УДК 616.613-053.2:575:616.61-07

ROLE OF IMMUNOGENETIC FACTORS IN EARLY DIAGNOSIS OF UROLITHIASIS IN CHILDREN

Sh. A. Yusupov, L.R. Khakimova

Samarkand State Medical University, Samarkand, Uzbekistan

РОЛЬ ИММУНОГЕНЕТИЧЕСКИХ ФАКТОРОВ В РАННЕЙ ДИАГНОСТИКЕ МОЧЕКАМЕННОЙ БОЛЕЗНИ У ДЕТЕЙ

Ш. А. Юсупов, Л. Р. Хакимова

Самаркандский государственный медицинский университет, Самарканд, Узбекистан

Abstract. In recent years, one of the main aspects of studying the problem of urolithiasis in the world is the search for genetic risk factors for the development of urolithiasis: identification of its association with polymorphic variants of a particular gene. **Objective.** To study the association of interleukin-1β and interleukin-18 genes with the development of urolithiasis in children. **Materials and methods**. Clinical examination of 100 children with urolithiasis and 100 practically healthy children was carried out, as well as collection and analysis of statistical data on urolithiasis in the group of children of different age groups to identify sex, age, family frequency of urolithiasis, peculiarities of lifestyle and nutrition, as well as seasonality of this pathological process. Conducting ultrasound and radiological examination of urinary organs of patients. The selection of patients for the study of gene polymorphism was carried out on the basis of the diagnosis made in the clinic and written consent of the proband. Blood samples were collected from patients with USD (100 samples) and a control group of practically healthy children (100 samples). The results obtained in this study indicate that the IL-1β gene contributes to the determination of disorders contributing to the development of USD in children. In particular, in children of Uzbek population genetic markers of predisposition to USD are genotype CC of IL-1β gene. It is considered reasonable to include testing of this genotype in a comprehensive USD prevention programme in Uzbekistan. Conclusion. The results obtained in this study indicate that the genetic variant CC genotype of IL-1β gene affects manifestation, duration of course, recurrences and clinical and functional features of the course of urolithiasis and can be used for early diagnosis and early diagnosis of the disease in children of Uzbek population.

Key words: *urolithiasis in children, urolithiasis, gene polymorphism, genetics, prediction of urolithiasis.*

Аннотация. В последние годы одним из основных аспектов изучения проблемы мочекаменной болезни в мире является поиск генетических факторов риска развития мочекаменной болезни: выявление его ассоциации с полиморфными вариантами того или иного гена. Цель. Изучить ассоциацию генов интерлейкина-1β и интерлейкина-18 с развитием мочекаменной болезни у детей. Материалы и методы. Проведено клиническое обследование 100 детей с мочекаменной болезнью и 100 практически здоровых детей, а также сбор и анализ статистических данных по мочекаменной болезни в группе детей разных возрастных групп для выявления пола, возраста, семейной частоты мочекаменной болезни, особенностей образа жизни и питания, а также сезонности данного патологического процесса. Проведение ультразвукового и рентгенологического исследования органов мочевыделения пациентов. Отбор пациентов для исследования полиморфизма генов осуществлялся на основании диагноза, поставленного в клинике, и письменного согласия пробанда. Образцы крови были собраны у пациентов с УСД (100 образцов) и контрольной группы практически здоровых детей (100 образцов). Результаты, полученные в ходе данного исследования, свидетельствуют о том, что ген IL-1β вносит вклад в определение нарушений, способствующих развитию УСД у детей. В частности, у детей узбекской популяции генетическими маркерами предрасположенности к УСД являются генотип CC гена IL-1β. Считается целесообразным включение тестирования данного генотипа в комплексную программу профилактики УСД в Узбекистане. Заключение. Результаты, полученные в ходе данного исследования, свидетельствуют о том, что генетический вариант генотипа CC гена IL-1β влияет на манифестацию, длительность течения, рецидивы и клинико-функциональные особенности течения мочекаменной болезни и может быть использован для ранней диагностики и раннего выявления заболевания у детей узбекской популяции.

Ключевые слова: *мочекаменная болезнь у детей, уролитиаз, полиморфизм генов, генетика, прогнозирование мочекаменной болезни.*

For citation: Shukhrat A. Yusupov., Leyla R. Khakimova. ROLE OF IMMUNOGENETIC FACTORS IN EARLY DIAGNOSIS OF UROLITHIASIS IN CHILDREN. INNOVATIVE SURGERY ON THE SILK ROAD. 2024. 1.

