Cukurova Medical Journal

Araştırma Makalesi / Research Article

MicroRNA Gene Polymorphisms in Congenital Anomalies of the Kidney and Urinary Tract

Doğumsal Böbrek ve İdrar Yolları Anomalilerinde MicroRNA Gen Polimorfizmleri

Özlem Tezol[1], Ali Delibaş[2], Özlem İzci Ay[3], Ümit Karakaş[3], Bahar Taşdelen[4], Mehmet Emin Erdal[3]

[1]Kars State Hospital, Clinics of Pediatrics, KARS

[2]Mersin University Faculty of Medicine, Department of Pediatric Nephrology, [3]Department of Medical Biology, [4]Department of Statistics, MERSİN

Cukurova Medical Journal 2015;40(3):439-451.

ABSTRACT

Purpose: Pathogenesis of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) is unknown. A strong genetic contribution is emphasized. In this study we investigated the role of microRNA gene polymorphism in CAKUT.

Material and Methods: 147 patients with CAKUT [(Ureteropelvic junction obstruction n: 39, vesicoureteral reflux (VUR) (n: 37), renal parenchymal malformations (n:43), anomalies of renal embryonic migration (n: 28)] and 51 healthy children were enrolled in the study. RNASEN, DGCR8, XPO5, RAN, DICER1, GEMIN3 gene polymorphisms were studied.

Results: When the patient and control group were compared by polymorphisms no statistically significant difference was found. But GEMIN3 mutant allel frequency was significantly higher in VUR group than renal parenchymal malformation group.

Conclusions: Mutant alleles of the GEMIN3 gene might be related to VUR pathogenesis within the context of the CAKUT spectrum. But studies with larger number of patients are required to delineate the association between CAKUT pathogenesis and microRNA gene polymorphisms.

Key words: Congenital Anomalies of the Kidney and Urinary Tract, microRNA, GEMIN3

ÖZET

Amaç: Doğuştan böbrek ve üriner traktus anomalilerinin (CAKUT) patogenezi bilinmemektedir. Etiyolojide güçlü bir genetik yatkınlık vardır. Bu çalışmada CAKUT'da mikroRNA sentez yolağında görevli gen polimorfizmlerinin rolü araştırıldı.

Materyal ve Metod: Mersin Üniversitesi Tıp Fakültesi Çocuk Nefrolojisi Polikliniği'nde izlenen CAKUT tanılı 147 hasta [Ureteropelvik bileşke darlığı: (n: 39), vezicoureteral reflü (VUR) (n: 37), renal parankimal malformasyonlar (n:43), böbreğin embriyonik migrasyon anomalileri (n: 28)] ve 51 sağlıklı çocuk çalışmaya dahil edildi. RNASEN, DGCR8, XPO5, RAN, DICER1, GEMIN3 gen polimorfizmleri Mersin Üniversitesi Tıp Fakültesi Tıbbi Biyoloji Anabilim Dalı'nda çalışıldı.

Bulgular: Hasta ve kontrol grubu arasında gen polimorfizmleri karşılaştırıldığında istatistiksel olarak anlamlı bir fark bulunmadı. GEMIN3 geni mutant allel sıklığı VUR grubunda renal parankimal malformasyon grubuna oranla daha yüksek saptandı.

Sonuç: CAKUT'da miRNA oluşum yolağında görevli gen polimorfizmlerinin patogenezi açıklayabilmesi için daha fazla sayıda hasta içeren çalışmalara ihtiyaç vardır. GEMIN3 geni mutant allelleri CAKUT spektrumu içinde VUR ile ilişkili olabilir.

Anahtar kelimeler: Doğuştan Böbrek ve İdrar Yollarının Anomalileri, mikroRNA, GEMIN3

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) are a group of diseases seen in 1:500 live births with a remarkable neonatal deaths rate of 1:2000[1]. They usually lead to progressive chronic kidney disease and end-stage renal failure as the most common cause of renal replacement therapy in childhood[2,3].

CAKUT may involve kidneys, collecting system or both indicating a common pathogenetic mechanisms and genetic basis. They might be related with maternal, placental, fetal, environmental and genetic factors affecting nephronogenesis. Under the influence of intrauterine milieu; mutations, epigenotype, urinary flow obstruction or abnormal interaction between mesenchyme, ureteric bud and bladder anlage may end up with CAKUT[4].

