Genetic Etiology Investigation in Treatment-Resistant Nocturnal Enuresis Children: A Descriptive Study

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Purpose: Our study aimed to evaluate the genetic etiology of treatment-resistant nocturnal enuresis in children who did not respond to at least six months of behavioral therapy, urotherapy, alarm therapy, and medical treatment.

Materials and Methods: A total of 21 children, aged 5-18 years, diagnosed with treatment-resistant enuresis according to International Children's Continence Society (ICCS) guidelines were included. The capture-based Sophia Hereditary Disease Panel by Sophia Genetics was used to analyze a panel of 19 genes associated with nocturnal enuresis (AGXT, AQP2, AVPR2, BNC2, CLCNKB, DLG3, ELN, FA2H, FAM20A, FOXP1, HPSE2, KCNJ10, MLXIPL, NPHP3, RNF168, SLC12A3, SLC25A13, SLC5A2, SMARCA2).

Results: No pathogenic changes that could explain the etiology of the disease were detected in 20 patients. One patient exhibited a variant in the AQP2 gene (hg19:Chr12:50344908, exon 1, c.295G>A), which was classified as a Variant of Uncertain Significance (VUS) according to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines. The AQP2 gene is associated with autosomal dominant and autosomal recessive inherited nephrogenic diabetes insipidus (type 2) in the OMIM database.

Conclusion: Our findings support previous studies indicating that nocturnal enuresis does not have a monogenic etiology but rather results from multifactorial effects, with a weak correlation between genotype and phenotype.

Keywords: children; enuresis; genetics; treatment-resistant

INTRODUCTION

Enuresis is defined as recurrent, spontaneous urine leakage during sleep that persists beyond the age of normal urinary control maturation in a child.[1] The etiology and pathogenesis of nocturnal enuresis are not fully understood. Currently, nocturnal enuresis is generally considered a multifactorial disease resulting from the complex interaction of somatic, psychosocial, and environmental factors. Various hypotheses have been proposed to explain this condition, including genetic predisposition, detrusor overactivity, altered vasopressin circadian rhythm, nocturnal polyuria, decreased secretion of antidiuretic hormone during sleep, and renal dysfunction. Additionally, sleep disorders, difficulty awakening, stress, neurological or psychological disorders, and delays in brain development also influence the development of nocturnal enuresis.[2-4] The familial risk component of nocturnal enuresis has long been recognized, and studies have been conducted since the early 1990s to identify its genetic causes.[5] Studies have shown that approximately 45% of enuretic cases are consistent with autosomal dominant inheritance. Linkage analysis studies have identified four gene loci associated with nocturnal enuresis (8q, 12q, 13q13-13q14.2, 22q.11).[6,7] Based on this evidence, we aimed to evaluate the genetic etiology of patients who were unresponsive to all treatments applied for at least six months.

MATERIALS AND METHODS

Study Population

The study participants were patients diagnosed with treatment-resistant nocturnal enuresis between 2023 and 2024.

Study Design

A total of 21 patients were included in the study. Inclusion criteria comprised children aged 5-18 years diagnosed with treatment-resistant enuresis according to the International Children's Continence Society (ICCS) guidelines. Treatment resistance was defined as no improvement in enuretic attacks or less than a 50% decrease from baseline after at least six months of continuous treatment with good patient compliance. Treatment protocols included behavioral therapy, urotherapy, biofeedback, alarm therapy, and medical treatment (anticholinergics, desmopressin analogue). Peripheral blood samples were taken after obtaining written consent from the patients. Genomic DNA was extracted from peripheral blood anticoagulated with EDTA using standard methods (Qiagen). The concentration and quality of DNA samples were determined by fluorometry (Qubit v3.0) and UV spectrophotometry. Amplification of disease-associated gene regions was performed by polymerase chain reaction (PCR), and sequencing was performed using next-generation sequencing technology. The capture-based Sophia Hereditary Disease Panel

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Table 1. Demographic data, ultrasonography, treatment protocols, and family history of patients