Relevance. The prevalence of urolithiasis has recently tended to increase. Being a metabolic disease, in world practice there are cases of urolithiasis even in infants. Taking into account multifactoriality of the disease, in recent years, one of the main aspects of studying the problem of urolithiasis in the world is the search for genetic risk factors for the development of urolithiasis: identification of its association with polymorphic variants of a particular gene [2, 4, 9]. The realisation of hereditary predisposition to urolithiasis is associated with genetically determined structural and functional features of metabolism, neurohumoral regulation, and local factors [6, 10]. In their epidemiological or clinical studies, foreign scientists note the participation of genetic factors in the occurrence of urolithiasis, which suggests the existence of specific genes responsible for the occurrence of urinary stone disease (USD) [1, 3, 11].

The balance between the production, expression, and inhibition of IL-1 family protein synthesis plays one of the key roles in the development, regulation, and outcome of the inflammatory process [7]. IL-18 is considered as one of the key factors of the body's anti-infective defence. In addition, there is evidence that IL-18 in some cases can act as a pathogenetic factor in the formation of some diseases accompanied by acute and chronic inflammation [8].

Studying the association of genes with the development of urolithiasis in children and identifying their polymorphisms will contribute to the early diagnosis and early diagnosis of urolithiasis in children. In cases of already existing disease, the study of the association of molecular genetic markers with recurrent forms of urolithiasis, as well as the establishment of pharmacogenetic interactions will contribute to a more effective postoperative metaphylaxis of urolithiasis. Reduction in the incidence of urolithiasis due to early effective detection of predisposition to it, as well as more effective postoperative metaphylaxis of urolithiasis will lead to a significant reduction in material costs for the organisation and implementation of treatment measures [5].

Research objectives: to investigate the association of interleukin-1β and interleukin-18 genes with the development of USD in children

Materials and methods. In order to fulfil the set objectives, we conducted a clinical examination of 100 children with USD and 100 practically healthy children, as well as collection and analysis of statistical data on urolithiasis in the group of children of different age groups to identify sex, age, family frequency of urolithiasis, lifestyle and nutrition peculiarities, as well as seasonality of this pathological process. Conducting ultrasound and radiological examination of urinary organs of patients.

The selection of patients for the study of gene polymorphism was carried out on the basis of the diagnosis made in the clinic and written consent of the proband. Blood samples were collected from patients with USD (100 samples) and the control group of practically healthy children (100 samples). Venous blood in the amount of 1 ml was collected in 0.5 ml of sodium citrate solution and stored at -20°C.

The material was collected at the Samarkand branch of children's surgery of the Republican Specialised Scientific and Practical Medical Centre for Paediatrics.

Table 1 presents data on the age distribution of patients in the groups under consideration.

Table 1

Age distribution of patients with USD and healthy children

Age	Control group (n = 100)		Main group (n = 100)		Total (n = 200)	
	Abs	%	Abs	%	Abs	%
0-3 year	17	17	31	31	48	24
4-11 year	43	43	43	43	86	43
12-17 year	46	46	26	26	72	36

Table 1 shows that school-age children prevailed among the patients - 69 (69%). This is due to the fact that it is at this age that metabolic disorders associated with the transition of children to general nutrition, violation of drinking regime, etc. are most often manifested, while in the younger group nutrition remains relatively rational and metabolic changes are manifested to a lesser extent. Table 2 shows the distribution of patients in both groups according to sex.

Table 2

Distribution of patients depending on sex

Nosology	Control group (n = 100)		Main group (n = 100)		Total (n = 200)	
	Abs	%	Abs	%	Abs	%
Girl	3	3	32	32	35	17.5
Boy	97	97	68	68	165	82.5

The data presented in Table 2 show that according to the sex distribution of patients, USD is more common among boys - 68 (68%) children than among girls - 32 (32%).

The most characteristic complaints of patients with urolithiasis were pain in the lumbar region, increased body temperature, haematuria, urine turbidity, increased or decreased frequency of urination. In some cases, acute urinary retention and spontaneous discharge of concrements were noted. In cases of dislocation of concrements in the lower third of the ureter, pain was localised in the iliac region on the corresponding side. Headache, poor appetite, drowsiness were characteristic symptoms for patients with renal failure.

Pain syndrome on admission was noted in 89 (89%) patients. At that, a pronounced pain syndrome - renal colic was observed in 15 (15%) patients. The localization and character of the pain syndrome depended on the age of the child, the presence of combined concrements in the urinary tract. Abdominal pain was characteristic in patients of the younger age group. Older patients more often complained of pain in the lumbar region, sometimes indicating pain irradiation along the course of the ureter.