Many genes involved in nephronogenesis have been revealed. But full understanding of this complex process underlying the formation of embryonic kidney and urinary tract has not been accomplished yet[5,6].

More than two thousand MicroRNAs(miRNA) are defined in humans. They are single-stranded RNA molecules with approximately 20-23 nucleotides not coding protein. They are functional RNA molecules transcribed from exons and introns on the genome but not translated to proteins.

miRNAs are effective in the control of gene expression since they can lead to messenger RNA (mRNA) degradation and translational inhibition by binding to target gene mRNA with low specificity[7,8]. They suppress target genes and play a role in important biological processes such as growth, differentiation, proliferation, cell death and apoptosis. Since they are involved in many normal processes of eukaryotic cells, defects in miRNAs can cause a variety of diseases including cancer.

Kidney and urinary tract development in mammals require complex interactions and signaling processes between embryonic tissues. Kidney induction and differentiation ensue at the end of multiple interactive processes involving transcription factors, cell adhesion molecules, growth factors, cell polarity molecules, Wnt signaling pathway, renin-angiotensin system (RAS) components and additional stimulating factors (9-12). MicroRNAs support the survival of nephron progenitor mesenchymal cells by suppressing the expression of miR-10a, miR-106b, miR-17-5p and proapoptotic protein Bim and PEP. They also protect and support nephron progenitors and enable to reach final nephron number by increasing the number of nephrons (13).

The Formation of miRNAs takes place in three steps;

- **Step 1:** Transcription of primary miRNAs (pri-miRNA) from miRNA genes.
- **Step 2:** Intranuclear conversion of pri-miRNAs into precursor miRNA (pre-miRNA).
- **Step 3:** Formation of mature miRNA in the cytoplasm.

miRNAs are synthesized from DNA as primary transcripts (pri - miRNA) by the enzyme RNA-polymerase. Pri-miRNA, having a "cap" and a "poly A" tail, is in a structure of "stem and loop". In the nucleus, via *"Drosha"*, an endonuclease of the RNAse III enzyme family, and its cofactor *"Pasha"* (DGCR8, double-stranded RNA -binding protein) they are converted into pre-miRNAs. Complexes formed by Drosha are called microprocessor complexes.

Pre-miRNA molecule is transported to the cytoplasm bound to a nuclear transport receptor "exportin 5" and a nuclear protein "Ran-GTP". In the cytoplasm, pre- miRNAs are translated into miRNA duplex with a length of 18-24 nucleotides, being cut by an endonuclease called *"Dicer"*

belonging to RNase III enzyme family. Dicer also initiates RNA-induced silencing complex (RISC) formation. After disconnection of the stem and loop of pre- miRNAs by Dicer, only one strand of miRNA duplex joins the RISC complex. With the effect of Argonaut, that is an RNAse within the RISC complex, one of the two strands with more stable 5' end is selected and included in the complex. This strand is called the guide strand. The other anti-guide or passenger strand is digested by the RISC complex. miRNAs, after integration into the active RISC complex, lead either to mRNA degradation with the help of Argonaute proteins or suppression of the protein translation[7,8,14].

Single nucleotide polymorphisms (SNPs) in MicroRNA and miRNA formation pathways may affect the formation of mature miRNA and probably affecting miRNA expression also. In this study we aimed to investigate whether SNPs in the RNASEN, D GCR8, XPO5, RAN, DICER1, GEMIN3 genes are related with CAKUT or not.

MATERIALS and METHODS

2.1. Study Population and DNA Extraction:

A total of 147 patients (85 male/62 female) with CAKUT, followed in Mersin University Medical Faculty Department of Pediatric Nephrology between June 2007- October 2012 were included in the study. As the control group 51 healthy children (17 male/ 34 female) were included in the study.

Inclusion criteria for the study were;

- Being between the ages of 0-18
- Getting a diagnosis of CAKUT

Exclusion criteria for the study were;

- Presence of acquired abnormalities of the kidney and urinary tract (stones, hydronephrosis, etc.)
- Syndromic patients
- Presence of other chronic systemic diseases

Patients were divided into four groups as those with ureteropelvic junction obstruction (UPJO), vesicoureteral reflux (VUR), renal parenchymal malformations (RPM) and those with renal localization, rotation, fusion anomalies (RLRF).

Peripheral blood samples were collected from 147 patients (mean age 6.3 ± 4.7 years) and 51 healthy children (mean age 9.7 ± 4.5 years). Written informed consent was taken from all participants (parents) according to the recommendations of the Mersin University Ethical Committee for Clinical Studies. Genomic DNA was isolated from whole blood by salting out procedure[15].