DPatient	Age (years)	Gender	Family History	USG	Medication/Treatment Protocol	Additional Information / Enuretic Family Members
1	14	M	+	Normal	Oxybutynin, tolterodine, desmopressin	Cupping therapy / Father
2	12	M	+	Right kidney with duplex collecting system, no HN	Oxybutynin, tolterodine, desmopressin / Standard urotherapy, pelvic floor muscle rehabilitation	Mother
3	15	F	+	Normal	Oxybutynin, desmopressin, alarm therapy	Mother
4	11	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy, alarm therapy	Father
5	12	M	+	Normal	Oxybutynin, tolterodine, desmopressin / Standard urotherapy	Father
6	8	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Father
7	13	F	+	Normal	Oxybutynin, desmopressin / Standard urotherapy, alarm therapy	Mother
8	9	F	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Father
9	10	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Mother
10	7	F	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Mother
11	10	F	+	Normal	Oxybutynin, desmopressin / Standard urotherapy, pelvic floor rehabilitation	Mother
12	7	M	+	Left Grade 1 HN	Oxybutynin, desmopressin / Standard urotherapy, alarm therapy, pelvic floor rehabilitation	Father
13	7	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy, alarm therapy, pelvic floor rehabilitation	Father
14	12	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Father
15	10	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Mother
16	13	F	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Mother
17	7	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Sibling, Father
18	8	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Sibling
19	11	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Sibling, Mother
20	10	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy, alarm therapy	Mother
21	11	M	+	Normal	Oxybutynin, desmopressin / Standard urotherapy	Mother

Abbreviations: HN, Hydronephrosis; USG, Ultrasonography.

by Sophia Genetics was used, consisting of a panel of 19 genes (AGXT, AQP2, AVPR2, BNC2, CLCNKB, DLG3, ELN, FA2H, FAM20A, FOXP1, HPSE2, KCNJ10, MLXIPL, NPHP3, RNF168, SLC12A3, SLC25A13, SLC5A2, SMARCA2). Sequencing reactions were performed using the Illumina NextSeq® system.

Data Analysis

Raw data were analyzed through the Sophia DDM® data analysis platform. Alignment and variant calling were performed using Pepper®, Sophia Genetics' proprietary algorithm based on the hg19 human genome reference. Variant annotation was carried out using Sophia Genetics' MOKA® software, and various population frequencies (1000G, ESP, ExAC, gnomAD) and prediction algorithms (SIFT, PolyPhen) were used to determine the variant's effect. Copy number variation (CNV) detection was performed using Sophia Genetics' MUSKAT® software. For variant classification, expert working groups from the ClinVar database[8,9] and a database created by Maxwell et al.[10] were used as references. The classification of variants was based on the guidelines of the American College of Medical Genetics and Genomics (ACMG).[11] The allele frequency threshold for PM2 evidence was set at 0.0001.[12]

Table 2. Genes selected for nocturnal enuresis panel

Gene	Inheritance	Phenotype (OMIM)
AGXT	AR	Hyperoxaluria, primary, type 1
AQP2	AD, AR	Diabetes insipidus, nephrogenic, 2
AVPR2	XLR	Diabetes insipidus, nephrogenic, 1; Nephrogenic syndrome of inappropriate antidiuresis
BNC2	AD	Lower urinary tract obstruction, congenital
CLCNKB	AR, DR	Bartter syndrome, type 3; Bartter syndrome, type 4b, digenic
DLG3	XLR	Intellectual developmental disorder, X-linked 90
ELN	AD	Cutis laxa, autosomal dominant; Supravalvar aortic stenosis
FA2H	AR	Spastic paraplegia 35, autosomal recessive
FAM20A	AR	Amelogenesis imperfecta, type IG (enamel-renal syndrome)
FOXP1	AD	Intellectual developmental disorder with language impairment with or without autistic features
HPSE2	AR	Urofacial syndrome 1
KCNJ10	AR	Enlarged vestibular aqueduct, digenic; SESAME syndrome
MLXIPL	-	-
NPHP3	AR	Meckel syndrome 7; Nephronophthisis 3; Renal-hepatic-pancreatic dysplasia 1
RNF168	AR	RIDDLE syndrome
SLC12A3	AR	Gitelman syndrome
SLC25A13	AR	Citrullinemia, adult-onset type II; Citrullinemia, type II, neonatal-onset
SLC5A2	AD, AR	Renal glucosuria
SMARCA2	AD	Blepharophimosis-impaired intellectual development syndrome; Nicolaides-Baraitser syndrome

