

Increased potassium excretion in children with monosymptomatic nocturnal enuresis: could it be related to Kir 4.1- KCNJ10 gene polymorphism?

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ABSTRACT

Background and objectives. There are controversial results in the literature regarding urinary electrolytes, especially potassium, in enuretic children. KCNJ10 channel protein, a member of the Kir 4.1 family is expressed in renal distal tubules and has an important function in renal ion transport. We investigated whether KCNJ10 gene polymorphisms are associated with clinical and laboratory findings of a group of Turkish children with monosymptomatic primary nocturnal enuresis (MNE).

Method. Ninety-seven MNE children and 100 healthy controls were tested for three single nucleotide polymorphisms (SNPs) in the KCNJ10 gene. The transversions in SNPs were G to A for intron 1 (SNP1), G to A for exon 2 (SNP2), and T to C transition for promoter (SNP3). All SNPs were genotyped by PCR-restriction fragment length polymorphism.

Results. SNP3 in promoter of KCNJ10 gene showed strong association with MNE children for distribution of genotype and allele frequency, while SNP1 in intron 1 and SNP2 in exon 2 were noninformative.

The distribution of TT, TC, and CC genotypes for SNP3 was 66%, 26.8% and 7.2% respectively in MNE compared with 38%, 59% and 3% respectively in controls ($p < 0.0001$). In enuretic children, TT genotype was higher and there was an increased potassium excretion in children with TT genotype ($P < 0.05$).

Conclusion. We conclude that KCNJ10 gene promoter polymorphism may have a role on potassium excretion in Turkish MNE children. This is the first study in literature evaluating KCNJ10 gene polymorphism in this patient population. Future studies investigating the other SNPs, mutations or altered regulation of Kir4.1 in larger samples would help clarify the role (s) of KCNJ10 gene in enuresis.

Key words: KCNJ10 gene, Kir 4.1 family, monosymptomatic nocturnal enuresis, primary nocturnal enuresis, polymorphism.

Monosymptomatic nocturnal enuresis (MNE) is the involuntary urination during the night in the absence of an organic disease in a child

over 5 years of age.¹ It is a common situation in children and accounts for about 85% of primary enuresis.² Although there are a lot of data concerning the epidemiology, pathophysiology, and management of MNE, etiology remains unclear. Recent researches indicate that it is mostly due to physiological reasons.³ Diurnal pattern of urinary sodium (Na^+) and potassium (K^+) in normal children, with a marked reduction from daytime to night time, has not been found

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in enuretics having polyuria, natriuresis, and kaliuresis, despite normal levels of plasma atrial natriuretic peptide.⁴ A few studies showed that fractional sodium (FENa) and potassium (FEK) excretions were higher in enuretic children.^{5,6} These studies suggested a possible benign hereditary and/or postural disorder in renal tubular handling of Na⁺ and K⁺ in those children.^{4,6}

There are various K⁺ channels in the kidney. The voltage-gated K⁺ channels are important in stabilization of cell membrane potential, and expressed in a variety of nephron segments. The Ca-activated maxi K⁺ channel plays a role in flow-dependent K⁺ secretion in the distal nephron,⁷⁻⁹ while the other K⁺ channel, the renal outer medullary K⁺ channel (ROMK), is also found along the collecting duct in principal cells, where ROMK mediates K⁺ secretion into urine to remove it from the body.¹⁰

Kir 4.1 and Kir 1.1, potassium transporter members, are expressed in distal tubules and they function as the key molecules for renal ion transport.¹¹ KCNJ10 channel protein is a member of Kir 4.1 family.¹² KCNJ10 gene (MIM602208) which is located on chromosome 1q22-23, consists of two exons, spans 33 kb, and several single nucleotide polymorphisms (SNPs) including exons, introns and promoter region.¹³ Functional studies demonstrated that a few of these SNPs in KCNJ10 gene could affect the protein expression.⁸

As we mentioned above, urinary electrolyte levels, especially K⁺, have been found different in enuretics,^{4,6,14,15} whereas some other studies have found similar Na⁺ and K⁺ excretion compared to controls.^{16,17} In the present study, we firstly investigated whether KCNJ10 gene polymorphisms are associated with MNE in Turkish children, and searched the relationship between studied polymorphisms, and Na⁺ / K⁺ excretion, FENa, FEK, frequency of bedwetting, duration of enuresis, the presence of wake-up problem (deep sleep), and family history.