2.2. Genotype Analysis of microRNA Machinery Genes polymorphisms:

Genotypes were determined by using a TaqMan™ fluorogenic 5′-nuclease assay with TaqMan Probes. The specific primers and fluorogenic probes for the microRNA Machinery Genes polymorphisms were designed by using Primer Express 3.0 software (Applied Biosystems) and are listed in Table 1. Primers and nh. probes were purchased from Metabion International AG, D-82152 Martinsried/Deutschland. Single nucleotide polymorphism amplification assays were performed according to the manufacturer's instructions. In brief, 25µl of reaction solution containing 30 ng of DNA was mixed with 12.5µl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems) and 900 nmol of each primer, 200 nmol of each probe. Reaction conditions consisted of preincubation at 60ºC for 1 minute and at 95ºC for 10 minute, followed by 40 cycles at 95ºC for 15 second and at 60ºC for 1 minute. Amplifications and analysis were performed in an ABI Prism 7500 Real-Time PCR System (Applied Biosystems), using the SDS 2.0.6 software for allelic discrimination (Applied Biosystems).

Table1. Primer/probe sequences of the microRNA Machinery Genes polymorphisms analyzed by Real-Time PCR.

Gene Name *Gene / **SNP ID	Primer Sequences	Probe Sequences1
DICER1 23405 rs13078	F:5'-TTAAATTCTGCCTTCAACTCATTCC-3' R:5'-CCCAATAGCTGAAACCGCTTT-3'	PRA:5'-FAM-CT(pdC)A(pdC)TAACAA(pdC)TTTAAGT(pdC)TT(pdC)CTT-BHO-1-3' PRT:5'-YakimaYellowTM-CT(pdC)A(pdC)TATCAA(pdC)TTTAAGT(pdC)TT(pdC)CTT-BHQ-1-3'
RNASEN (DROSHA) 29102 rs10719	F:5'-CATCCAGCTAAAAACAGATCATTAAAAC-3' R:5'-TGACTGTTGTCTATTGAGACCTAGCCT-3'	PRG:5'-FAM-CTTCGTT(pdC)ATTGT(pdC)TG(pdC)AGGABHQ-1-3' PRA:5'-YakimaYellowTM-CTT(pdC)ATT(pdC)ATTGT(pdC)TG(pdC)AGGA-BHQ-1-3'
GEMIN3 (DDX20) 11218 rs197388	F:5'-CCCAGCACTCTCTTGTTTTGC-3' R:5'-AGACAGAATAGGTTCTTGTCCTCATAGAGT-3'	PRT:5'-FAM-TATATGT(pdC)TT(pdC)TGC(pdC)TGT(pdC)T(pdC)C-BHQ-1-3' PRA:5'-YakimaYellowTM-ATTATATGT(pdC)TA(pdC)TGC(pdC)TGT(pdC)T(pdC)C-BHQ-1-3'
RAN 5901 rs14035	F:5'-TGCCATCCACTGATGTTCCA-3' R:5'-TGACCTGTCAGAATAAAAATGTGGTT-3'	PRA:5'-FAM-C(pdC)TGTTTGAAGTT(pdC)TA(pdC)ATTAAAA(pdC)AT-BHQ-1-3' PRG:5'-YakimaYellowTM-C(pdC)TGTTTGAGGTT(pdC)TA(pdC)ATTAAAA(pdC)A-BHQ-1-3'
XPO5 57510 rs11077	F:5'-TCATGGAAGGGCAAGATGTGT-3' R:5'-CCATGGTACAGGCTACTGCTAAACT-3'	PRT:5'-FAM-A(pdC)TAAAGA(pdC)TTCC(pdC)AG(pdC)C(pdC)T-BHQ-1-3' PRG:5'-YakimaYellowTM-A(pdC)TAAAGA(pdC)TGCC(pdC)AG(pdC)CCT-BHQ-1-3'
DGCR8 54487 rs1640299	F:5'-TGGCCTCCTAGGGTCCCTT-3' R:5'-AAGGCAGAGAGGGCCTCAGT-3'	PRG:5'-FAM-TCTTAATGC(pdC)CTAAAAG(pdC)GCC-BHQ-1-3' PRT:5'-YakimaYellowTM-T(pdC)TAATTC(pdC)CTAAAG(pdC)GCCT-BHQ-1-3'

* www.ncbi.nlm.nih.gov/gene, ** http://www.ncbi.nlm.nih.gov/SNP [1]**pdC:** Substitution of C-5 propynyl-dC (pdC) for dC is an effective strategy to enhance base pairing. Using these base substitutions, duplex stability and melting temperatures are raised by C-5 propynyl-C 2.8° per substitution.

