



Research paper

Frequency of polymorphic loci of *GSTM1* and *GSTT1* modifier genes in the genotype of children with pyelonephritis and congenital urinary malformations

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ABSTRACT

Introduction: The growing number, prevalence, numerous complications, and deaths in patients with congenital anomalies of the kidney and urinary tract (CAKUT) indicate the high relevance of the declared topic. Currently, clinical medicine is actively engaged in research on the cellular and molecular mechanisms that cause the appearance of these diseases.

Aim: The aim of the work is to study genetic markers of CAKUT and the tendency to a more severe course of pyelonephritis in young children.

Material and methods: Using the multiplex polymerase chain reaction method, 50 children with pyelonephritis were examined for the presence of deletion alleles of the glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*) genes.

Results and discussion: As a result, 35 children were diagnosed with certain CAKUT. A statistically significant associative relationship between the development of pyelonephritis in a child and the presence of a null allele *GSTM1* 0/0 in its genotype and a high probability of CAKUT with quantitative and positional anomalies and impaired formation and differentiation of renal tissue in carriers of null alleles *GSTT1* 0/0, *GSTM1* 0/0 in their combination was revealed.

Conclusions: The fact that different forms of abnormalities are detected in members of the same family suggests that certain genetic mutations can potentially lead to CAKUT syndrome, but the final phenotype of the renal system depends either on the genetic background or on environmental factors.

1. INTRODUCTION

Pyelonephritis frequently complicates the clinical course of children with congenital anomalies of the kidney and urinary tract (CAKUT) worldwide. CAKUT accounts for 40% of all congenital malformations.^{1,2} Numerous studies confirm the influence of epigenetic and environmental factors on kidney development and the natural course of CAKUT, suggesting that the pathogenesis of this syndrome is multifactorial.³ The high prevalence, severity of complications, and often mortality of children with congenital malformations of the urinary system make us consider the problem as very relevant and encourage the search for new methods of early diagnosis and treatment.⁴ Current trends in the development of clinical medicine are determined by the study of cellular and molecular mechanisms of the occurrence and course of diseases with the widespread introduction into practice of methods of molecular, cellular and biochemical genetics, morphology, biochemistry, physiology and other fundamental disciplines.⁵ Under the influence of environmental factors in the process of evolution on the one hand and constantly emerging new environmental factors on the other, a genetic and phenotypic polymorphism is formed of populations and individuals.^{6,7} Detection of biomarkers and bioindicators allows us to establish the sensitivity and susceptibility of genomic structures to external aggression, as well as its early consequences.⁸ Most of the toxins that enter the body do not have direct biological effects, but are subject to various transformations, the so-called biotransformation,⁹ which includes activation (phase 1), detoxification (phase 2) and elimination (phase 3). The main purpose of biotransformation phase 2 is to neutralize (deactivate, detoxify) hydrophilic and often toxic products of phase 1 with the help of hydrolases and transferases that carry out or complete detoxification. This phase involves glutathione transferases, glucuronyl transferases, sulfotransferases, acetyltransferases, and methyltransferases, which convert toxic products of phase 1 metabolism into polar, water-soluble, non-toxic compounds that are eliminated from the body.¹⁰

Phase 2 detoxification enzymes include glutathione transferases (GST) that catalyze conjugation reactions of reduced glutathione with electrophilic substrates.¹¹ GST synthesis is controlled by genes (*GSTM*, *GSTT*, *GSTR*), which are located on different chromosomes. Functionally defective variants of these genes create a predisposition to various diseases.¹² Testing predisposition genes allows us to establish a link between functionally defective alleles and the body's response to exposure to external toxins, including infectious ones.¹³ *GSTM1* they act as a modifier and risk factor for many diseases, such as various tumors (lung, bladder, barley, skin, breast cancer),¹⁴ benign neoplasms (endometriosis, rectal adenoma), and chronic diseases.¹⁵ The null genotype is a risk factor for reduced lung function during smoking, which leads to chronic obstructive pulmonary disease, as well as asthma.^{16,17} A correlation was established between the frequency of chromosomal aberrations and the presence of *GSTM1 0/0* genotype.¹⁸ An increased predisposition to

bronchial asthma and oncopathology was recorded in homozygous individuals with *GSTT1 (GSTT1 0/0)* null allele, in which a significant part of the gene structure was deleted, as in the case of *GSTM1 (GSTM1 0/0)* and to the birth of children with congenital malformations in women.^{19,20}

