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BRIEF REPORT

Prevalence of *MTHFR* Polymorphisms in Patients With Hypermobile Ehlers-Danlos Syndrome and Hypermobile Spectrum Disorders in a US Hypermobility Clinic

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Objective. Hypermobile Ehlers-Danlos syndrome (hEDS) and hypermobility spectrum disorders (HSD) are characterized by joint hypermobility, joint subluxations and dislocations, hyperextensible skin, and chronic and progressive multiorgan comorbidities. Diagnosing hEDS and HSD is difficult because of variable phenotypes and unknown genetic etiology. In our clinic, we observed many patients with hEDS and HSD with a high serum level of unmetabolized folate, which suggests that hypermobility may be linked to methylenetetrahydrofolate reductase (*MTHFR)*–mediated folate metabolism. The present study aims to examine the prevalence of *MTHFR* polymorphisms, C677T and A1298C, among patients with hEDS and HSD.

Methods. Clinical and demographic information of patients visiting our hypermobility clinic from January 2023 to July 2023 were retrospectively reviewed. Continuous variables were reported as mean ± SD and range, whereas categorical variables were reported as total count and percentage.

Results. Among 157 patients, 93% of patients were female patients, 52.2% were diagnosed with hEDS, and 47.8% were diagnosed with HSD. Interestingly, 85% of the patients had *MTHFR* C677T and/or A1298C polymorphisms in heterozygous or homozygous state. *MTHFR* 677CT/TT genotype was present in 52.9% of cases, and 49.7% of patients had 1298AC/CC genotype. In addition,14% of patients with hypermobility exhibited *MTHFR* 677TT genotype, 10.2% showed 1298CC genotype, and 17.2% displayed combined heterozygosity, collectively representing 41.4% hypermobile patients with two copies of *MTHFR* variant alleles.

Conclusion. There is a high prevalence of *MTHFR* polymorphisms among patients with hypermobility, which supports the hypothesis that hypermobility may be dependent on folate status.

INTRODUCTION

Hypermobile Ehlers-Danlos syndrome (hEDS) and hypermobility spectrum disorders (HSD) are two distinct but related connective tissue disorders, characterized by joint hypermobility, joint subluxations or dislocations, hyperextensible skin, chronic pain, and progressive multiorgan comorbidities.[1] Patients often experience debilitating physiological and psychological impairments.[2] It is estimated that around 3.4% of the population worldwide have hEDS or HSD.[3] Notably, individuals with HSD do not meet all the criteria necessary for EDS diagnosis. On the other hand, hEDS is 1 of 13 EDS subtypes, each subtype distinguished by specific mutations in collagen and extracellular matrix synthesis and maintenance genes.[4] Although hEDS represents the most prevalent subtype within EDS, the genetic cause for this, as well as HSD, remains unknown. Overall, the considerable variability in clinical presentations of patients with hEDS and HSD, coupled with the lack of definitive genetic test, makes distinct diagnosis difficult.

Folate, also known as vitamin B9, is known to affect multiple physiological processes like nucleotide synthesis, amino acid homeostasis, homocysteine metabolism, epigenetic

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regulation via S-adenosyl methionine, and antioxidant defense.[5] Methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in folate metabolism, which irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulating form of folate. There are two common polymorphisms in the *MTHFR* gene, C677T and A1298C, which are associated with reduced enzyme activity. The C677T polymorphism causes an alanine to valine substitution at codon 222 in the N-terminal catalytic domain, resulting in thermolabile enzyme.[6] As a result, individuals who are heterozygous and homozygous for the C677T polymorphism have 65% and 30% of normal enzyme activity, respectively, and higher homocysteine levels.[6] *MTHFR* A1298C causes glutamic acid to an alanine change at codon 429 in the C-terminal regulatory domain and results in 85% and 60% of normal enzyme activity in individuals with 1298AC and CC genotype, respectively, and with normal homocysteine level.[7] In addition, the combined heterozygous genotype, *MTHFR* 677CT1298AC, leads to reduced enzyme activity and homocysteine levels comparable to those observed in individuals with *MTHFR* 677TT genotype.[7]

At the Tulane Fascia Institute and Treatment Center, we observed many patients with hEDS and HSD with a high serum level of unmetabolized folate. We have previously proposed a mechanistic hypothesis linking hypermobility to *MTHFR*-dependent folate status via regulation of extracellular matrix proteases.[8] In this study, we report the frequencies of *MTHFR* polymorphisms, C677T and A1298C, among the patients with hEDS and HSD evaluated in our clinic, providing clinical data in support of our theory on folate-based hypermobility disorders.

