Determination of the relationship between the polymorphic genes of metaloproteinases MMP9 (A-8202G) RS11697325 and the level of cystatin c in children with chronic nephritic syndrome

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Abstract. An analysis of the scientific literature in recent years reliably indicates the importance of molecular diagnostics in the field of nephrology. Research contains information on the role of genetic factors in the chronicization of the process of nephritic syndrome, ongoing research in this area. The pathology of development is explained by the fact that the chronicity of the process occurs primarily due to genetic inferiority of parts of the nephron, which leads to changes in the structure of the protein. The results of the study show the relationship between changes in the genotype of MMP9 (Matrix metalloproteinase-9) (A-8202G) rs11697325 with the development of the chronicization process. Our results provide evidence that cystatin C is an accurate diagnostic and prognostic marker of chronic nephritic syndrome in the pediatric population. At the same time, chromosomal polymorphic MMP9 genes affect the glomerular filtration rate by increasing the amount of Cystatin C, which is a gold marker in the diagnosis of chronic nephritic syndrome in children, determining the prognosis of the disease, which makes it possible for timely treatment and further tactics of the doctor.

1 Introduction

Today, chronic nephritic syndrome is one of the most severe kidney diseases in children, characterized by rapid progression and the development of many complications [1]. According to the World Health Organization (WHO), "... chronic nephritic syndrome ranks 2nd among the main forms of kidney pathology characteristic of childhood, and in the last 10 years, the incidence of chronic nephritic syndrome has been steadily progressing and accounts for 36.8% of all kidney diseases ..." Currently, due to the complexity of early diagnosis of the disease in patients with chronic nephritic syndrome, it is difficult to predict the duration, identify them in a timely manner and select the most adequate method for the full implementation of the diagnostic and treatment process, determine the optimal timing of their use and treat patients with these data diseases. The concept itself does not allow either making a correct diagnosis, prescribing pathogenetic treatment, or determining the

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morphological basis of the disease. These differences are likely due to genetic predisposition [2].

Evaluation of results is a problem that needs to be solved in the practice of nephrology. If this pathology is not diagnosed and treated in time, it can lead to serious consequences, disability and huge costs, dialysis and kidney transplantation. Any clinical variant, including nephritic and nephrotic syndromes, can reveal severe chronic kidney disease leading to end-stage renal failure [3].

Chronic nephritic syndrome in children has medical and social significance, and different ideas arise about the pathogenesis of the disease, the relationship of the body's immune system, poor prognosis, and difficulties in diagnosis and treatment. [4].

A number of scientific studies are being conducted all over the world aimed at improving the mechanisms of development of chronic nephritic syndrome in children, methods of diagnosis, treatment and prevention at an early stage. Currently, the problem of introducing new approaches to classification, assessment of severity, and diagnosis of its early stages, based on new data on the morphofunctional state of the kidneys, remains unresolved [5].

According to the authors, the pathogenetic role of MMPs in acute and chronic kidney diseases has been proven [6].

In this regard, determining the clinical and laboratory characteristics of chronic nephritic syndrome in children, timely diagnosis, assessment of factors, determining the role of the allele and genotype of polymorphic genes belonging to the group of metalloproteinases, and conducting studies that study the relationship between the concentration of Cystatin C in blood serum is timely.

Goal of the work. To determine the relationship between the polymorphic genes of metaloproteinases MMP9, (A-8202G) rs11697325 and the level of Cystatin C in children with chronic nephritic syndrome.

2 Materials and methods of research

Our study was conducted at the Samarkand Regional Children's Multidisciplinary Medical Center and at the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan.

The subject of the study was venous blood, serum and urine of patients for general clinical, laboratory, biochemical and PCR (Polymerase chain reaction) genotyping methods. The study used turbidometric, molecular genetics and statistical methods.

DNA extraction was carried out by the standard nucleosorb method using Diatom™ DNAPrep 200 kits (IsoGen Laboratory, Moscow, Russia). The amount of Cystatin C was determined in the SWISS-LAB laboratory using the KonelabT-Series CYSTATIN-C kit (Finland). Statistical processing of the study results was carried out in the automated Microsoft Excel Windows 10 program and in the software packages for statistical analysis Statistica 8 and Statistics 17.0. SPSS, SISA9.17®, Arlequin 3.5.2.

The selection of patients for the study was carried out according to inclusion/exclusion criteria.

Criteria for inclusion in the study:

- Signed informed consent from the patient.
- Age <18 years.
- CNS confirmed by clinical, laboratory and functional methods.
- Exclusion criteria:
- Refusal of the patient to sign informed consent for the study.

