response. Cell Cycle. 2009; 8(11):1659-1664.
13. Hailemariam K, Iwasaki K, Huang BW, Sakamoto K, Tsuji Y. Transcriptional regulation of ferritin and antioxidant genes by HIPK2 under genotoxic stress. J Cell Sci. 2010; 123(22): 3863-3871.
14. Nardinocchi L, Puca R, D'Orazi G. HIF-1α antagonizes p53-mediated apoptosis by triggering HIPK2 degradation. Aging (Albany NY). 2011; 3(1): 33-43.
15. Chalazonitis A, Tang AA, Shang Y, Pham TD, Hsieh I, Setlik W, et al. Homeodomain interacting protein kinase 2 regulates postnatal development of enteric dopaminergic neurons and glia via BMP signaling. J Neurosci. 2011; 31(39): 13746-137457.
16. Zhang J, Pho V, Bonasera SJ, Holtzman J, Tang AT, Hellmuth J et al. Essential function of HIPK2 in TGFbeta-dependent survival of midbrain dopamine neurons. Nat Neurosci. 2007; 10(1): 77-86.
17. Doxakis E, Huang EJ, Davies AM. Homeodomain-interacting protein kinase-2 regulates apoptosis in developing sensory and sympathetic neurons. Curr Biol. 2004; 14(19): 1761-1765.
18. Aikawa Y, Nguyen LA, Isono K, Takakura N, Tagata Y, Schmitz ML et al. Roles of HIPK1 and HIPK2 in AML1- and p300-dependent transcription, hematopoiesis and blood vessel formation. EMBO J. 2006; 25(17): 3955-3965.
19. Ohtsu N, Nobuhisa I, Mochita M, Taga T. Inhibitory effects of homeodomain-interacting protein kinase 2 on the aorta-gonad-mesonephros hematopoiesis. Exp Cell Res. 2007; 313(1): 88-97.
20. Puca R, Nardinocchi L, D'Orazi G. Regulation of vascular endothelial growth factor expression by homeodomain-interacting protein kinase-2. J Exp Clin Cancer Res. 2008; 27: 22.
21. Nardinocchi L, Puca R, Givol D, D'Orazi G. HIPK2-a therapeutical target to be (re)activated for tumor suppression: role in p53 activation and HIF-1α inhibition. Cell Cycle. 2010; 9(7): 1270-1275.
22. Puca R, Nardinocchi L, Givol D, D'Orazi G. Regulation of p53 activity by HIPK2: molecular mechanisms and therapeutical implications in human cancer cells. Oncogene. 2010; 29(31): 4378-4387.
23. Wei G, Ku S, Ma GK, Saito S, Tang AA, Zhang J et al. HIPK2 represses beta-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis. Proc Natl Acad Sci U S A. 2007; 104(32): 13040-13045.
24. Cao Z, Yoon JH, Nam SW, Lee JY, Park WS. Mutational analysis of the HIPK2 gene in keratoacanthoma and squamous cell carcinoma of the skin. APMIS. 2011; 119(6): 399-401.
25. Krieghoff-Henning E, Hofmann TG. HIPK2 and cancer cell resistance to therapy. Future Oncol. 2008; 4(6): 751-754.
26. Nardinocchi L, Puca R, Sacchi A, D'Orazi G. HIPK2 knock-down compromises tumor cell efficiency to repair damaged DNA. Biochem Biophys Res Commun. 2007; 361(1): 249-255.