Slota Diana, Wasilewski Tadeusz Paweł, Goniewicz Mariusz, Długoborska Klaudia. The expression level of the CLDN14 gene in patients with urolithiasis - a preliminary report. Journal of Education, Health and Sport. 2019;9(2):145-155. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2560054
http://ojs.ukw.edu.pl/index.php/johs/article/view/6571

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 25.01.2019. Revised: 30.01.2019. Accepted: 08.02.2019.

The expression level of the CLDN14 gene in patients with urolithiasis - a preliminary report

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Summary

Aim: An attempt to assess the expression of the CLDN14 gene at the mRNA level in patients diagnosed with urolithiasis in relation to healthy persons constituting a comparative group.

Material and methods: The material for examinations was peripheral blood from 23 patients, including 12 women and 11 men with diagnosed kidney stones disease and 6 donors (control group), including 2 women and 4 men who did not have kidney stones.

Results and conclusions: The studies showed differences in the level of CLDN14 gene expression in both the examined and the comparative group.

Key words: CLDN14, gene expression, urolithiasis

Introduction

In recent years, the role of genetic factors in the emergence of numerous diseases is underlined. The etiopathogenesis of kidney stones disease depends on many factors, including: place of residence, diet, lifestyle, racial affiliation and even gender.

Studies carried out so far prove that the incidence of renal stones is also associated with genetic factors. Scientific publications, among others, inform about the polymorphism of the CLDN14 gene (claudin14) encoding the clavivin protein, which is important in predisposition to nephrolithiasis. Getting to know all the genetic variants of this gene that affect urolithiasis may be a way to solve many problems related to it in the future [1].

In one of the studies conducted in 2009 in Reykjavik, the effect of the CLDN14 gene variant on the occurrence of nephrolithiasis was discovered. The results of these studies conducted on a group of over 40,000 patients suggested the need for further research in this respect [2].

Our studies show the level of expression of the CLDN14 gene in the group of patients with urolithiasis, as well as in the group of healthy people. Analysis of the expression of this gene is intended to assess whether there is a relationship between overexpression / reduced expression of CLDN14 and the incidence of patients with nephrolithiasis. In the future, such assessment could be used as a predisposing and diagnostic factor for many patients, for which reasons for kidney stones are found in genetic factors.

No research results have been documented in Poland on the association of CLDN14 gene expression with urolithiasis.

Claudins were first described in 1998 by Japanese scientists Mikio Furuse and Schoichiro Tsukita from the University of Kyoto. Their name comes from the Latin word "claudere" meaning "closure".

Claudins are a family of proteins which, along with occludin, are the most important components of the tight junctions. Tight junctions establish the paracellular barrier that controls the flow of molecules in the intercellular space between the cells of an epithelium. They have four transmembrane domains, with the N-terminus and the C-terminus in the cytoplasm [5].

Claudins are small (20–27 kilodalton (kDa)) transmembrane proteins which are found in many organisms, ranging from nematodes to human beings, and are very similar in their structure [3, 4].

Claudins span the cellular membrane 4 times, with the N-terminal end and the C-terminal end both located in the cytoplasm, and two extracellular loops which show the highest degree of conservation. The first extracellular loop consists on average of 53 amino acids and the second one, being slightly smaller, of 24 amino acids. The N-terminal end is usually very short (4–10 amino acids), the C-terminal end varies in length from 21 to 63 and is necessary for the localisation of these proteins in the tight junctions [5].

These are proteins that, thanks to adhesion, close intercellular spaces creating close connections between neighboring cells, oclaudin, tricellulin and so-called JAMM (Junctional adhesion molecules). They also function in the regulation of intercellular permeability [6,7].

An example may be claudin-16 (formerly known as paracelin-1), which participates in the resorption of magnesium ions in the kidneys [8].

Numerous studies confirm the impact of claudins defects on the formation of hereditary diseases, eg mutation of the CLDN16 gene leads to familial hypomagnesaemia, while the mutation in the CLDN1 gene leads to sclerosing cholangitis in newborns [9].