Pyelonephritis (PN) and CAKUT syndrome are multifactorial diseases that result from the additive effect of many genetic loci and many environmental factors. Their sum determines the predisposition of a particular person to a particular disease.²¹ The complex pathogenesis, as well as the variability of clinical manifestations of PN against the background of CAKUT, suggest the influence of many modifier candidate genes in the development of these diseases. At the same time, the contribution of each of them may vary to the development of the disease.²² Xenobiotic metabolism genes are one of the potential modifier genes for CAKUT and secondary PN. The metabolic system of xenobiotics, including infectious agent toxins, is involved both in protecting the body from the consequences of developing inflammatory reactions, and can act as genes for predisposition to congenital anomalies.^{23,24}

2. AIM

The aim of work is to study genetic markers of congenital anomalies of the kidney and urinary tract and the tendency to a more severe course of pyelonephritis in young children, namely, to establish the distribution of *GSTM1* and *GSTT1* polymorphic gene among young children.

3. MATERIAL AND METHODS

This is a pilot study. Using the multiplex polymerase chain reaction (MPCR) method,²⁵ 50 children (aged 6–12 years) with pyelonephritis were examined for deletion alleles of *GSTM1* and *GSTT1* genes. All children had a kidney and bladder urinary system (US). Of these, 35 were diagnosed with certain abnormalities of the kidneys and urinary tract, and 15 were not found to have congenital anomalies of the US. Children with CAKUT were divided into three groups according to the type of anomaly detected: group I (I-VR) included children with vesicoureteral reflux (VR) – 7 males, 8 females; group II (II-RA) included children with quantitative and positional renal abnormalities (RA) – 5 males, 7 females, and group III (III-ART) included children with abnormal renal tissue (ART) formation and differentiation – 3 males, 5 females. The data obtained were compared with the distribution frequency of deletion *GSTM1* and *GSTT1* alleles in randomly selected children from Western Ukraine (29 children), who made up the general population control (GPC) group.²⁶

The most important finding in the general analysis of urine, which confirmed the diagnosis of PN is the presence of bacteriuria on the background of leukocyturia. Deoxyribonucleic acid (DNA) was the material for the study, isolated from peripheral blood leukocytes of the patients. DNA iso-

Table 1. Distribution of *GSTT1* and *GSTM1* alleles among children with secondary pyelonephritis and CAKUT and in the control group.

Distribution of alleles	Groups of children											
	All examined with CAKUT		I-VR		II-RA		III-ART		Pyelonephritis without CAKUT		GPCG	
	<i>n</i> = 35	%	<i>n</i> = 15	%	<i>n</i> = 12	%	<i>n</i> = 8	%	<i>n</i> = 15	%	<i>n</i> = 29	%
Slippery road	12	34.3	5	33.3	4	33.3	3	37.5	4	26.7	9	31.0
Road curves	18	51.4*	4	26.7**	8	66.7*	6	75.0*	9	60.0*	9	31.0
Night	8	22.9*	2	13.3**	4	33.3*	2	25.0*	0	–	3	10.3

Comments: * significant difference in the indicator compared to healthy children from the GPCG; $P < 0.01$; ** likely difference in the indicator in children with different CAKUT; $P < 0.01$.