STATISTICAL ANALYSIS

The age distribution of the patients and controls were given as mean and standard deviation and were compared with Student's t-test. Gene distributions in the groups were given as number and percentages. Chi-square test was used for controlling the relationship between the genes and the disease investigated. The Statistical Package for Social Science for Windows 11.5 (SPSS Inc, Chicago, IL) program was used. P values <0.05 were accepted as statistically significant.

RESULTS

In our study 147 children(85 boys; 57.8%) with CAKUT and 51 healthy children (17 boys; 33.3%) as controls were included.

According to the diagnosis, 39 (26.5%) patients with UPJO, 37 (25.1%) with VUR, 43 (29.2%) with RPM and 28 (19 %) with RLRF were present. In RPM group, 4 (2.7%) renal hypoplasia, 5 (3.4%) renal dysplasia, 23 (15.6%) renal agenesis, 6 (4%) multicystic kidney, 2 (1.3%) multicystic dysplastic kidney and 3 (2%) polycystic kidney patients were present. In RLRF group; 8 (5.4%) horseshoe kidney, 18 (12.2%) ectopic kidney and 2 (1.3%) cross-ectopic adherent kidney patients were present (Table 2).

Allelic distributions of the patients and controls for RNAS, DGCR8, XPO5, RAN, DICER1 and GEMIN3 genes were similar ($p > 0.05$).

Genotype and allele frequencies of each group compared with controls are shown in Table 3-7.

Table2: The number of patients with CAKUT

GROUPS	NUMBER (n=147) (%)
Ureteropelvic junction obstruction (UPJO)	39 (26.5)
Vesicouretheral reklux (VUR)	37 (25.1)
Renal parenchimal malformations (RPM)	43 (29.2)
Renal hypoplasia	4 (2.7)
Renal dysplasia	5 (3.4)
Renal agenesis	23 (15.6)
Multicystic kidney	6 (4)
Multicystic dysplastic kidney	2 (1.3)
Polycystic kidney	3 (2)
Renal localisation, rotation, fusion anomaly (RLRF)	28 (19)
Horseshoe kidney	8 (5.4)
Ectopic kidney	18 (12.2)
Cross fusioned kidney	2 (1.3)

Table 3. Genotype and allel frequency distributions of the CAKUT and control group

	GENE NAME	CAKUT		
		Patient	Control	p
Genotype	**RNASEN *rs10719*** (n_p:147/n_c:51) CC/CT/TT	79/51/17 %53.7/37.7/11.6	26/20/5 %51.0/39.2/9.8	0.829
Allele frequency	C>T	209>85	72>30	0.97
Genotype	**DGCR8*rs1640299*** (n_p:146/n_c:51) GG/GT/TT	42/69/35 %28.8/47.2/24.0	17/25/9 %33.3/49.0/17.7	0.617
Allele frequency	G>T	153>139	59>43	0.404
Genotype	**XPO5*rs11077*** (n_p:145/n_c:51) AA/AC/CC	61/69/15 %42.0/47.6/10.4	19/28/4 %37.3/54.9/7.8	0.649
Allele frequency	A>C	191>99	66>36	0.928
Genotype	**RAN*rs14035*** (n_p:143/n_c:50) CC/CT/TT	67/59/17 %46.9/41.2/11.9	24/21/5 %48.0/42.0/10.0	0.837
Allele frequency	C>T	193>93	69>31	0.877
Genotype	**DICER1*rs3742330*** (n_p:141/n_c:51) AA/AG/GG	102/35/4 %72.34/24.82/2.84	38/12/1 %74.5/23.5/1.96	0.923
Allele frequency	A>G	239>43	88>14	0.835
Genotype	**GEMIN3*rs197388*** (n_p:143/n_c:51) TT/TA/AA	102/37/4 %71.3/25.9/2.8	36/15/0 %70.6/29.4/0	0.270
Allele frequency	T>A	241>45	87>15	0.931