Material and Methods

Forty-seven male and 50 female children with MNE (mean age 9.5 ± 2.7 years) referred to the pediatric outpatient clinic of Gaziantep University, Department of Pediatrics and Pediatric Nephrology were included in the study. Healthy controls comprised of 100 age- and sex-matched volunteers with no known disease affecting electrolyte concentrations, renal functions, and none of them were relatives of the enuretics. A patient is considered to have MNE if the involuntary voiding at least 2 nights per week occurs, beyond the age at which bladder control was normally achieved in the absence of congenital or acquired defects of the urinary tract.

The study was approved by the local ethical committee (report# 02.07.2007/40), and informed consents were obtained from the patients and/or parents.

All patients and controls had detailed physical examination, tested negative for urinary protein, blood, and nitrate by dipstick and urine culture. None of the enuretics had ever been dry, and none had daytime incontinence or symptoms suggesting bladder-bowel dysfunction. Parents were asked about family history and the presence of wake-up problem (deep sleep). Although the subjects had similar eating habits, the diet was checked over 5 days before specimen collection to avoid excessive Na⁺ and K⁺ intake. None of the enuretics was taking any medication during the study period. All children had normal blood urea nitrogen (BUN) and creatinine levels, normal urinary ultrasonographic findings, and were normotensive at the time of study. The families not willing to give urine and blood samples were excluded (18 of 115 families). Blood and urine samples, *first urine in the morning*, were obtained to determine BUN, creatinine, phosphorus, Na⁺, K⁺, and urinary creatinine and electrolytes.

BUN, creatinine, electrolytes, as well as urinary creatinine, electrolytes, and urinary density

were determined by routine methods. FE_{Na} and FE_K were calculated from below formulae:

$$FE_{Na} (\%) = [(Urine Na^+/Plasma Na^+) / (Urine creatinine / Plasma creatinine)] \times 100$$

$$FE_K (\%) = [(Urine K^+/Plasma K^+) / (Urine creatinine / Plasma creatinine)] \times 100$$

Genotyping

All children (97 MNE patients and 100 healthy controls) were analyzed for three SNPs in KCNJ10 gene. These SNPs were G to A transversion in intron 1 (SNP1) and G to A transversion in exon 2 (SNP2), and T to C transition in promoter (SNP3). Genomic DNA was extracted from peripheral blood samples.¹⁸ All SNPs were genotyped by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) using the appropriate primers and restriction enzymes.¹³ The digested products were resolved on 3% agarose gels and visualized under U.V.

Statistical Analysis

Results are given as mean \pm SD, while allele frequencies and the distribution of genotype are given as %. Clinical and laboratory data of study groups compared by using Independent

sample t and Mann-Whitney U tests. Genotype frequencies among patients and controls were calculated by using Graphpad InStat Version 3, and deviation from Hardy-Weinberg equilibrium (HWE) was examined by De-finetti program. We calculated odds ratios and 95% confidence intervals using binary logistic regression. The analyses of data were performed by use of a statistical software (SPSS for Windows, version 11.5). Max type-I error was accepted as 0.05.

Results

Since we found no statistically significant differences in serum and urinary electrolyte levels and the distribution of SNPs between boys and girls ($p > 0.05$), we evaluated all children without sex differences.

BUN, creatinine, electrolytes, and tubular reabsorption of phosphorus were within normal limits in enuretics and controls. Urinary densities of enuretics and controls were 1019.7 ± 5.6 and 1021.1 ± 4.8 , respectively ($p > 0.05$). Serum K values were within normal limits in the patient and control groups (4.46 ± 0.35 mEq/L vs 4.50 ± 0.47 mEq/L, $p > 0.05$), while urinary K^+ excretion was higher in enuretics than controls

Table I. The demographic features of enuretic children and controls.

		Enuretics (n=97)	Controls (n=100)	p
Mean age (years)		9.5 \pm 2.7	9.8 \pm 2.5	>0.05
Gender	Male	47	45	>0.05
	Female	50	55	>0.05
Enuretic relatives	Present	77	17	<0.01
	Absent	20	83	<0.01
Frequency of bedwetting (night per week)		5.9 \pm 1.7	-	-

Table II. Urinary electrolytes of enuretic children and controls.