lation and purification were performed by enzymatic cleavage and subsequent phenolic extraction or by efflorescence.²⁷ The polymerase chain reaction (PCR) method was performed automatically on a Tertsik thermal cyclor (DNA technology, Russian Federation), and oligonucleotide primers were used (Fermentas, Vilnius, Lithuania), a set of reagents for amplification GenePak PCR Core (Limited Liability Company Iso-Gen Laboratory, Moscow, RF), which is lyophilized dry mixes containing Taq DNA polymerase inhibited for 'hot start,' deoxynucleoside triphosphates and magnesium chloride with final concentrations, respectively – 1 U, 200 μ and 2.5 mm, as well as an optimized buffer system.

PCR products were analyzed by electrophoresis in 2.0% agarose gel. Electrophoresis was performed for 30–40 minutes at 100 V, and an electrophoregram was scanned on an ultraviolet transilluminator. The resulting signals were compared with length markers, and based on this; the researchers determined the size of the resulting fragments. Homozygous and heterozygous carriers of functional *GSTT1* '+' and *GSTM1* '+' alleles were detected by the presence of an amplification product with a size of 470 bps for *GSTT1* and 271 bps for *GSTM1* on the electrophorograms. The absence of a suitable fragment indicates homozygosity for the deletion variant of *GSTT1* and/or *GSTM1*. A fragment of the albumin gene of 330 bps was used as an internal control.

For statistical analysis, all data was entered into Excel spreadsheets. The obtained digital data was processed using mathematical statistics with variance and alternative analyses. The Student's *t*-test were used to compare the groups. The results were analyzed using the licensed statistical programs Statistika 6.0 and Microsoft Excel. Blood was taken for the study from a peripheral vein on an empty stomach in the morning.

4. RESULTS

Percentage distribution was the same of gene polymorphism of *GSTM1* 0/0 and *GSTT1* 0/0 allele in children of the general population group, i.e. 31.0% for each (Table 1). Their combination was detected in 10.0% of cases. These data corresponded to the frequency of null alleles, which is established for the Caucasian race: *GSTM1* 30.0%–45.0%, *GSTT1* 15.0%–30.0%.²⁸

In the group of children with PN on the background of CAKUT, *GSTT1* 0/0 null allele was registered in 34.3% of the

surveyed. It was registered in 26.7% of children with PN without CAKUT, which statistically did not differ from the data of children from the general population control group. The frequency of *GSTM1* 0/0 deletion allele was accurate in children with pyelonephritis, and significantly different from the data of the children from the GPCG in three groups (II-RA – 66.7%, III-ART – 75.0%, IV-GPC – 60.0%), except for the I-VR, where it was 26.7%. Therefore, there was the association of pyelonephritis with *GSTM1* 0/0 deletion allele in the child's genotype. A statistically significant difference was revealed in the frequency of a combination of *GSTM1* 0/0 and *GSTT1* 0/0 null alleles in the children from the I-VR and III-ART groups compared with the IV-GPC group. This combination was not recorded in the children with primary pyelonephritis. The obtained results allow us to conclude that the combination of functionally defective *GSTM1* 0/0 and *GSTT1* 0/0 alleles is associated with the I-VR and III-ART groups. Consequently, statistically significant patterns were revealed of pyelonephritis association with *GSTM1* 0/0 null allele in the child's genotype and a high probability of I-VR and III-ART in carriers of *GSTT1* 0/0 and *GSTM1* 0/0 null alleles in their combination.