Table 4.Genotype and allel frequency distribitions of VUR and control group

	Patient Genotype	Control Genotype	p
RNASEN rs10719 CC/CT/TT	19/14/4 %51.4/37.8/10.8	26/20/5 %51.0/39.2/9.8	0.984
C>T	52>22 (n=37)	72>30 (n=51)	0.903
DGCR8 rs1640299 GG/GT/TT	11/18/7 %3.5/50.0/19.5	17/25/9 %33,3/49/17,7	0.955
G>T	40>32 (n=36)	59>43 (n=51)	0.885
XPO5 rs11077 AA/AC/CC	13/19/5 %35.1/51.4/13.5	19/28/4 %37,3/54,9/7,8	0.687
A>C	45>29 (n=37)	66>36 (n=51)	0.711
RAN rs14035 CC/CT/TT	17/15/4 %47.2/41.7/11.1	24/21/5 %48,0/42,0/10,0	0.986
C>T	49>23 (n=36)	69>31 (n=50)	0.972
DICER1 rs3742330 AA/AG/GG	25/10/1 %69.4/27.8/2.8	38/12/1 %74,5/23,5/2,0	0.867
A>G	60>12 (n=36)	88>14 (n=51)	0.749
GEMIN3 rs197388 TT/TA/AA	20/14/2 %55.5/38.9/5.6	36/15/0 %70,6/29,4/0	0.089
T>A	54>18 (n=36)	87>15 (n=51)	0.131

Table 5. Genotype and allel frequency distribitions of UPJO and control group

GENE NAME	UPJO		
	Patient Genotype	**Control Genotype**	**p**
RNASEN rs10719 CC/CT/TT	20/12/7 %51.3/30.8/17.9	26/20/5 %51.0/39.2/9.8	0.462
C>T	52>26 (n=39)	46>30 (n=51)	0.689
DGCR8 rs1640299 GG/GT/TT	8/21/10 %20.5/53.8/25.7	17/25/9 %33.3/49/17.7	0.354
G>T	37>41 (n=39)	59>43 (n=51)	0.216
XPO5 rs11077 AA/AC/CC	17/16/4 %46.0/43.2/10.8	19/28/4 %37.2/55.0/7.8	0.552
A>C	50>24 (n=37)	66>36 (n=51)	0.815
RAN rs14035 CC/CT/TT	24/8/5 %64.9/21.6/13.5	24/21/5 %48.0/42.0/10.0	0.137
C>T	56>18 (n=37)	69>31 (n=50)	0.425
DICER1 rs3742330 AA/AG/GG	26/11/0 %70.3/29.7/0	38/12/1 %74.5/23.5/2	0.483
A>G	63>11 (n=37)	88>14 (n=51)	0.996
GEMIN3 rs197388 TT/TA/AA	29/8/1 %76.4/21.0/2.6	36/15/0 %70.6/29.4/0	0.301
T>A	66>10 (n=38)	87>15 (n=51)	0.939

Table 6. Genotype and allel frequency distribitions of RPM and control group

GENE NAME	RPM		
	Patient Genotype	Control Genotype	p
RNASEN rs10719 CC/CT/TT	22/16/5 %51.2/37.2/11.6	26/20/5 %51/39.2/9.8	0.952
C>T	60>26 (n=43)	72>30 (n=51)	0.970
DGCR8 rs1640299 GG/GT/TT	16/17/10 %37.2/39.5/23.3	17/25/9 %33.3/49.0/17.7	0.627
G>T	49>37 (n=43)	59>43 (n=51)	0.977
XPO5 rs11077 AA/AC/CC	20/19/4 %46.5/44.2/9.3	19/28/4 %37.2/55.0/7.8	20/19/4 %46.5/44.2/9.3
A>C	59>27 (n=43)	66>36 (n=51)	0.682
RAN rs14035 CC/CT/TT	14/24/4 %33.3/57.2/9.5	24/21/5 %48.0/42.0/10.0	14/24/4 %33.3/57.2/9.5
C>T	52>32 (n=42)	69>31 (n=50)	0.393
DICER1 rs3742330 AA/AG/GG	31/8/1 %77.5/20.0/2.5	38/12/1 %74.5/23.5/1.96	0.912
A>G	70>10 (n=40)	88>14 (n=51)	0.983
GEMIN3 rs197388 TT/TA/AA	35/7/0 %83.3/16.7/0	36/15/0 %70.6/29.4/0	0.150
T>A	77>7 (n=42)	87>15 (n=51)	0.266g

Table 7. Genotype and allal frequency distribitions of RLRF and control group.