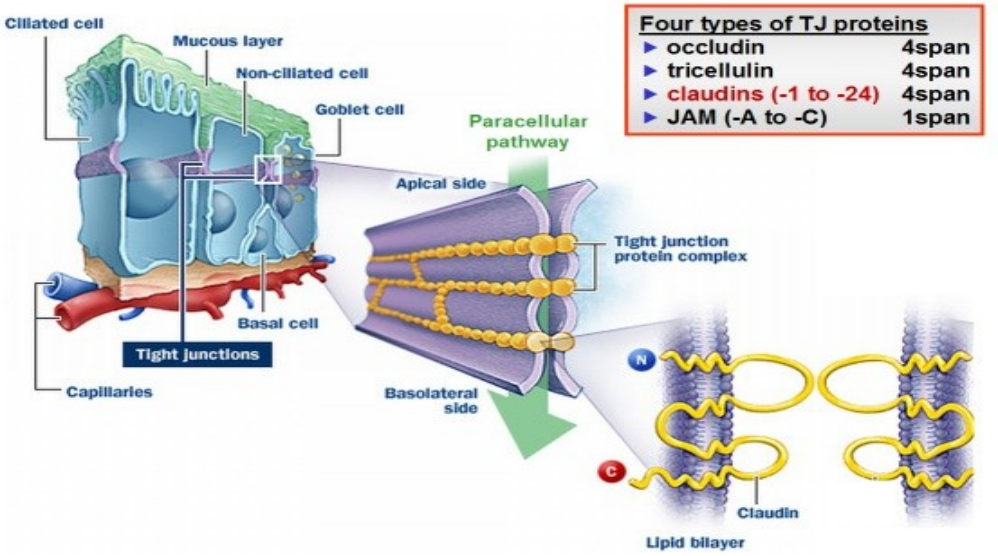

Figure 1. The function of claudines
[http://schmieder.fmp-berlin.info/research/tight_junctions.html]

The CLDN14 gene is found in the long arm of chromosome 21 and codes the clavine-14 protein.

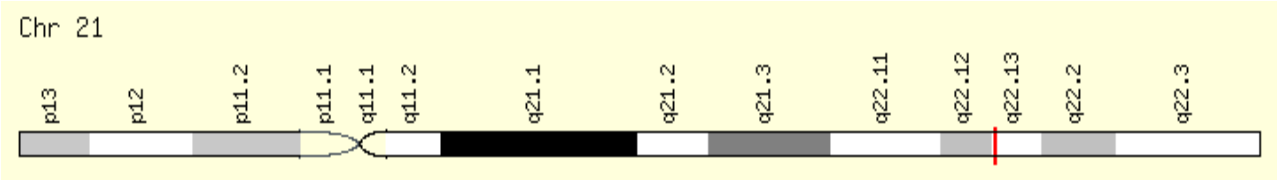

Figure 2. Localization of the CLDN14 gene on chromosome 21

Claudin-14 is found in the cells of many organs, eg in the intestines, the inner ear of liver tissue and kidneys. In the inner ear it probably plays an essential role in the processing of sound waves to nerve impulses. It has been shown that CLDN14 gene mutation causes perceptual deafness called a sensorineural or sensory deafness in a different way, i.e. a form of hearing loss related to the auditory nerve or auditory pathway that leads impulses to the cerebral cortex [http://ghr.nlm.nih.gov/gene/CLDN14; 9]

Baker et al. [9] published an article about the role of claudin-14 in tumor angiogenesisin mice. It turned out that the removal of a single copy of this gene leads to a defect of tight intercellular connections and leakage of blood vessels, promoting tumor formation.

Thorleifsson et al. [2] demonstrated the correlation of CLDN14 gene mutation with urolithiasis. In connection with the above, it was decided to carry out a study of the expression of this gene in the population of patients with diagnosed kidney stones in the Lublin province.