5. DISCUSSION

In VR, retrograde urine enters ureters and kidneys from the bladder. VR is the most common type of urinary tract abnormality in children. Genetic risk factors may be related to the etiology of VR. As multifunctional enzymes, the role of glutathione s-transferases is to combat cellular oxidative stress.²⁹ The clinical spectrum of congenital anomalies of the kidney and urinary tract covers a common birth defect that has a significant impact on the long-term survival of patients. The evidence suggests that approximately 20% of patients suffer from a genetic disorder that was not detected by standard clinical assessment, suggesting many mutation mechanisms and pathogenic pathways. In particular, between 10% and 15% of CAKUT patients have an unpredictable genomic disorder that increases the risk of neurocognitive disorders, early recognition of which may affect clinical care.³⁰

Vesicoureteral reflux is a congenital developmental anomaly in which the ureterovesical junction is impaired, and retrograde vesicoureteral reflux occurs from the bladder back into the ureter and collecting system. Reflux can

occur even when the canal is normally developed, with increased pressure in the bladder caused by infravesical obstruction or dysfunctional urination. Functional disorders of urination include infrequent urination, coprostatics, or both, which may be evidence of VR development. Reflux contributes to urinary tract infection (UTI) and often recurs.³¹ Primary reflux is formed with congenital disorders of the ureteral anatomy, as well as with insufficient valve development. Complications of reflux are especially dangerous and can cause impaired kidney function. Pyelonephritis can develop due to impaired fluid excretion and accumulation of toxins.

The results of radionuclide kidney scans show that the vast majority of infants and young children with febrile UTIs suffer from acute pediatric pyelonephritis.³² In children with UTIs, VR occurs in approximately 25%–40% of cases.³³ It should be noted that there are two types of VR primary and postprimary. Primary reflux, as mentioned earlier, is caused by a congenital anomaly caused by a developmental disorder (rudiment) of the muscular apparatus of the trigonum vesicae, and is perceived as a congenital anatomical defect. Postprimary reflux is the result of urinary tract infections or infravesical obstruction (diseases impairs the outflow of urine from the bladder). Inflammatory processes, ureteral ectopia, duplicated ureters, neurogenic bladder dysfunction, and iatrogenic consequences can also cause postprimary vesicoureteral reflux.³⁴

There is a genetic predisposition to recurrent UTIs and kidney scars. Genes that cause patients to relapse UTIs and renal scarring include angiotensin-converting enzyme insertion/deletion gene (ACE I/D), interleukin receptor (IL) 8 CXCR1 and CXCR2 genes, IL-10-1082(G/A) gene, heat shock protein family A of gene 72, which transforms platelet derived growth factor, Toll-like receptor and vascular endothelial growth factor gene.³⁵ Inheritance of urethral vesicular malformation is associated with one or more genes. With a probability of 50%–70%, the pathology manifests itself in children born to parents who have VR. This fact indicates the dominant nature of the gene. According to various authors, 30%–50% of twins have a risk of inheritance.²³ Upper urinary tract and kidney infections cause more serious symptoms and can lead to permanent kidney damage. Kidney damage is suspected if there are signs of inflammation, fever and increased content of C-reactive protein or procalcitonin, as well as if the general condition is disturbed. However, specific symptoms are difficult to identify in children, as there is not always a change in general condition and fever.^{36,37}

6. CONCLUSIONS

Based on the results of the study, the following conclusions were formulated:

- (1) Functional inferiority of genetically determined body detoxification processes in infant with CAKUT led to the manifestation of pyelonephritis in those children whose genotype has *GSTM10/0 null allele*.
- (2) A statistically significant associative relationship and a high probability of CAKUT formation with quantitative and positional abnormalities and impaired renal tissue formation and differentiation were found in children with *GSTT1 0/0* and *GSTM1 0/0* null alleles in their combination.
- (3) Detection of urinary tract defects in relatives of patients with reflux indicates the presence of a hereditary predisposition. However, the transmission mechanism has not yet been identified.

Conflict of interest

None declared.

Funding

None declared.

Ethics

The study of biological material was carried out taking into account the main provisions of the Helsinki Declaration on biomedical research, in which a person acts as their object, the Belmont report and the International Conference on harmonization-Good Clinical Practice (CCI-GCP). The study provided for compliance with the principle of confidentiality and respect for the child's personality, as a person incapable of self-defense.

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