Abbreviations: AD, Autosomal dominant; AR, Autosomal recessive; XLR, X-linked recessive; DR, Digenic recessive; OMIM, Online Mendelian Inheritance in Man®.
Note on MLXIPL: The MLXIPL gene is one of the OMIM genes whose inheritance model and phenotype relationship are uncertain in the OMIM database; however, it is among the genes associated with enuresis in the HPO (The Human Phenotype Ontology) database.

RESULTS

Fifteen (71.4%) of the patients were male and six (28.5%) were female. The average age was 8.8 ± 2.4 years. No pathogenic changes that could explain the etiology of the disease were detected in 20 patients. Demographic data, medications used, and additional interventions are summarized in (**Table 1**). A heterozygous missense variant, c.295G >A p.(Ala99Thr), in the AQP2 gene was detected in one patient (a 14-year-old male). This variant was classified as a Variant of Uncertain Significance (VUS) according to the ACMG 2015 guidelines. The AQP2 gene is associated with autosomal dominant and autosomal recessive inherited diabetes insipidus (nephrogenic, type 2) in the OMIM database. The genes screened in the study are presented in (**Table 2**). Family screening was planned for the patient with the heterozygous finding.

DISCUSSION

Nocturnal enuresis is a common developmental disorder affecting approximately 15-20% of children around the age of 5 and nearly 2% of young adults.[13] It was first documented in the Ebers Papyrus around 1500 BCE.[14] It often leads to various degrees of psychosocial stress and self-esteem issues in affected children, which can contribute to depression, social maladjustment, and reduced sleep quality. Although the heritability of enuresis has been observed since the 19th century, this has not invalidated the widespread belief that enuresis arises from psychological mechanisms.[15] Therefore, understanding its etiological factors is important for treatment. The tendency for enuresis to occur in families has long been recognized and supported by epidemiological studies, twin studies, and formal genetics.[16,17] Many candidate "enuresis genes" were later identified,[18,19] but it also became apparent that no single gene explains all cases and that a weak correlation exists between genotype and phenotype.[20,21] This means that although multiple individuals in a family may experience enuresis, their prognosis and underlying pathogenetic mechanisms may vary significantly. The enuresis gene loci identified so far have not provided much useful information about pathogenic mechanisms. Genetic factors have been reported to increase the incidence of nocturnal enuresis by 77% if both parents have a history of enuresis (an 11.3-fold higher risk), by 44% if one parent has a history (a 5-7-fold higher risk), and by 15% if neither parent has a history.[22,23] All of our patients had a positive family history in first- and/or second-degree relatives. Three of our patients were siblings. In our cohort, one patient who had received pelvic floor muscle rehabilitation had a right duplex collecting system anomaly, and another had grade 1 hydronephrosis. The patient with the VUS in the AQP2 gene had undergone cupping therapy, an alternative medical treatment.

Our study has shown that even in cases of treatment-resistant nocturnal enuresis, where all modalities such as behavioral therapy, diet, anticholinergics, desmopressin, and urotherapy have failed to achieve a complete response after at least six months, the etiology remains multifactorial, with a weak correlation between genotype and phenotype, similar to non-resistant cases.

CONCLUSIONS

In our study, 19 genes associated with enuresis were analyzed, and only one VUS was observed in the AQP2 gene. These results support the existing literature, suggesting that treatment-resistant nocturnal enuresis does not have a monogenic etiology but occurs due to multifactorial effects with a weak genotype-phenotype correlation. We believe our study will pave the way for more comprehensive research on the etiology of nocturnal enuresis.

SUMMARY

This study investigated the genetic causes of bedwetting in 21 children who did not respond to standard treatments. A genetic link was not found in most cases, suggesting that treatment-resistant enuresis is caused by multiple factors, not a single gene.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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