A Regional Genetic Study of Primary Monosymptomatic Nocturnal Enuresis Using Chromosomal Microarray Analysis

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Purpose: This study aimed to investigate the genetic heterogeneity of primary monosymptomatic nocturnal enuresis (PMNE) and assess potential genetic variants contributing to its etiology.

Materials and Methods: A total of 92 children aged 5–15 years with a positive family history of PMNE were evaluated. All patients underwent detailed urological and nephrological assessments to exclude organic causes. Genetic testing was performed using high-resolution chromosomal microarray technology to identify potential pathogenic variants.

Results: No pathogenic or likely pathogenic copy number variations were identified. A small number of patients exhibited variants of uncertain significance (VUS), none of which were conclusively linked to PMNE after parental segregation analysis. Our findings challenge previous studies that reported significant genetic markers and highlight the complex genetic architecture of PMNE.

Conclusion: This study reinforces the genetic heterogeneity of PMNE and suggests it follows a polygenic and multifactorial inheritance pattern. Further research using whole-exome and whole-genome sequencing is needed to explore potential genetic contributors alongside environmental factors.

Keywords: primary monosymptomatic nocturnal enuresis; genetic analysis; chromosomal abnormalities; microarray; genetic heterogeneity

INTRODUCTION

Nocturnal enuresis (NE) is a common childhood disorder characterized by involuntary urination during sleep. The DSM-5 defines NE as recurrent nighttime urinary incontinence in children aged five years and older, occurring at least twice per week and impairing daily functioning, without an underlying medical cause.[1] Similarly, the International Children's Continence Society (ICCS) defines enuresis as nighttime urinary incontinence occurring at least once per month for at least three consecutive months. The ICCS further classifies enuresis into monosymptomatic nocturnal enuresis (MNE), which occurs without daytime symptoms, and non-monosymptomatic (NMNE), which involves additional lower urinary tract symptoms. This study focuses exclusively on primary monosymptomatic nocturnal enuresis (PMNE), which refers to cases where nocturnal bladder control has never been achieved after five years of age and daytime symptoms are absent.[2,3]
Nocturnal enuresis rates vary from 5.5% to 16.8% globally and from 11.9% to 19.3% in Turkiye. About 15% of cases resolve spontaneously each year, and the prevalence decreases with age.[4,5]
Nocturnal enuresis is a complex disorder with multiple contributing factors, including somatic, psychosocial, and environmental influences. Proposed etiological factors include disruption of the circadian rhythm of vasopressin, impaired renal sodium function, genetic predisposition, and delayed brain maturation. Neurophysiological studies suggest a lack of maturation in the brainstem and cerebral cortex of children with NE.[6] Low serum levels of vitamin B12 and folic acid, which are necessary for nervous system development, have been observed in children with PMNE, supporting the theory of maturational delays.[7]
Family studies consistently demonstrate the strong familial nature of NE. Children with one or both parents affected by the disorder have a 43% to 77% risk of developing NE themselves.[8] Twin studies provide further support for the hereditary nature of NE.[9] The co-occurrence of NE with attention-deficit/hyperactivity disorder (ADHD) and other neurological symptoms raises the possibility of shared etiological pathways or complex interactions between genetic and environmental factors.[10] Formal genetic research on NE has been conducted since the 1930s, with molecular genetic research commencing in 1995.[11] Many loci or chromosomal regions have been associated with NE; however, no conclusive results have been obtained (**Table 1**).
In a Danish study, Eiberg et al. identified 11 large families with autosomal dominant inheritance and high penetrance (90%) for PMNE. Five of these families showed linkage to chromosome 13q (13q13-13q14.2), but the arginine vasopressin gene (chromosome 20p13) was excluded.[12,14] Later reports identified linkage to

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Table 1. Summary of genetic loci and genes previously studied in relation to nocturnal enuresis.