PATIENTS AND METHODS

This retrospective observational study presents data from the medical records of the Tulane Fascia Institute and Treatment Center. Demographic and clinical details of the patients with HSD and patients with hEDS who visited the clinic during January 2023 to July 2023 were collected. Continuous variables were reported as mean ± SD and range, whereas categorical variables were reported as total count and percentage. This study was approved by the Biomedical Institutional Review Board at Tulane University (2020-1543) on June 26, 2023.

RESULTS

A total of 157 patients who visited the hypermobility clinic during January 2023 to July 2023 were included in the study. The majority of patients (93%) were female. About 48% of patients were diagnosed with HSD, and 52% of patients were diagnosed with hEDS (Table 1). The mean age of patients was 37.09 ± 14.60 years, and the age of patients ranges between 13 to 70 years (Table 1).

We evaluated the two most common *MTHFR* polymorphisms, 677C>T and 1298A>C, in our patients. Interestingly, we observed that approximately 85% of patients had *MTHFR* C677T and/or A1298C polymorphisms in heterozygous or homozygous state. *MTHFR* 677CT or TT genotype was present in 52.9% of all patients, 57.3 % of patients with hEDS, and 48.0% of patients with HSD. At the allele level, *MTHFR* 677T allele was observed in 33.4% of all patients, 35.4% of patients with hEDS, and 31.3% of patients with HSD. Similarly, *MTHFR 1298AC* or *CC* genotype was observed in 49.7% of all cases, 46.3% of patients with hEDS, and 53.3 % of patients with HSD. The variant C allele of *MTHFR 1298* polymorphism was found in 30.3% of total patients, 28.7% of patients with hEDS, and 32.0% of patients with HSD (Table 2). In addition, we found that the *MTHFR* 677CT genotype was more prevalent in patients with hEDS (43.9%) compared with patients with HSD (33.3%), whereas the *MTHFR* 1298AC genotype frequency was higher in patients with HSD (42.7%) compared with patients with hEDS (35.4%, Table 2).

We further evaluated the combined genotype frequencies of *MTHFR* 677C>T and A1298A>C polymorphisms in our patients. There are nine possible genotype combinations for *MTHFR* 677and 1298 polymorphisms. Among them, 677CT1298AA and 677CC1298AC were present at approximately 21% frequency each. Whereas 677CT1298AC, the combined heterozygous genotype, was observed in 17.2% of patients (Table 2), the frequencies of 677CC1298CC and 677TT1298AA genotypes were 10.2% and 14.0%, respectively. The 677CT1298CC genotype was very rare and observed in only 0.6% of total cases, whereas 677TT1298AC

Table 1. Characteristics of patients included in the study*

Case type	N (%)	Age range, mean age ± SD, y	Sex	
			Male patients, n (%)	Female patients, n (%)
Total number of cases	157 (100)	13–70 (37.09 ± 14.60)	11 (7.0)	146 (93.0)
HSD	75 (47.8)	13–70 (37.17 ± 14.18)	5 (6.7)	70 (93.3)
hEDS	82 (52.2)	13–65 (37.01 ± 15.07)	6 (7.3)	76 (92.7)

*hEDS, hypermobile Ehlers-Danlos syndrome; HSD, hypermobile spectrum disorders.

Table 2. Distribution of *MTHFR* polymorphisms in patients visiting our clinic during January 2023 to July 2023*