GENE NAME	RLRF		
	Patient Genotype	Control Genotype	p
RNASEN rs10719 CC/CT/TT	18/9/1 %64.3/32.1/3.6	26/20/5 %51.0/39.2/9.8	0.395
C>T	45>11 (n=28)	72>30 (n=51)	0.250
DGCR8 rs1640299 GG/GT/TT	7/13/8 %25.0/46.4/28.6	17/25/9 %33.3/49.0/17.7	0.487
G>T	27>29 (n=28)	59>43 (n=51)	0.319
XPO5 rs11077 AA/AC/CC	11/15/2 %39.3/53.6/7.1	19/28/4 %37.2/55/7.8	0.982
A>C	37>19 (n=28)	66>36 (n=51)	0.998
RAN rs14035 CC/CT/TT	12/12/4 %42.9/42.9/14.2	24/21/5 %48.0/42.0/10.0	0.822
C>T	36>20 (n=28)	69>31 (n=50)	0.671
DICER1 rs3742330 AA/AG/GG	20/6/2 %71.4/21.4/7.2	38/12/1 %74.5/23.5/1.9	0.532
A>G	44>10 (n=28)	87>15 (n=51)	0.771
GEMIN3 rs197388 TT/TA/AA	18/8/1 %66.7/29.6/3.7	36/15/0 %70.6/29.4/0	0.339
T>A	77>7 (n=27)	87>15 (n=51)	0.698

DISCUSSION

Congenital anomalies of kidney and urinary tract (CAKUT), through multisystem complications, may lead to growth retardation, severe impairment in cognitive and psychosocial adjustment and increased morbidity and mortality. The need for lifelong expensive treatments and issues regarding the employment of both patients and their parents create a major economic burden on the healthcare and insurance system. Such results of CAKUT indicate the need for experimental and clinical studies with diagnostic, preventive and therapeutic purposes. In our study, it was aimed to define whether there is an association between the polymorphisms of the genes involved in the miRNA formation pathway and CAKUT, to determine the genetic predisposition and to contribute to the efforts for early diagnosis and development of new treatment approaches in CAKUT.

DGCR8 is required for initial stages in the creation of primary transcripts in mature miRNA formation pathway. It has been reported that DGCR8 deletion leads to cardiomyopathy and early death and is associated with an increased incidence of neuropsychiatric diseases[16]. It has

also been reported that when DGCR8 is suppressed, morphological abnormalities and spatial memory-dependent learning disorders arise in the central nervous system[17]. In our study, no significant difference was detected for CAKUT in DGCR8 rs1640299 genotype.

XPO5 protein is a specific pre-miRNA carrier. It is probable that the SNPs of XPO5 affect the expression levels of proteins. Boni et al. in their study conducted on patients with metastatic cancer, detected that SNPs of XPO5 gene changed the disease control rate[18]. Ryan et al. in a study on cancer research reported that polymorphisms occurring in RAN SNP, rs14035 increased significantly the risk of esophageal cancer (19). In our study, although the allelic and genotype data of XPO5 and R genes did not show significance, we think that their functions in the formation of mature miRNA are sill noteworthy.

Horikawa et al. reported an association between renal cell carcinoma and miRNA SNPs. In their study, they emphasized that a change in three SNPs belonging to GEMIN3 and GEMIN4 had significantly reduced the risk of renal cell carcinoma[20]. In our study, we could not show a correlation between GEMIN3 polymorphisms and CAKUT. But heterozygous (TA) and homozygous (AA) mutant GEMIN3 alleles were significantly higher in VUR group compared to RPM group ($p=0.005$). This can be interpreted that carriage of GEMIN3 mutant alleles might especially be associated with VUR development. Since the proper ureteric bud growth and branching are known to be dependent on mesenchymal factors it may be argued that GEMIN3 gene might be one of the hereditary characteristics influencing the leaving position of ureteric bud on Wolfian duct during nephronogenesis. It may be considered that, the actual mechanism in the pathogenesis of VUR might be mutant GEMIN3 alleles causing ectopic ureter budding and ultimately ureterovesical valve insufficiency rather than the interactions between metanephric blastema and ureteric bud. It is also known that GEMIN3 inhibits apoptosis by p53 suppression. Suppression of p53 which is known to play a role in Metanephric development, migth be affected by GEMIN3 polymorphism or mutant allele carriage and this may influence ureteric budding during nephronogenesis. In the literature, GEMIN3 gene is not among the genes proven to have a role in VUR pathogenesis[10,21]. It has been shown that homozygous GEMIN3 knock-out mice died in the very early stages of embryonic development; but heterozygous mice were healthy and fertile having minor anomalies in steroidogenic tissues. These findings support the fact that GEMIN3 is essential in early embryonic development[22]. Our results regarding the potential role of GEMIN3 in the development of VUR also support the probable role of GEMIN3 in nephronogenesis.