In 3 (4.5%) children with a concrement in the lower third of the right ureter, pain syndrome was the reason for an unjustified appendectomy, which was performed at the place of residence. Ureterolithotomy in these patients was associated with certain technical difficulties due to the presence of adhesions.

Dysuric phenomena were noted in 28 (28%) patients and were most often characterised by frequent, painful urination. Acute urinary retention was noted in 5 (5%) patients. Dysuric phenomena in younger patients were almost 2 times more frequent. Acute urinary retention in all patients was resolved by insertion of Ad'mer catheter, and spontaneous detachment of the concrement was noted in 3 (3%) patients after catheter removal.

The localisation of the concrements revealed the following: kidney stones in 43 patients (right - 21, left - 13, both sides - 9); ureteral stones in 12 patients (upper one-third ureter - 1, mid-urethra - 1, lower urethra - 10); bladder stones - 9 patients (1 of them recurrent bladder stone); urethral hanging stone in 4 patients; multilocular urolithiasis in 10 patients. The combination of urolithiasis with developmental anomalies of the urinary system was detected in 22 children.

Molecular genetic studies were carried out in the laboratory of genomics at the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan (AS RUz). DNA extraction from whole blood was performed using Diatom™ DNA Prep 200 reagent kit (Izogen Laboratory, Moscow, Russia). Primers were designed and restrictionases were selected for polymorphisms of interleukin-1β (IL-1 β) and interleukin-18 (IL-18) genes.

Statistical processing of the study results was carried out using online calculator openepi. Conformity of the observed genotype frequency distribution of the studied genes in the control group, theoretically expected by Hardy-Weinberg equilibrium, was evaluated by the χ^2 criterion. The calculation was performed using an online calculator: http:// www.oege.org/software/hwe-mr-calc.shtml.

Results. The study of IL-1 gene polymorphism showed its association with the development of urolithiasis in children. The frequency of IL-1 β genotypes is presented in Figure 1.

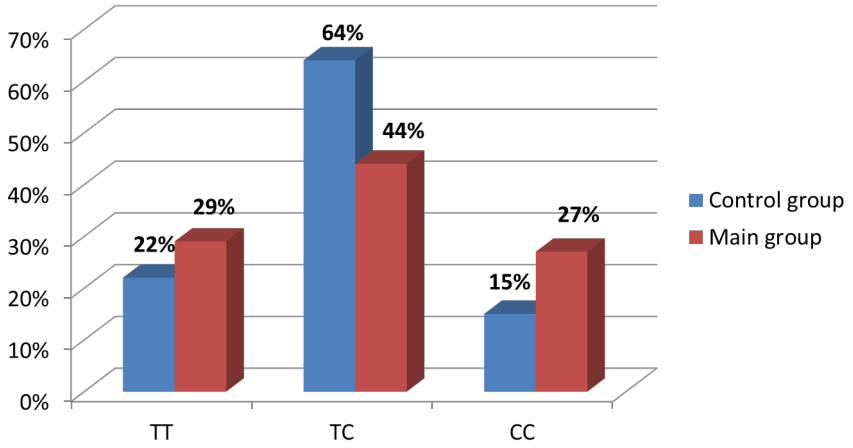

Fig. 1. Distribution of IL-1 gene genotype frequencies in the control group and in patients with urolithiasis

As shown in the chart, the prevalence of frequencies of TT, TS and SS genotypes in children with USD were 29%, 44% and 27%, respectively, whereas in the control group 22%, 64% and 15%, respectively.

The distribution frequencies of the CC heterozygote genotype were 27% and 15% in the control group, respectively (χ^2 =0.47; p=0.24; OR=1.35; 95% CI 0.57-3.17; df=1). Detection of the C allele increased the risk of USD in children 1.4-fold, compared with the presence of the T allele (95% CI = 0.68-2.93; df=1).

The analysis of IL-1 gene genotype frequencies showed that the distribution of the analysed genotypes in our population corresponds to the Hardy-Weinberg distribution (χ^2=6.77; p=0.03) (Table 3).

Table 3

General inheritance model (χ^2 test, df=2)

Genotypes	Case	Control	χ^2	p	OR	
	n=100	n=94			Sign.	95% CI
Genotype C/C	0.410	0.585	6.77	0.03	0.49	0.28-0.87
Genotype C/T	0.490	0.372			1.62	0.91-2.87
Genotype T/T	0.100	0.043			2.50	0.76-8.27

Analysis of the dominant inheritance frequency showed that the frequency of the Hardy-Weinberg distribution genotype distribution in the group of patients has no statistically significant deviation between the common and dominant inheritance model (Table 4).