Polymorphisms	All patients, n (%)	Patients with hypermobile EDS, n (%)	Patients with hypermobile spectrum disorders, n (%)
MTHFR C677T and A1298C polymorphisms			
Present	133 (84.7)	69 (84.1)	64 (85.3)
Absent	24 (15.3)	13 (15.9)	11 (14.7)
MTHFR C667T			
Genotypes			
CC wild homozygous	74 (47.1)	35 (42.7)	39 (52.0)
CT heterozygote	61 (38.9)	36 (43.9)	25 (33.3)
TT mutant homozygous	22 (14.0)	11 (13.4)	11 (14.7)
CT+TT heterozygote+mutant homozygous	83 (52.9)	47 (57.3)	36 (48.0)
Allele			
C	209 (66.6)	106 (64.6)	103 (68.7)
T	105 (33.4)	58 (35.4)	47 (31.3)
MTHFR A1298C			
Genotypes			
AA wild homozygous	79 (50.3)	44 (53.7)	35 (46.7)
AC heterozygote	61 (38.9)	29 (35.4)	32 (42.7)
CC mutant homozygous	17 (10.8)	9 (10.9)	8 (10.6)
AC+CC heterozygous+mutant homozygous	78 (49.7)	38 (46.3)	40 (53.3)
Allele			
A	219 (69.7)	117 (71.3)	102 (68.0)
C	95 (30.3)	47 (28.7)	48 (32.0)
MTHFR C677T and A1298C combined genotypes			
677CC1298AA wild homozygous	24 (15.3)	13 (15.9)	11 (14.7)
677CC1298AC heterozygous	34 (21.7)	13 (15.9)	21 (28.0)
677CC1298CC mutant homozygous	16 (10.2)	9 (10.9)	7 (9.3)
677CT1298AA heterozygous	33 (21.0)	20 (24.4)	13 (17.3)
677CT1298AC combined heterozygous	27 (17.2)	16 (19.5)	11 (14.7)
677CT1298CC combined heterozygous and mutant homozygous	1 (0.6)	0 (0)	1 (1.3)
677TT1298AA mutant homozygous	22 (14.0)	11 (13.4)	11 (14.7)
677TT1298AC combined heterozygous and mutant homozygous	0 (0)	0 (0)	0 (0)
677TT1298CC combined homozygous mutant	0 (0)	0 (0)	0 (0)

*EDS, Ehler-Danlos syndrome.

and 677TT1298CC genotypes were not detected in hypermobile patients (Table 2).

DISCUSSION

In this retrospective study, we observed that the majority of the patients with HSD and patients with hEDS seen at the Tulane Fascia Institute and Treatment Center were either heterozygous or homozygous for *MTHFR* C677T or A1298C polymorphisms. Only 15.3% of patients were not polymorphic at these two *MTHFR* loci. In addition, an increased prevalence of *MTHFR* 677T allele (33.4%) and 1298C allele (30.3%) was observed among patients with hypermobility compared with global frequencies of 677T allele (24.5%) and 1298C allele (24.9%) reported in 1000G database.[9] At the genotype level, we observed that 14% of patients with hypermobility exhibited *MTHFR* 677TT homozygosity, 10.2% showed 1298CC homozygosity, and 17.2% displayed combined heterozygosity for the *MTHFR* 677 and 1298 loci, collectively representing 41.4% of patients with hypermobility with two copies of *MTHFR* variant alleles (Table 2). Overall, the high prevalence of *MTHFR* polymorphisms in patients with hypermobility suggests significant involvement of *MTHFR* in the development of HSD and hEDS. In addition, we observed that *MTHFR* 677CT genotype was more prevalent in patients with hEDS compared with patients with HSD, whereas *MTHFR* 1298 AC frequency was higher in patients with HSD compared with patients with hEDS (Table 2). Because the *MTHFR 677*CT genotype is associated with a considerable decrease in enzymatic activity compared with the 1298AC genotype,[6,7] increased frequency of *MTHFR* 677CT could be one of the possible reasons for the severe phenotype observed in patients with hEDS.

Previous studies suggest that hypermobility is generally more prevalent in female patients compared with male patients.[10–12] Consistent with these reports, we found that the majority of patients with hEDS and HSD in our study were female. This gender discrepancy in hypermobility could be because of several factors,[13] including differences in hormone levels between male and female patients, particularly estrogen, which may affect the laxity of connective tissues and may contribute to increased joint flexibility in female patients[14] and biomechanical differences in joint structure and musculature between sexes.[15,16] However, the exact interplay of these factors and their contribution to the

higher prevalence of hypermobility in female patients compared with male patients requires further research for a comprehensive understanding.

In conclusion, the high prevalence of *MTHFR* polymorphism in patients with hypermobility underscores the gene's potential role in the maintenance of connective tissue integrity and potentially supports our hypothesis that hypermobility may be dependent on folate status. However, further research is needed to comprehensively understand the mechanisms involved and potential therapeutic implications.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr Bix had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Courseault, Bix.

Acquisition of data. Bordnick, Simons, Volic.

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