DICER is the responsible enzyme in the conversion and processing of pre-miRNAs into mature miRNAs. As a result of the genetic ablation of DICER, effects of miRNAs are eliminated totally. DICER knock-out rat embryos might die during embryonic stages. These findings have revealed that miRNAs play a crucial role in normal renal development. In a recent study the effect of global miRNA suppression were examined by tissue specific removal of DICER. Experimental studies in the kidney have been performed by DICER removal in podocytes, proximal tubules and juxtaglomerular cells. At the end of these experimental models different phenotypes have emerged. Based on these different results it was reported that miRNAs played role not only in kidney development and maintaining normal kidney functions but also in the pathogenesis of kidney disease[23-31]. As a limitation of our study, due to small sample size our results might not reflect the actual relation between DICER gene frequency and CAKUT.

As a result, in our study we did not detect a significant difference in the allelic distribution of studied genes in patients with CAKUT and healthy controls. However a significant difference was detected for GEMIN3 gene allelic distribution

between VUR and RPM groups. This result can be interpreted that mutant GEMIN3 alleles might be related with VUR rather than RPM in the spectrum of CAKUT. But the need for confirmation of our results with larger studies is obvious.

Acknowledgements

This study is supported by Mersin University Scientific Research Projects Unit.

(Project number:BAP-TF DTB (ÖT) 2012-3 TU)

REFERENCES

1. Loane M, Dolk H, Kelly A, Teljeur C, Greenlees R, Densem J; EUROCAT Working Group. Paper 4: EUROCAT statistical monitoring: identification and investigation of ten years trend of congenital anomalies in Europe. Birth Defects Res A Clin Mol Teratol. 2011;91:31-43.
2. Sanna-Cherchi S, Ravani P, Corbani V, Parodi S, Haupt R, Piaggio G, Innocenti ML, Somenzi D, Trivelli A, Caridi G,et al. Renal outcome in patients with congenital anomalies of the kidney and urinary tract. Kidney Int. 2009;76:528-33.
3. Mansoor O, Chandar J, Rodriguez MM Abitbol CL, Seeherunvong W, Freundlich M, Zilleruelo G. Long term risk of chronic kidney disease in unilateral multicystic dysplastic kidney. Pediatr Nephrol. 2011;26:597-603.
4. Yosypiv IV. Congenital anomalies of the kidney and urinary tract: a genetic disorder? Int J Nephrol. 2012;90:9083-93.
5. Uetani N, Bouchard M. Plumbing in the embryo: developmental defects of the urinary tracts. Clin Genet. 2009;75:307-17.
6. Rumballe B, Georgas K, Wilkinson L, Little M. Molecular anatomy of the kidney: what have we learned from gene expression and functional genomics? Pediatr Nephrol. 2010;25:1005-16.
7. Ambros V. The functions of animal microRNAs. Nature. 2004;431:350-5.
8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism and function. Cell. 2004;116:281-97.
9. Schedl A. Renal abnormalities and their developmental origin. Nat Rev Genet. 2007;8:791-802.
10. Woolf AS. A molecular and genetic view of human renal and urinary tract malformations. Kidney Int. 2000;58:500-12.
11. Vainio S, Lin Y. Coordinating early kidney development: lessons from gene targeting. Nat Rev Genet. 2002;3:533-43.
12. Sanna-Cherchi S, Caridi G, Weng PL, Scolari F, Perfumo F, Gharavi AG, Ghiggeri GM. Genetic approaches to human renal agenesis/hypoplasia and dysplasia. Pediatr Nephrol. 2007;22:1675-84.
13. Ho J, Pandey P, Schatton P, Sims-Lucas S, Khalid M, Frank MH, Hartwig S, Kreidberg JA. The pro-apoptotic protein BIM is a microRNA target in kidney progenitor. J Am Soc Nephrol. 2011;22:1053-62.
14. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005;123:631-40.
15. Miller SA,Dykes DD, Polesky HI. A Simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res.1988;16:12-5.
16. Wilker EH, Baccarelli A, Suh H et al. Black caorbon exposures, blood pressure and interactions with SNP in microRNA processing genes. Environ Health Perspect. 2010;118:943-8.
17. Gascon E, Gao FB. Cause or effect: misregulation of microRNA pathways in neurodegeneration. Front Neurosci. 2012;6:48.
18. Boni V, Zarate R, Villa JC, Bandrés E, Gomez MA, Maiello E, Garcia-Foncillas J, Aranda E. Role of primary miRNA polymorphic variants in metastatic colon cancer patients treated with 5-FLU and irinotecan. Pharmacogenomics J 2011;11:429-36.
19. Ryan BM, Robles AL, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer. 2010;10:389-402.
20. Horikawa Y, Wood CG, Yang H. SNP of microRNA machinery genes modify the risk of renal cell carcinoma. Clin Cancer Res. 2008;14:7956-62.