	Enuretics (n=97)	Controls (n=100)	p
Spot Urine (first urine in the morning)			
Na^+ (mmol/ L)	130.82 \pm 68.20	146.10 \pm 71.36	>0.05
K^+ (mmol/ L)	55.72 \pm 42.08	36.44 \pm 22.30	<0.01
FE_{Na} (%)	0.67 \pm 0.58	0.75 \pm 0.83	>0.05
FE_K (%)	7.87 \pm 7.70	5.55 \pm 4.38	<0.01

FE_{Na} : fractional sodium excretion, FE_K : fractional potassium excretion.

($p < 0.01$). The demographic characteristics of children are shown in Table I and the urinary excretions of Na^+ and K^+ are shown in Table II.

KCNJ10 gene SNP1 in intron 1 and SNP2 in exon 2 were noninformative for Turkish children. SNP3 in promoter was informative and digestion of SNP3 PCR product by *AciI* enzyme generated two fragments for T allele (Fig. 1).

SNP3 in promoter showed a strong association in enuretic children for either distribution of genotype and allele frequency (Table III).

For SNP3, the distribution of TT, TC, and CC genotypes was 66%, 26.8% and 7.2% respectively in MNE compared with 38%, 59% and 3% respectively in the controls ($p < 0.0001$), and TT genotype was higher in enuretics. The allele frequencies of T /C were 79.4% / 20.6% in MNE and 67.5% / 32.5 % in the controls ($p =$

0.003). The observed genotype counts were not deviated from those expected according to the HWE in patient and control groups ($p = 0.561$, and $p = 0.339$, respectively).

Potassium excretion (mmol/L), and FE_{K} (%) in the first urine sample of enuretics with TT genotype were higher than controls (58.07 ± 42.87 vs 35.21 ± 22.79 , $P < 0.01$, and 7.65 ± 7.49 vs 5.53 ± 4.42 , $p = 0.034$, respectively).

There was no correlation between the bedwetting episodes per week and duration of enuresis with FE_{Na} , FE_{K} , urinary Na^+ and K^+ excretion, and the distribution of genotypes, and allele frequencies ($P > 0.05$). No statistically significant relationship was found between the gender and presence of deep sleep ($p = 0.758$). Because of the small number of enuretics with CC genotype, we compared the relationship between the presence of deep sleep and TT and 'TC +CC' genotypes. Enuretic children with TT

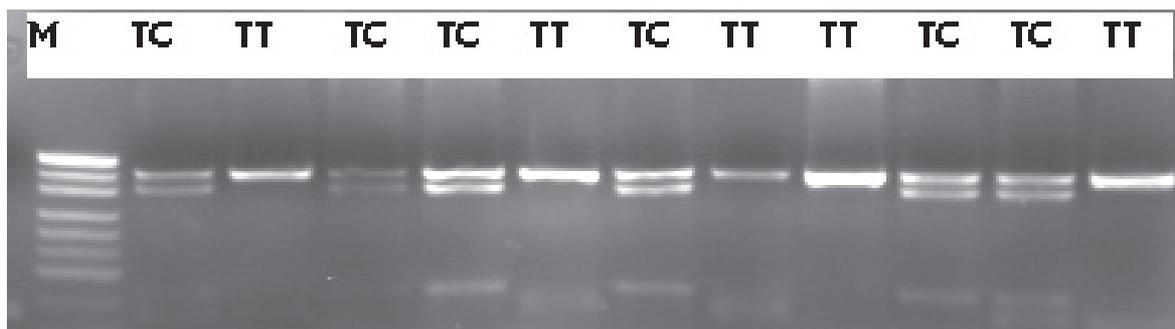


Fig. 1. KCNJ10 gene promoter polymorphism (M: pUC 19 MspI DNA leader).

Table III. The genotype distributions and allele frequencies of KCNJ10 gene promoter polymorphism in enuretic children and controls.

	Enuretics n (%)	Controls n (%)	Odds Ratio (95% CI)	P value
Genotype				
TT	64 (66)	38 (38)	3.16 (1.76-5.66)	<0.0001
TC	26 (26.8)	59 (59)	0.25 (0.13-0.46)	<0.0001
CC	7 (7.2)	3 (3)	2.51 (0.63-10.02)	0.08*
Total	97	100		
Allele				
T	154 (79.4)	135 (67.5)	1.85 (1.17-2.92)	0.003
C	40 (20.6)	65 (32.5)	0.53 (0.34-0.85)	0.003

*Fisher exact test

genotype had 4 times higher increased risk for the presence of deep sleep (odds=4,015 95%CI [1,562-10,320], while patients with T allele had 3.8 times higher risk compared to having C allele (odds=3,813, 95%CI [1,834-7,926]).