Aim

The aim of the study was an attempt to evaluate the expression of the CLDN14 gene at the mRNA level in patients who have or have been diagnosed with renal calculi in the past or in relation to healthy persons constituting a comparative group.

Material and methods

The consent of the Bioethics Commission of the Medical University in Lublin was obtained for conducting this project. All participants in the study were informed about their purpose and expressed their written consent to carry them out. The study material was peripheral blood from 23 patients, including 12 women and 11 men between the ages of 21 and 67 years with diagnosed kidney stones and 6 donors (control group), including 2 women and 4 men from 24 up to 48 years of age with no kidney stones. In the group of patients suffering from kidney stones, the duration of the disease ranged from one year to 20 years.

Table 1 presents information on the examined group (age, sex, place of residence, other people in the family suffering from urolithiasis, co-morbidities and duration of kidney stones) as well as high level of CLDN gene expression marked with pink. Table 2 shows the proportionality of the comparative group in a commensurate manner.

The following methods were used successively: isolation of peripheral blood lymphocytes, isolation of total RNA from peripheral blood lymphocytes, quantitative and qualitative RNA analysis, cDNA synthesis in reverse transcription reaction (RT-PCR), gene expression analysis using the Real-time PCR method.

The collected results were analyzed further in the Expression Suite (Life Technologies) to establish the relative expression (RQ) of the CLDN14 gene in the test group and the control group against endogenous GAPDH control including the internal calibrator. The calibrator was the median of the ΔCT values of the CLDN14 gene of the comparative group lymphocytes.
The expression level was calculated according to the formula RQ = 2- ΔΔCT, where Δ ΔCT = Δ CT of the gene - Δ CT of the calibrator.

Table 1. Information on the group under study (the color was marked with people with high gene expression CLDN14)

no.	sample number	gender	age	place of residence	people suffering from urolithiasis in the family	comorbidities	the length of the duration of urolithiasis
1	**K1**	F	40	city<100000	-	neurological diseases, respiratory system diseases	6-10 years
2	**K2**	M	58	city >100000	-	neurological diseases,	1-5 years
3	**K3**	F	24	city >100000	mother	respiratory system diseases	11-20 years
4	**K4**	M	58	city<100000	mother	hypertension	1-5 years
5	**K5**	F	44	village	-	neurologiczne, overweight, respiratory system diseases	11-20 years
6	**K6**	M	30	city<100tys	grandparents	-	6-10 years
7	**K7**	M	58	city >100tys	mother	overweight	11-20 years
8	**K9**	F	55	city >100tys	father	overweight	11-20 years
9	**K10**	M	34	city>100tys	-	-	6-10 years
10	**K11**	M	54	city >100tys	mother	-	11-20 years
11	**K12**	F	62	city<100tys	father	hypertension, neurological diseases, diabetes	1-5 years
12	**K13**	M	62	village	mother	hypertension, neurological diseases	1-5 years
13	**K14**	F	67	village	-	hypertension, neurological diseases	11-20 years
14	**K15**	M	74	city>100tys	father, grandparents	hypertension, neurological diseases,	1-5 years
15	**K16**	M	54	city<100tys	-	neurological diseases, overweight, hypertension	6-10 years
16	**K17**	M	50	village	-	cancer, neurological diseases	6-10 years
17	**K18**	F	50	city>100tys	siblings	hypertension	11-20 years
18	**K19**	F	22	city<100tys	mother, father	overweight	1-5 years
19	**K20**	F	21	city<100tys	father, grandparents	respiratory system diseases	1-5 years
20	**K21**	F	82	city>100tys	-	hypertension	11-20 years
21	**K22**	F	41	city >100tys	father	-	11-20 years
22	**K23**	M	60	village	mother	hypertension	11-20 years
23	**K24**	F	24	village	grandparent	-	1-5 years