We examined 129 sick children, aged 5 to 15 years, who were treated in the nephrology department in the period from 2020 to 2022.

The main group in this study included 102 patients with chronic nephritic syndrome. The average age of patients with CNS was 10.28±3.76 with a range of 4–15 years. Of these, 65 (63.7%) patients were male and 37 (36.3%) patients were female. (Table 1). Among male children with CNS, 24 (36.9%) patients were aged 5–7 years, 18 (27.7%) were aged 8–11 years, and 23 (35.4%) patients were aged 12–15 years. Among female children with CNS, 13 (35.1%) patients were aged 5–7 years, 13 (35.1%) were aged 8–11 years and 11 (29.9%) patients were aged 12–15 years. Among male and female children with CNS, when compared by age groups, no statistically significant differences were found (p=0.13, p=0.36 and p=0.07, respectively).

The control group consisted of 27 relatively healthy patients. The average age of healthy children in the control group was 7.96±2.28. The age of healthy patients ranged from 5–13 years. Among healthy male children in the control group, 8 (61.5%) patients were aged 5–7 years, 4 (30.8%) were aged 8–11 years and 1 (35.4%) boy was aged 15 years. Among healthy female children in the control group, 6 (42.8%) patients were aged 5–7 years, 6 (42.8%) were aged 8–11 years and 2 (14.4%) patients were aged 12– 15 years. Among male and female children in the control group, when compared by age groups, no statistically significant differences were found (p=0.27, p=0.51 and p=0.48, respectively).

General characteristics of the patients included in the study are presented in Table. 1.

Table 1. Demographic characteristics of patients included in the study.

Children examined	Floor	Age, years			Total
		5–7	8–11	12–15	
With CNS	Boys	24(36,9%)	18(27,7%)	23(35,4%)	65(100%)
	Girls	13(35,1%)	13(35,1%)	11(29,8%)	37(100%)
Control group	Boys	8(61,5%)	4(30,8%)	1(7,7%)	13(100%)
	Girls	6(42,8%)	6(42,8%)	2(14,4%)	14(100%)
Total		51(39,5%)	41(31,8%)	37(28,7%)	129(100%)

Patients with CNS (Chronic Nephritic Syndrome) were conditionally divided into 3 groups depending on the form of CNS. The first group consisted of 36 patients (35.3%) with the proteinuric form of CNS, the second and third groups – 35 (34.3%) and 31 (30.4%) patients, respectively, with macrohematuric and mixed forms of CNS. Differences were considered statistically significant at $p<0.05$.

3 Research results

According to the purpose and objectives of our study, we analyzed the genotypes of MMP9 (A-8202G) rs11697325 depending on the level of Cystatin C. Judging by the results, in the general group of patients the level of cystatin C was 1.56 times higher in genotype AG and 2.06 times higher times higher in the pathological GG genotype compared to patients in whom the AA genotype was identified. Compared with the control group, significantly high average levels of cystatin C were found in patients with genotypes AG and GG, while in patients with genotype AA, the average level of cystatin C was not statistically different from the control group (Table 2).

Table 2. Average cystatin C values depending on the genotypes of the MMP9 (A-8202G) rs11697325 gene in patients with CNS in the general group and in healthy children.

MMP9 (A-8202G) rs11697325	Main group (n =102)	Control group, (n=27)	p-value
AA	1.64±2.31	1.23±2.55	0.57
AG	2.56±2.38	1.22±1.47	<0.001
GG	3.38±2.46	1.12±2.04	<0.001

When comparing cystatin C values depending on the genotypes of the TIMP2 (C536T) rs11551797 gene in patients in the general group and in healthy children, no significant differences were found

An analysis of the level of Cystatin C was carried out depending on the genotypes of MMP9 (A-8202G) rs11697325 and TIMP2 (C536T) rs11551797 and various forms of CNS. According to the results, in patients with proteinuric and mixed forms of glomerulonephritis, the level of cystatin C was 1.57 and 1.92 times higher with the AG genotype and, respectively, 2.42 and 2.27 times higher with the pathological GG genotype compared with patients with of whom the AA genotype was identified. At the same time, in patients with the macrohematuric form of the disease, as well as among healthy children in the control group, no significant differences were detected in the levels of cystatin C depending on the genotypes of MMP9 (A-8202G) rs11697325 (Table 3).

Table 3. Average cystatin C values depending on the genotypes of the MMP9 (A-8202G) rs11697325 gene in patients with CNS and healthy children.