Table 4

Dominant inheritance model (χ^2 test, df=2)

Genotypes	Case	Control	χ^2	p	OR	
	n=100	n=94			Sign.	95% CI
Genotype C/C	0.410	0.585	5.94	0.01	0.49	0.28-0.87
Genotype C/T+T/T	0.590	0.415			2.03	1.15-3.60

Comparative analysis of the genotype frequency distribution of the ±105A/C polymorphism of the IL-18 gene did not reveal any significant differences between the group of patients and the control group (Fig. 2), whereas CC in sick children was 2 times more frequent and had a significant deviation (p<0.05). As can be seen from the diagram, the prevalence of frequencies of AA, AC and CC genotypes in children suffering from USD was 52%, 43% and 5%, respectively, whereas in the control group it was 57%, 41% and 2%, respectively.

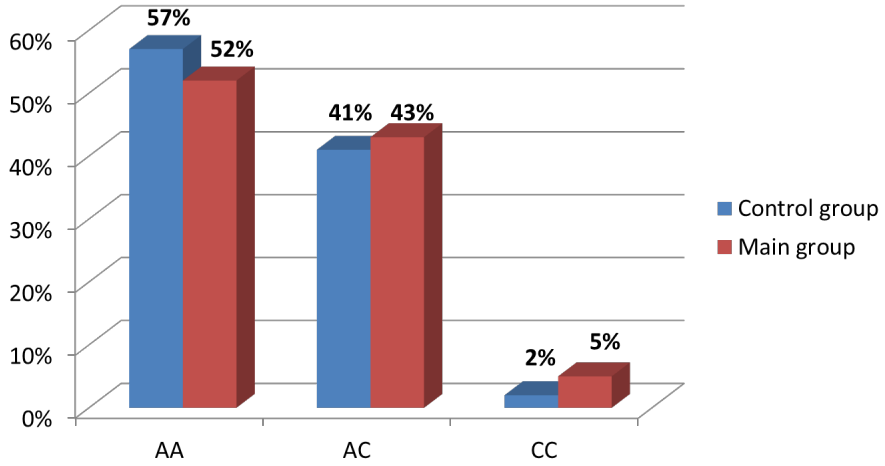

Fig. 2. Distribution of genotype frequencies of +105A/C polymorphism of IL-18 gene in control group and in patients with urolithiasis

Analysis of the dominant inheritance frequency showed that the frequency of the Hardy-Weinberg genotype distribution in the group of patients has no statistically significant deviation between the common and dominant inheritance pattern (Table 5).

Table 5

Common inheritance pattern of IL-18 gene genotypes in children with urolithiasis and practically healthy children

Genotypes	Case	Control	X^2	p	OR	
	n=100	n=94			Sign.	95% CI
Genotype A/A	0.570	0.521	1.72	0.42	1.22	0.69-2.14
Genotype A/C	0.410	0.426			0.94	0.53-1.66
Genotype C/C	0.020	0.053			0.36	0.07-1.92

Analysis of the dominant inheritance frequency showed that the frequency distribution of IL-18 genotypes in children with urolithiasis by the frequency of the Hardy-Weinberg in the patient group did not have a statistically significant deviation between the common and dominant inheritance pattern (Table 6).

Table 6

Genotypes	Case	Control	X^2	p	OR	
	n=100	n=94			Sign.	95% CI
Genotype A/A+ A/C	0.980	0.947	1.53	0.22	2.75	0.52-14.55
Genotype C/C	0.020	0.053			0.36	0.07-1.92

Thus, the results obtained in this study indicate that the IL-1β gene contributes to the determination of disorders contributing to the development of urolithiasis in children. In particular, in the Uzbek population genetic markers of predisposition to USD are genotype CC of IL-1β gene. It is considered appropriate to include testing of this genotype in a comprehensive USD prevention programme in Uzbekistan.

Discussion.

Urolithiasis is a multifactorial disease based on the interaction of genotype and other factors, which in turn affect the disturbance of homeostasis, leading to changes in the complex physical and chemical processes occurring in the body [2-6].

When studying the molecular genetic status in children with urolithiasis, it became possible to clearly identify the main indications for surgical intervention and to develop a set of conservative measures for the prevention of recurrent urolithiasis after studying the genetic factor. The study of the association of molecular genetic markers with recurrent forms of urolithiasis, as well as the establishment of pharmacogenetic interactions will contribute to a more effective postoperative metaphylaxis of urolithiasis.