Discussion

Current data suggest that underlying mechanism of enuresis is mostly physiological reasons, rather than psychiatric factors. Some studies stressed a possible renal tubular maturation defect,^{4-6,14} and there are conflicting results in the literature on urinary electrolytes of enuretics. Some studies demonstrated higher FE_{Na} and FE_K excretions in enuretic children,⁵ and a significant increase in FE_{Na} and FE_K during the day and at night,⁶ while some others have found similar urinary Na^+ and K^+ values to controls.^{16,17} In this study, we also found significantly increased K^+ excretion in enuretics, similarly to the previous studies.^{5,6,14} Urinary K^+ predominantly derived from distal K^+ secretion, since filtered K^+ is reabsorbed almost entirely in proximal segments of the nephron.¹⁹ Therefore, increased K^+ excretion in those children suggests that there may be a problem in distal tubular regulation of K^+ , like a failure of some K^+ -regulating mechanisms. Excessive K^+ in the distal tubule together with the low ADH (which could not be shown in some studies) in enuretics may cause less tubular fluid reabsorption, and insufficiently reabsorbed K^+ remains in the distal tubule concomitantly with water. According to above hypothesis, all of these events may result in increased K^+ excretion (together with or without nocturnal polyuria) in those children,¹⁴ indicating that there may be a problem in renal regulation of K^+ in MNE. We investigated the relationship between urinary electrolytes, especially K^+ , and KCNJ10 gene promoter polymorphism which plays an important role in K^+ secretion in the distal nephron. This relationship has not been investigated previously.

Bockenbauer et al.²⁰ demonstrated that Kir 4.1 is expressed in the kidney with high specificity

only in the distal convoluted tubule (DCT) on the basolateral membrane, and speculated that Kir 4.1 is critical for K^+ recycling across the basolateral membrane of DCT cells. Potassium is taken up by the basolateral Na^+ , K^+ -ATPase and must be recycled to maintain Na^+ / K^+ pump activity, and loss of Kir 4.1 function may reduce the function of Na^+ , K^+ -ATPase.^{20,21} The exact role(s) of the inward-rectifying K channel Kir 4.1 in the distal nephron would be better understood by the continuing researches. Recently, an experimental study demonstrated that even modest reduction in Kir 4.1 expression results in impaired K conservation, which appears to be mediated by reduced expression of activated Na/Cl cotransporter expression.²²

In the present study, SNP3 in promoter of KCNJ10 gene was strongly associated with either distribution of genotype and allele frequency in enuretics, and TT genotype was associated with higher urinary K^+ excretion. We suggest that TT genotype and T allele of KCNJ10 gene may have an important role on renal tubular handling of K^+ in nephron. It would be better to determine also the relationship between KCNJ10 gene polymorphism and polyuria in enuretic children. Unfortunately, this parameter could not be evaluated because the families mostly refused to weigh overnight diaper(s) + the amount of first voided urine. This is one of the limitations of our study.

Although there are only a few studies regarding the sleep patterns of enuretic children,^{23,24} it is generally believed that they are deep-sleepers with impaired arousability.²⁵ We found that enuretic children with TT genotype had 4 times higher increased risk in terms of deep-sleep. KCNJ10 channel plays an important role in the central nervous system functioning.^{26,27} It is a possible candidate gene for Autism Spectrum Disorders, and also linked to seizure susceptibility both in humans and in experimental studies.²⁸⁻³² However, there is no study evaluating the possible relationship between sleep disorders and KCNJ10 in literature. One of the limitation of this study is that the presence of deep sleep is based on only

anamnesis, and polysomnography could not be performed. This may be an interesting finding to be tested in the future.

The present study demonstrated that KCNJ10 gene promoter polymorphism may be associated with MNE in Turkish children, and enuretics with TT genotype are prone to high urinary K⁺ excretion, and the presence of deep sleep. However, this does not mean that KCNJ10 gene polymorphism is the cause of MNE in children, but it may partially explain the different urinary K⁺ excretions found in several studies. Future studies investigating the other SNPs, mutations or altered regulation of Kir4.1 in larger samples may help to learn the main role of KCNJ10 gene in enuresis.

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