Chromosome Location	Gene Name / Region	Gene Function / Description	Association Status
20p13	AVP	Arginine Vasopressin hormone	Excluded[14]
13q14.3	GUCY1B2	Guanylate Cyclase 1 Soluble Subunit Beta 2 (Pseudogene)	Excluded[18]
22q11	GNAZ	G Protein, α-z Polypeptide	Excluded[19]
12q13.12	AQP2	Aquaporin-2, collecting tubule protein	Excluded[20]
11p14.1	BDNF	Brain-derived neurotrophic factor	Excluded[21]
17q21.33	NGFR	Nerve Growth Factor Receptor	Excluded[21]
13q13-13q14.2	ENUR1 Locus	-	Linkage suggested, unproven[14]
8q	ENUR Locus	-	Linkage suggested, excluded in other families[13]
12q	ENUR2 Locus	-	Linkage suggested[16]
11p15.5	DRD4	Dopamine Receptor D4	Excluded[22]
12q24.22	NOS1	Nitric Oxide Synthase 1	Excluded[23]
7q36.1	NOS3	Nitric Oxide Synthase 3	Excluded[23]
13q14.2	HTR2A	5-Hydroxytryptamine Receptor 2A	Excluded[24]

chromosome 8q in two families unrelated to 13q, but linkage to 12q could not be verified in any of the families studied.[13] A Swedish study of 392 children with PMNE found that 43% of cases showed an autosomal dominant inheritance pattern.[15] From this cohort, 16 multigenerational families were selected for further linkage research. Three families displayed positive LOD scores for markers on chromosome 13q, while six families demonstrated linkage to a region on chromosome 12q.[16,17]

The genetic heterogeneity of NE is highlighted by these investigations, which show linkage and exclusion of linkage to distinct chromosomes (13q, 12q, or 8q) in various families. Methodological limitations, such as incompletely described phenotypes and potential recall bias in retrospectively acquired clinical data, are common. This study aims to characterize the clinical aspects of PMNE and uncover related genetic changes using high-resolution microarray analysis.

MATERIALS AND METHODS

Study Population

This observational study incorporated both retrospective and prospective elements. Patients aged 5–15 years with a diagnosis of non-organic enuresis (ICD-10: F98.0) were included. Patients were evaluated at the child and adolescent psychiatry outpatient clinic of Erzurum Regional Training and Research Hospital between January 2019 and January 2024. Prior to inclusion, all patients underwent a comprehensive pediatric assessment, including urological and nephrological evaluations, to rule out structural or functional abnormalities of the urinary tract. Only those with no identified organic pathology were referred to child psychiatry for their first psychiatric evaluation due to enuresis. The sample size of 92 children was determined based on feasibility constraints, primarily the cost of genetic testing and the availability of eligible participants.

Study Design

A child psychiatrist used the Affective Disorders and Schizophrenia Interview Schedule for School-Aged Children - Present and Lifetime Version (K-SADS-PL) to assess urinary incontinence and co-occurring psychiatric disorders. DSM-5 criteria were used to screen for other conditions. All participants had a documented family history of NE and were evaluated in the medical genetics outpatient clinic. The study comprised both treatment-naïve patients and those with prior pharmacological treatment. Enuresis frequency was assessed using voiding diaries. Patients were exclusively treated with behavioral interventions, and no pharmacological treatment for PMNE was administered. However, disorder-specific pharmacological treatments were prescribed for patients with comorbid psychiatric conditions.

Study Limitations

1. Limited Family Genetic Testing: Due to cost constraints, genetic testing was performed only on affected individuals. Parental testing was conducted only in cases with variants of uncertain significance (VUS) to assess segregation.
2. Lack of a Control Group: Since no clinically significant pathogenic mutations were identified, a control group was not included.
3. Genetic Testing Methods: The study utilized microarray analysis, but advanced techniques such as whole-exome sequencing (WES) or whole-genome sequencing (WGS) were not employed.
4. Sample Size: The relatively small sample size was primarily due to the high cost of genetic testing.

Ethical Considerations

Written informed consent was obtained from all patients or their legal guardians. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Health Sciences University, Erzurum Faculty of Medicine (BAEK 2024/03-70).

Genetic Analysis

A typical G-banding karyotype analysis was performed on each patient. For array comparative genomic hybridization (aCGH) analysis, genomic DNA was extracted from peripheral blood. Copy number variations (CNVs) were detected using Affymetrix CytoScan Optima 315K arrays (Thermo Fisher Scientific, Waltham, MA, USA). The aCGH data were assessed using Chromosome Analysis Suite 3.1.0 (Thermo Fisher Scientific). All CNVs were named in compliance with GRCh37 (hg19).

The chromosomal abnormalities identified were validated in the index cases and/or parents using available FISH probes. Detected CNVs were evaluated using the American College of Medical Genetics (ACMG) criteria and categorized as pathogenic, VUS, or benign based on size, gene content, inheritance pattern, and frequency in population databases. The Online Mendelian Inheritance in Man (OMIM), DECIPHER, Database of

Genomic Variants (DGV), ClinGen, and PubMed were consulted to assess the pathogenicity of novel CNVs.