Table 2. Information on the comparison group (people with high CLDN14 expression were marked with the color)

no.	gender	age	place of residence	people suffering from urolithiasis in the family	comorbidities	the length of the duration of urolithiasis
PK1	F	25	city < 100000	-	overweight	0
PK2	M	27	city< 100000	grandparents	-	0
PK3	M	25	village	grandparents	overweight	0
PK4	F	24	city < 100000	-	-	0
PK5	M	28	city >100000	Mother, father, grandparents	-	0
PK6	M	48	village	daughter, wife	hypertension	0

Results

The obtained results showed a different level of expression of the CLDN14 gene at the mRNA level in both the test and the comparison group (Fig. 3, 4). Expression of CDLN14 mRNA in six patients was at the level of the mean, comparative group. In ten patients, the expression was at a level much lower than the average of the comparative group. Particularly noteworthy are the obtained results of six subjects (five women and one man) at different ages (from 21 to 82 years), in whom CDLN14 mRNA expression was extremely high (Fig. 4). The results were analyzed in comparison to clinical symptoms other than urolithiasis. The cells linking the subjects with the overexpression of the CLDN14 gene seem to be genetic strain in the family and the duration of the disease. Analysis of the test group and comparative people with elevated CLDN14 gene expression showed that in the family history there is kidney stones in almost all individuals (the 82-year-old person did not remember the history of his family's disease). It seems that overexpression of the CLDN14 gene may depend on the duration of the disease (duration less than 5 years or over 11 years). An analysis of the clinical results and symptoms of patients in whom the expression was lower than the average in the control group was also performed. No relevant links found.

Table 3. List of the research and comparison group (green color) of people with high expression of the CLDN14 gene

no.	gender	age	place of residence	people suffering from urolithiasis in the family	comorbidities	the length of the duration of urolithiasis
K3	F	24	city >100000	mother	respiratory system diseases	11-20 years
K4	M	58	city<100000	mother	hypertension	1-5 years
K19	F	22	city<100000	mother, father	overweight	1-5 years
K20	F	21	city<100000	Father, grandparents	respiratory system diseases	1-5 years
K21	F	82	city>100000	-	hipertension, overweight, neurological disease, respiratory system diseases	11-20 years
K22	F	41	city >100000	father	-	11-20 years
PK3	M	25	village	grandparents	-	0
PK5	M	28	city >100000	mother, father, grandparents	-	0