MMP9 (A-8202G) rs11697325	Macro form (n =35)	Mixed form (n = 31)	Proto-form (n =36)	Control group,n=27
AA	1.94±2.34	1.81±2.41	1.89±2.35	1.23±2.55
AG	2.04±2.41	3.49±2.56**	2.98±1.34**	1.22±1.47
GG	1.85±2.39	4.11±2.67**	4.58±2.72**	1.12±2.04

Note: * – $p<0.05$, ** – $p<0.01$, $p<0.001$

Thus, analysis of the data obtained indicates a relationship between the AG and GG genotypes of the MMP9 (A-8202G) rs11697325 gene and the level of cystatin C in patients with CNS; in particular, more pronounced changes are observed in patients with proteinuric and mixed forms of the disease. The identified associations can serve as an important diagnostic and prognostic marker of the pathology under study.

At the next stage, we analyzed the associations between the MMP9 (A-8202G) rs11697325 genes and increased levels of cystatin C (> 2.0 mg/l vs <2.0 mg/l) in patients with proteinuric and mixed forms of the disease. As can be seen from table. 4.3.5, in the group of patients with proteinuric and mixed forms of the disease, a significantly significant allelic variant G and genotype GG of the MMP9 gene (A-8202G) rs11697325 were identified. In the group of patients, the allelic variant G was detected 1.64 times more often than in the control group (OR= 1.647; $\chi2$=3.94 (p=0.046); 95% CI: 1.006 >1.647> 2.695). In addition, the GG genotype of this gene in the group of patients was detected 2.57 times more often than in the control group (OR= 2.578; $\chi2$=4.46 (p=0.034); 95% CI: 1.070 >2.578> 6.212). Our results also showed that the presence of the AA genotype of the MMP9 gene (A-8202G) rs11697325 has a protective effect and is more common in the control group (OR=0.387; $\chi2$=4.46 (p=0.034); 95% CI: 0.161 >0.387>0.934) and more often in patients with low levels of cystatin C.

According to the analyses, the presence of the AG genotype of the MMP9 gene (A-8202G) rs11697325 does not have a significant association with the disease, but occurs slightly more often in healthy controls (OR=1.551; $\chi2$=0.91 (p=0.337); 95% CI: 0.632 > 1.551>3.804).

MMP9 (A-8202G) rs11697325 genotypes were analyzed in relation to creatinine levels. According to the results, in the general group of patients, the creatinine level was 1.28 times higher with the AG genotype and 1.46 times higher with the pathological GG genotype compared to patients in whom the AA genotype was identified. Compared with the control group, significantly high average creatinine levels were established in patients with genotypes AG and GG, while in patients with the AA genotype, the average creatinine level was not statistically different from the control group (Table 4).

Table 4. Average creatinine values depending on the genotypes of the MMP9 (A-8202G) rs11697325 gene in patients with CNS in the general group and healthy children.

MMP9 (A-8202G) rs11697325	Main group (n =102)	Control group, (n=27)	p-value
AA	119.7±21.48	89.3±18.51	0.39
AG	153.6±25.27	86.47±17.14	<0.001
GG	174.82±20.91	93.12±15.03	<0.001

An analysis of creatinine levels was carried out depending on the genotypes of MMP9 (A-8202G) rs11697325 and various forms of CNS (Table 5). According to the results, in patients with proteinuric and mixed forms of the disease, the level of cystatin C was 1.57 and 1.92 times higher with the AG genotype and, respectively, 2.42 and 2.27 times higher with the pathological GG genotype compared with patients with of whom the AA genotype was identified. At the same time, in patients with the macrohematuric form of the disease, as well as among healthy children of the control group, no significant differences were detected in the levels of cystatin C depending on the genotypes of MMP9 (A-8202G) rs11697325.

Table 5. Average cystatin C values depending on the genotypes of the MMP9 (A-8202G) rs11697325 gene in patients with CNS and healthy children.

MMP9 (A-8202G) rs11697325	Macrohemanic form (n =35)	Mixed form (n = 31)	Prot form (n =36)	Control group,n=27
AA	116.2±12.34	124.5±15.41	132.7±21.3	87.7±14.8
AG	114.84±13.71	152.93±25.76**	168.11±20.1**	92.67±16.47
GG	119.95±12.48	173.21±21.77**	172.85±28.7**	93.2±19.24

Note: * – p<0.05, ** – p<0.01

Thus, analysis of the data obtained indicates a relationship between the AG and GG genotypes of the MMP9 (A-8202G) rs11697325 gene and creatinine levels in patients with CNS; in particular, more pronounced changes are observed in patients with proteinuric and mixed forms of the disease. The identified associations can serve as an important diagnostic and prognostic marker of the studied

4 The discussion of the results

Recently, much attention has been drawn by researchers to the alternative marker cystatin C, which can be used to assess the filtration function of the kidneys. Everyone knows that GFR (glomerular filtration rate) is calculated using serum creatinine, an amount that depends on body weight and gender, since this does not give reliable test results. Cystatin C does not have similar properties, so this assay has a wide range of applications [7].