21. Marrone AK, Ho J. MicroRNAs: potential regulators of renal development genes that contribute to CAKUT. Pediatr Nephrol 2014;29:565–74.

22. Mouillet JF, Yan X, Ou Q, Jin L, Muglia LJ, Crawford PA, Sadovsky Y. DEAD-Box Protein-103 is essantial for early embriyonic development and modulates ovarian morphology and function. Endocrinology. 2008;149:2168-75.

23. Harvey SJ, Jarad G, Cunningham J, Goldberg S, Schermer B, Harfe BD, McManus MT, Benzing T, Miner JH. Podocyte spesific deletion of dicer alters cytoskeletal Dynamics and causes glomerular disease. J Am Soc Nephrol 2008;19:2150-8.

24. Ho J, Ng KH, Rosen S, Dostal A,Gregory RI, Kreidberg JA. Podocyte spesific loss of functional microRNAs leads to rapid glomerular and tubuler injury. J Am Soc Nephrol. 2008;19:2069-75.

25. Nagalakshmi VK, Ren Q, Pugh MM, Valerius MT, McMahon AP, Yu J. Dicer regulates the development of nephrogenic and ureteric compartments in the mammalian kidney. Kidney Int. 2011;79:317-30.

26. Sequeira Lopez ML, Weatherford ET, Borges GR, Monteagudo MC, Pentz ES, Harfe BD, Carretero O, Sigmund CD, Gomez RA. The microRNA processing enzyme dicer maintains juxtaglomerular cells. J Am Soc Nephrol. 2010;21: 460-7.

27. Shi S, Yu L, Chiu C, Sun Y, Chen J, Khitrov G, Merkenschlager M, Holzman LB, Zhang W, Mundel P, et al. Podocyte selective deletion of dicer induces proteinuria and glomerulosclerosis. J Am Soc Nephrol. 2008;19:2159-69.

28. Wei Q, Bhatt K, He HZ, Mi QS, Haase VH, Dong Z. Targeted deletion of Dicer from proximal tubules protects against renal ischemia-reperfusion injury. J Am Soc Nephrol. 2010;21:756-61.

29. Pastorelli LM, Wells S, Fray M, Smith A, Hough T, Harfe BD, McManus MT, Smith L, Woolf AS, Cheeseman M, et al. Genetic analyses reveal a requirement for Dicer1 in the Mouse urogenital tract. Mamm Genome. 2009;20:140-51.

30. Zhdanova O, Srivastava S, Di L, Li Z, Tchelebi L, Dworkin S, Johnstone DB, Zavadil J, Chong MM, Littman DR,et al. The inducible deletion of Drosha and microRNAs in mature podocytes results in a collapsing glomerulopathy. Kidney Int. 2011;80:719-30.

31. Vivante A, Kohl S, Hwang DW, Dworschak GC, Hildebrant F. Single-gene causes of congenital anomalies of the kidnet and urinary tract (CAKUT) in humans. Pediatr Nephrol. 2014;29:695-704.

Yazışma Adresi / Address for Correspondence:
Dr. Ali DELİBAŞ
Mersin Üniversitesi Tıp Fakültesi Hastanesi
Çocuk Nefrolojisi Bilim Dalı
MERSİN
Tel: 0 324 241 00 00-1486
Fax: 0 324 241 00 98
E-Mail: delibas@mersin.edu.tr

Geliş tarihi/Received on : 22.01.2015
Kabul tarihi/Accepted on: 25.02.2015