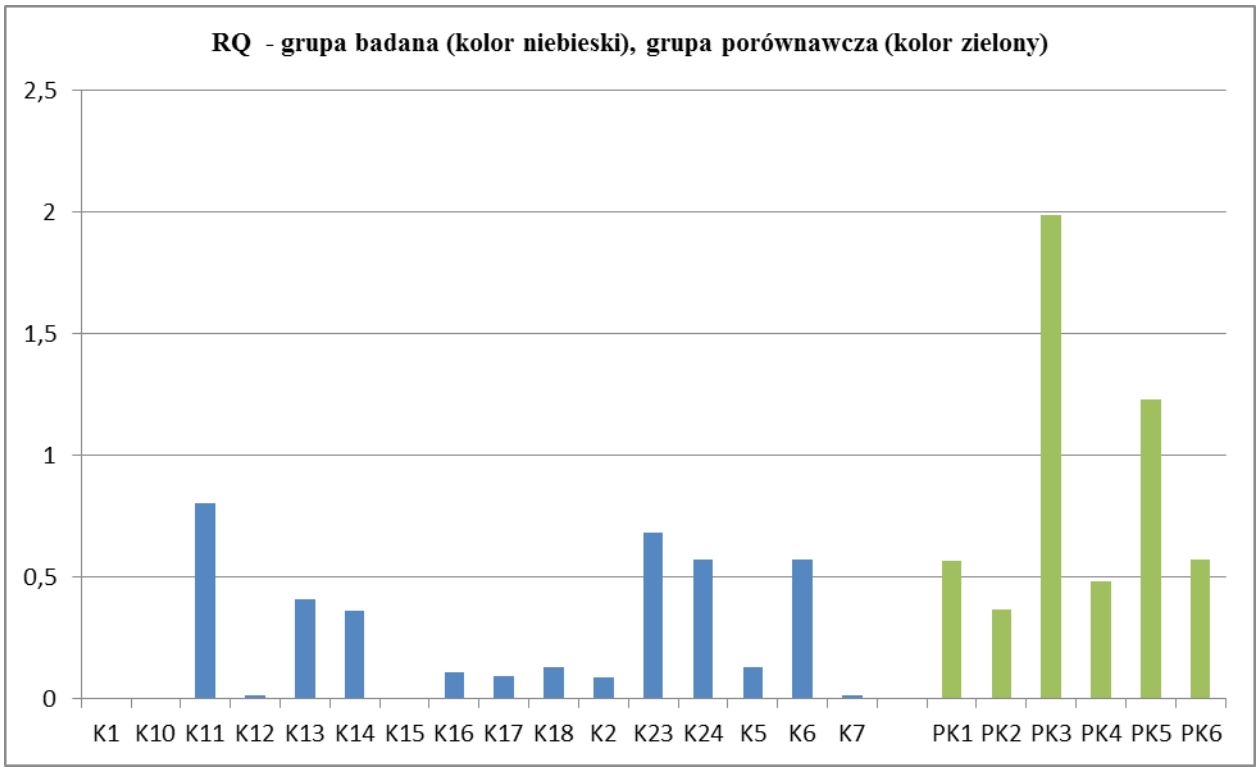

Figure 3. Comparison of CLDN14 gene expression in the stud group and comparison groups