The formation of urinary tract stones is a pathological condition that affects an increasing number of people worldwide. Urolithiasis is therefore a serious health care concern. The recurrent nature of the disease emphasises not only the removal of stones from the urinary tract and assistance with spontaneous stone egress, but also appropriate ways of correcting metabolic disorders, taking into account the biochemical and genetic status of patients with urolithiasis [4,5].

Consequently, the effectiveness of USD treatment is increased by the application of concrement removal and prevention of primary (for uric acid nephrolithiasis) and recurrent stone formation. Currently, the leading methods in the treatment of urolithiasis are aimed at elimination of the concrement. Conservative therapy, aimed at chemical dissolution of the stone and prevention of its further growth, is ineffective and is used in cases where surgical treatment for one reason or another cannot be performed or not achieved complete disintegration of the stone. Reducing the incidence of urolithiasis due to early effective detection of predisposition to it, as well as more effective postoperative metaphylaxis of urolithiasis will lead to a significant reduction in material costs for the organisation and implementation of treatment measures.

Conclusions:

1. One of the most informative methods of predicting urolithiasis in children is a molecular genetic method, which allows to detect predisposition to the disease at any age and even in the absence of clinical and laboratory-instrumental manifestations, that is, at the earliest preclinical stage of pathology development.
2. The results obtained in this study indicate that the genetic variant CC genotype of IL-1β gene influences the manifestation, duration of course, recurrences and clinical and functional features of the course of urolithiasis.
3. Identification of the polymorphic gene allowed to predict urolithiasis at the preclinical stage and, thus, to avoid organ-killing operations and recurrences of the disease, as well as such formidable complications of the disease as hydronephrosis and renal failure.

References

1. Apolikhin OI, Sivkov AV, Konstantinova OV, Slominsky PA, Tupitsyna TV, Kalinichenko DN. Association of urolithiasis in patients with different states of family history of urolithiasis with polymorphisms of its candida genes in the Russian population. Journal of Experimental and Clinical Urology. 2014; 3:50-52 (In Russ.).
2. Apolikhin OI, Sivkov AV, Konstantinova OV, Slominsky PA, Tupitsyna TV, Kalinichenko DN. Connection of unilateral and bilateral urolithiasis with genetic factors. Journal of Experimental and Clinical Urology. 2015; 2:68-70 (In Russ.).
3. Apolikhin OI, Sivkov AV, Konstantinova OV, Slominsky PA, Tupitsyna TV, Kalinichenko DN. Search for polymorphic variants of candidate genes of urolithiasis in the Russian population. Journal of Experimental and Clinical Urology. 2013; 3:56-60 (In Russ.).
4. Apolikhin OI, Sivkov AV, Moskaleva NG, Solntseva TV, Komarova VA. Analysis of urological morbidity and mortality in the Russian Federation over a ten-year period (2002-2012). Journal of Experimental and Clinical Urology. 2014; 2:4-12 (In Russ.).
5. Dutov VV. Modern aspects of diagnostics and treatment of urolithiasis in elderly and senile patients. Russian Medical Journal. 2014; 22(29):2100-2104 (In Russ.).
6. Limborskaya SA, Slominskiy PA. Molecular genetics of man: Medico-genetic and population studies. In book: Problems and prospects of molecular genetics. 2013; Vol. 1:307-371 (In Russ.).
7. Nasonov EL, Eliseeva MS. Role of interleukin 1 in the development of human diseases. Journal of Scientific and Practical Rheumatology. 2016; 56(12):60-76 (In Russ.).
8. Yakushenko EV, Lopatnikova YA, Sennikov SV. Interleukin-18 and its role in the immune response. Journal of Medical Immunology. 2015; T.7;4:355-364 (In Russ.).
9. Rendina D. Metabolic syndrome and nephrolithiasis: a systematic review and meta-analysis of the scientific evidence//Nephrology Journal. 2014. no. 27(4). P. 371-376.
10. Scales C., Smith A., Hanley J., Saigal C. Prevalence of kidney stones in the United States//Europa Urology Journal. 2021. no. 62(1). P.160-165.
11. Turk C., Knoll T., Petrik A., Sarica K., Skolarikos A., Straub M., et al. EAU Guidelines on Urolithiasis. 2015. availible from: http://uroweb.org/wp-content/uploads/22-Urolithiasis_LR_full.pdf