Determining the level of Cystatin C, alone or together with creatinine, allows you to more accurately determine GFR and assess the risk of developing renal failure. When determining GFR using creatinine, GFR with proteinuric form was 73.6%, with mixed form 72%, and when determining GFR with cystatin C, the level of indicators was lower than GFR with creatinine (56.6; 52.3). Determining the amount of cystatin C is a modern method for diagnosing nephritic syndrome in children and timely prognosis and treatment compared to similar diagnostic methods, therefore cystatin C is a more accurate marker of GFR [8].

MMP9 (A-8202G) rs11697325 and TIMP2 (C536T) rs11551797 genotypes were analyzed depending on cystatin C levels.

According to the results, in the general group of patients, the level of cystatin C was 1.56 times higher in the AG genotype and 2.06 times higher in the pathological GG genotype compared to patients in whom the AA genotype was identified. Compared with the control

group, significantly high average levels of cystatin C were found in patients with genotypes AG and GG, while in patients with genotype AA, the average level of cystatin C was not statistically different from the control group. According to the results, in patients with proteinuric and mixed forms of nephritic syndrome, the level of cystatin C was 1.58 and 1.93 times higher with the AG genotype and, respectively, 2.44 and 2.27 times higher with the pathological GG genotype compared with patients in whom the AA genotype was identified. At the same time, in patients with the macrohematuric form of the disease, as well as among healthy children in the control group, no significant differences were detected in the levels of cystatin C depending on the genotypes of MMP9 (A-8202G) rs11697325.

Our results provide evidence that cystatin C is an accurate diagnostic and prognostic marker of chronic nephritic syndrome in the pediatric population. In addition, in patients with proteinuric and mixed forms of CNS, the level of cystatin C was significantly higher if the patients had pathological genotypes. Unlike previous forms of CNS, in patients with the macrohematuric form of the disease, no significant differences were detected in the levels of cystatin C depending on the genotypes of MMP9 (A-8202G) rs11697325.

5 Conclusion

All chronic diseases, including chronic nephritic syndrome, manifest themselves weakly in the initial stages, when treatment is the cheapest and most effective, and in an advanced state they lead to a significant decrease in the quality of life and ultimately to early death. It is impossible to prevent all diseases at once, and there is no need for this - after all, each person has a chance to encounter only a few of them. And this set of diseases is not accidental. Information about our health and future risks is contained only in our genes. Each person has his own genetic health map. Determining the genetic risk of diseases is preventive prevention, the path to creating a genetic passport, predicting the development of various diseases [9]. Genes contain information about the structure of certain proteins that directly affect biochemical processes in the body. Mutations in genes can change the structure of proteins and affect physical performance.

DNA analysis will allow you to find out which disease has the most hereditary predisposition. Based on this information, a personalized program is developed to proactively take steps to prevent these diseases.

References

1. S. V. Bajko, Nefrologiya i dializ **22(1)**, 53–70 (2020)
2. B. A. Abeuova, G. N. Chingaeva, Pediatricheskaya nefrologiya **2**, 60-63 (2012)
3. V. A. Obuhova, Rossijskij vestnik perinatologii i pediatrii **59(4)**, 10-15 (2014)
4. N. P. Chesnokova, V. V. Morrison, T. N. Zhevak, M. N. Bizenkova, Medicinskie nauki **1**, 75-77 (2016)
5. A. A. Vyalkova, I. V. Zorin, S. A. Chesnokova, S. V. Plotnikova, Nefrologiya **23(5)**, 29 (2019)
6. A. S. Krutova, V. N. Luchaninova, O. V. Semeshina, A. Ni, O. G. Bykova, Tihookeanskij medicinskij zhurnal **1**,11-15 (2020)
7. N. S. Bazarova, Sh. H. Ziyadullaev, “Web of scientist: International scientific research journal” Indoneziya **3(5)**, 12-17 (2022)
8. N. B. Sobirdzhonovna, Journal of Universal Science Research **1(6)**, 778-782 (2023)

9. N. S. Bazarova, Sh. H. Ziyadullaev, European Journal of Molecular medicine **1**, 42-45 (2021)
10. N. B. Mukhamadieva, European Journal of Molecular and Clinical Medicine **7(11)**, 418-426 (2020)
11. F. Nurutdinova, Z. Tuksanova, Y. Rasulova, E3S Web Conf. **474**, 01002 (2024)
12. Sh. Oblokulov, E3S Web Conf. **474**, 01003 (2024)