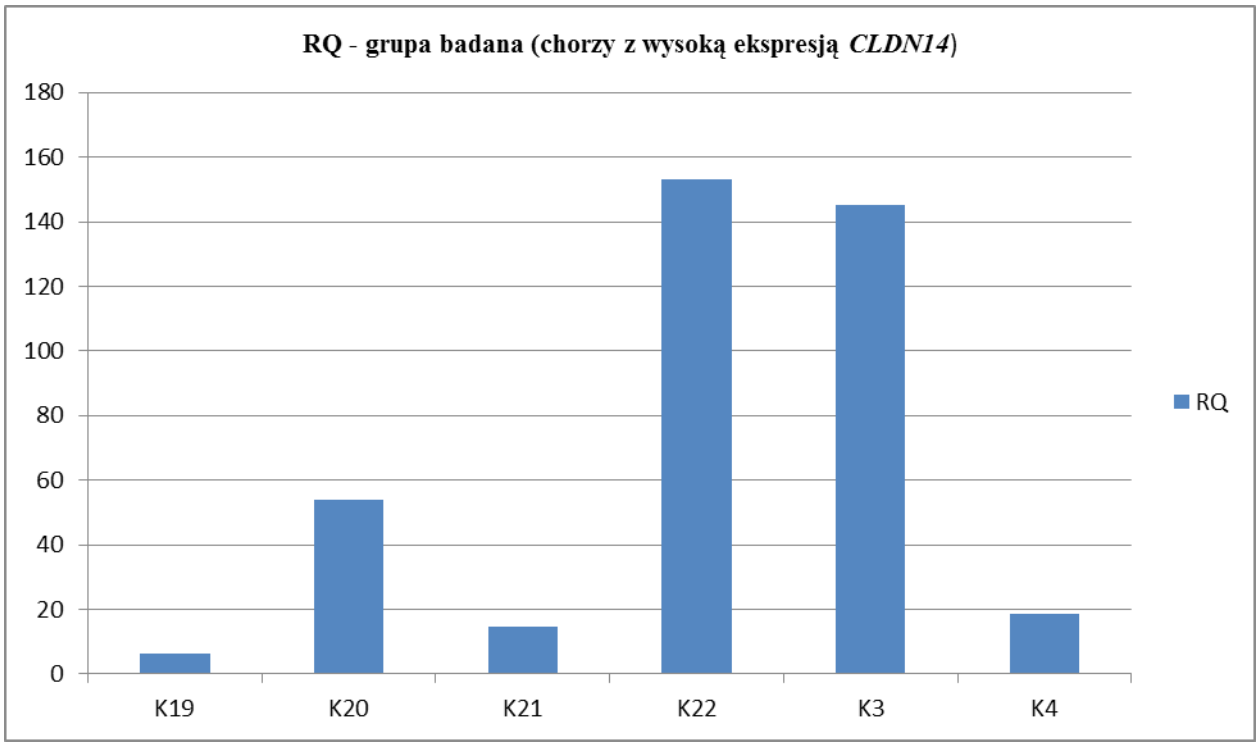

Figure 4. Selected patients with high expression of the CLDN14 gene

Discussion

Obtained results certainly provide an encouraging introduction to further research on the expression of the CLDN14 gene on a much larger population of patients. Our research included only 23 patients, which compared to the 40,000 examined by Thorleifsson et al. [2] is only a small percentage.

Therefore, no constructive conclusions can be drawn regarding the causal relationship: overexpression / decreased CLDN14 gene expression and nephrolithiasis. Undoubtedly, an important complement to the research would be the extension of the comparison group and observation of these donors of genetic material, in which a somewhat higher expression of the CLDN14 gene has been shown in comparison with the mean. It can not be ruled out that increased expression in PK3 and PK5 donors (Fig. 3) is a harbinger of currently unknown lesions. Especially that from the interview it appears that these families are burdened with kidney stones. Therefore, it would be important to monitor these people (PK3 and PK5) for the appearance of renal stones in the future.

Conclusions

1. Overexpression / decreased expression of the CLDN14 gene is not related to the patient's age.
2. Overexpression / reduced expression of the CLDN14 gene is not related to the patient's place of residence.
3. Overexpression / decreased expression of the CLDN14 gene is not associated with co-morbid diseases.
4. It seems that over-expression of the CLDN14 gene occurs more often in women than in men.
5. It seems that increased expression of the CLDN14 gene may have a family genetic basis.

References

1. Hewitt K.J., Agarwal R., Morin P.J.: The claudin gene family: expression in normal and neoplastic tissues. BMC Cancer, 2006, 6 (186)
2. Thorleifsson G. i współ.: Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. Nature Genetics, 2009, 41 (8).
3. Furuse M., Sasaki H., Fujimoto K., Tsukita S.: A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. J. Cell. Biol., 1998, 4.
4. Yamazaki Y., Tokumasua R., Kimurab H., Tsukitaa S.: Role of claudin species–specific dynamics in reconstitution and remodeling of the zonula occludens. Mol. Biol. Cell., 2011, 22 (9).
5. Rüffer C., Gerke V.: The C-terminal cytoplasmic tail of claudins 1 and 5 but not its PDZ-binding motif is required for apical localization at epithelial and endothelial tight junctions. Eur. J. Cell Biol., 2004, 83 (4).
6. Angelow S., Ahlstrom R., Yu A.S.L.: *Biology of cladins*, American Journal of Physiology Renal Physiology, 2008, 295 (4).
7. Fromm M., Schulzke J.D.: Molecular structure and function of the tight junction. Ann. N.Y. Acad. Sci., 2009, 165.
8. Simon D.B., Lu Y., Choate K.A.: Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. Science. 1999, 285.
9. Haj-Rabia S., Baala L., Vabres P., Hamel-Teillac D., Jacquemin E., Fabre M., Lyonnet S., De Prost Y., Munnich A., Hadchouel M.: Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology, 2004, 127.

10. Baker M., Reynolds L.E., Robinson S.D., Lees D.M., Parsons M., Elia G.: Stromal Claudin14-Heterozygosity, but Not Deletion, Increases Tumour Blood Leakage without Affecting Tumour Growth. PLoS ONE, 8 (5).

http://www.genecards.org,

http://schmieder.fmp-berlin.info/research/tight_junctions.htm

http://ghr.nlm.nih.gov/gene/CLDN14

https://www.ncbi.nlm.nih.gov/gene/23562