RESULTS

Children aged 5 to 15 years were enrolled in the study, with a mean age of 9.6 ± 2.3 years. Among the participants, 24 (26.0%) were female and 68 (74.0%) were male. The mean frequency of MNE episodes was 17.5 nights over a 30-day period.

A comorbid psychiatric condition was present in 60 children (65.2%), with ADHD being the most common (n = 26, 28.3%). Anxiety disorders (n = 14, 15.2%) and encopresis (n = 20, 21.7%) were other prevalent comorbidities.

The family history analysis indicated that NE affected 20 first-degree relatives (21.7%), 36 second-degree relatives (39.1%), and 10 third-degree relatives (10.9%).

Four patients had chromosomal alterations identified by aCGH: one with arr[GRCh37] 10q23.1(86,242,837_87,133,028)x3, one with arr[GRCh37] Xq21.3(94,185,517_94,471,709)x0, one with both arr[GRCh37] 21q22.12(35,964,093_36,380,852)x3 and arr[GRCh37] Xp22.31(6,516,735_8,131,442)x1, and one with arr[GRCh37] 22q12.3(34,144,498_34,251,544)x1. However, all these findings were classified as VUS. Family screening was performed in three patients with VUS. Parental analysis revealed that one variant was inherited from the mother and the other two from the father. As these variants were familial and did not segregate with the disease, they were not considered clinically significant.

DISCUSSION

This study aimed to investigate whether clinical features, family history, and genetic variations influence the genetic background of PMNE. The average age of our cohort was 9.6 years, and we observed a male predominance, consistent with previous reports.[4,25,26]

A strong familial component is evident in NE. Studies indicate that the incidence is 44.0% when one parent is affected and rises to 77.0% when both parents are affected, compared to 15.0% when neither is.[8] Our findings also show a high prevalence among first- and second-degree relatives. Genetic studies on NE face challenges, including high spontaneous recovery rates and recall bias due to social stigma.[5]

Previous genetic studies have suggested linkages on chromosomes 8, 12, and 13 using techniques like Multiplex Ligation-dependent Probe Amplification (MLPA) and Polymerase Chain Reaction (PCR).[28] However, these methods have limited resolution.[29] Our study utilized an advanced microarray platform with high-resolution screening. Despite this comprehensive approach, no pathogenic or likely pathogenic mutations were identified, challenging previous findings and emphasizing the genetic complexity of PMNE.[30]

The inconsistency of results across studies suggests that PMNE follows a polygenic and multifactorial model rather than a simple Mendelian inheritance pattern.[31] Our results reinforce the genetic and clinical heterogeneity of PMNE. The absence of pathogenic mutations in our cohort, despite high-resolution screening, highlights the need for larger-scale investigations using WES and WGS to further explore genetic contributors. Given the complexity of genetic inheritance and incomplete penetrance in enuresis, careful sample selection is crucial. In this study, we applied strict inclusion criteria, requiring a documented family history of NE in at least one first- or second-degree relative to maximize the likelihood of detecting relevant genetic variations.

CONCLUSIONS

This study underscores the genetic heterogeneity of PMNE and the absence of a single definitive genetic cause. Despite employing high-resolution microarray technology, no pathogenic or likely pathogenic mutations were detected. These findings question the reliability of prior genetic association studies and indicate that PMNE likely follows a polygenic and multifactorial inheritance pattern rather than being a single-gene Mendelian disorder. The inconsistencies across studies emphasize the need for larger-scale investigations using WES and WGS, integrating environmental factors to provide a more comprehensive understanding of PMNE.

SUMMARY

This study explored the genetic basis of primary monosymptomatic nocturnal enuresis (PMNE) in 92 children using advanced microarray analysis. No specific pathogenic gene mutations were found, suggesting that bedwetting is likely caused by a complex combination of multiple genes and environmental factors rather than a single gene.

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AUTHOR CONTRIBUTIONS

Oğuzhan Yaralı conceived and designed the study, performed the genetic analyses, and drafted the manuscript. Yüksel Sümeyra Naralan contributed to selecting patients, collecting clinical data, and critically revising the manuscript. Both authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

Qualified investigators can obtain anonymized data from the corresponding author upon reasonable request.

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