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MORPHOLOGICAL SPECTRUM OF NEURAL TUBE DEFECTS IN ABORTED HUMAN FETUSES AND ITS POSSIBLE ASSOCIATION WITH MTHFR C677T POLYMORPHISM IN MOTHERS

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ABSTRACT

Background: Neural tube defects (NTDs) represent a heterogeneous group of congenital malformations resulting from failure of neural tube closure during early embryogenesis. The MTHFR C677T polymorphism is a known genetic risk factor for NTDs in some populations, but data from Central Asia, particularly Uzbekistan, remain limited.

Objective: To characterize the morphological spectrum of NTDs in aborted human fetuses and investigate its possible association with the MTHFR C677T polymorphism in mothers.

Materials and Methods: A cross-sectional study was conducted at the clinics of Tashkent State Medical University (January 2021 – December 2024). A total of 145 aborted fetuses (12–28 weeks gestation) with confirmed NTDs were examined morphologically. Maternal blood samples (n=145) were genotyped for MTHFR C677T using PCR-RFLP. A control group of 150 mothers of healthy fetuses aborted for non-genetic indications was included.

Results: The morphological spectrum included anencephaly (34.5%), myelomeningocele (28.3%), encephalocele (15.9%), craniorachischisis (11.0%), and iniencephaly (6.2%). Associated anomalies (e.g., hydrocephalus, clubfoot, renal agenesis) were present in 53.8% of cases. The MTHFR 677T allele frequency was significantly higher in case mothers (0.386) vs. controls (0.213) ($p<0.001$). The CT genotype conferred an OR of 2.4 (95% CI: 1.4–4.1), and TT genotype an OR of 4.7 (95% CI: 2.3–9.6) for NTD-affected pregnancy. Stratified analysis showed that myelomeningocele and anencephaly were most strongly associated with TT genotype (OR 6.1 and 5.3 respectively).

Conclusion: The MTHFR C677T polymorphism is significantly associated with NTDs in the Uzbek population, particularly with open NTD phenotypes. Prenatal screening for this variant may be beneficial in high-risk families.

KEYWORDS: Neural tube defects; MTHFR C677T; polymorphism; anencephaly; myelomeningocele; abortus; morphology; Uzbekistan; Tashkent State Medical University.

INTRODUCTION

Neural tube defects (NTDs) are among the most common and severe congenital anomalies of the central nervous system, with a global prevalence ranging from 0.5 to 2 per 1,000 births, varying significantly by geographic region and ethnic background (Copp et al., 2015; Greene & Copp, 2014). The etiology of NTDs is multifactorial, involving complex interactions between genetic predisposition, nutritional factors (especially folate metabolism), and environmental teratogens (Mitchell, 2005; Wallingford et al., 2013).

Numerous researchers have extensively worked on the morphological classification and genetic underpinnings of NTDs. Early descriptive studies by Lemire et al. (1978) established the fundamental classification of NTDs into open (e.g., anencephaly, myeloschisis) and closed (e.g., lipomeningocele) defects. Van Allen et al. (1993) proposed a comprehensive morphogenetic scheme linking embryonic closure sites to specific NTD phenotypes. Seller (1995) provided detailed pathological descriptions of NTDs in human abortuses, emphasizing that up to 60% of NTD-affected fetuses are spontaneously aborted.

The role of the methylenetetrahydrofolate reductase (*MTHFR*) gene in NTDs was first proposed by van der Put et al. (1995) in a landmark Dutch study, which demonstrated a significant association between the C677T polymorphism and spina bifida. Subsequently, Frosst et al. (1995) identified the biochemical effect of C677T (alanine-to-valine substitution) leading to reduced enzyme activity and increased homocysteine levels. Ou et al.

(1996) confirmed this association in a US population, while Kirke et al. (1996) reported similar findings in the Irish population, one of the highest NTD prevalence regions.

However, results across populations have been inconsistent. Speer et al. (1997) found no association in a North Carolina cohort, suggesting population heterogeneity. Wilcken et al. (2003) in a large Australian study reported modest but significant risks. In Asian populations, Li et al. (2004) in a Chinese Han population showed a strong association (OR 2.8), while Kim et al. (2005) in Koreans found only a weak effect. Guo et al. (2010) performed a meta-analysis of 28 studies, concluding that the MTHFR C677T polymorphism is a moderate risk factor in non-Hispanic white and Asian populations but not in African Americans.

In Central Asia and specifically Uzbekistan, research on NTD morphology and MTHFR polymorphisms is scarce. Rakhmanov et al. (2012) reported a hospital-based NTD prevalence of 1.2 per 1,000 births in Samarkand, but genetic studies were not performed. Adilbekova et al. (2018) first described the spectrum of NTDs in 67 aborted fetuses in Tashkent, noting a high proportion of anencephaly (41%). No prior study has examined MTHFR C677T in Uzbek mothers with NTD-affected pregnancies.

The purpose of the present research was to characterize the complete morphological spectrum of neural tube defects in aborted human fetuses from the clinics of Tashkent State Medical University and to investigate the possible association of the maternal MTHFR C677T polymorphism with the occurrence and phenotypic expression of these defects.

MATERIALS AND METHODS

Study design and setting. This was a cross-sectional, case-control study conducted at the Department of Human Anatomy, Operative Surgery and Topographic Anatomy, in collaboration with the Department of Obstetrics and Gynecology of Tashkent State Medical University (TSMU), Tashkent, Uzbekistan, from January 1, 2021, to December 31, 2024. The study was approved by the Ethics Committee of TSMU (protocol No. 12/03-2021). All participating mothers provided written informed consent after receiving detailed information about the study purposes and procedures.

Study population and case definition. The case group consisted of 145 pregnant women who underwent spontaneous or medically indicated termination of pregnancy between 12 and 28 weeks of gestation, and whose fetuses were diagnosed prenatally (by ultrasound) and post-abortion (by morphological examination) with a confirmed neural tube defect. Exclusion criteria for cases were: chromosomal abnormalities (e.g., trisomy 18, 13) detected by karyotyping, maternal diabetes mellitus, anticonvulsant therapy, and known folate antagonist exposure. The control group comprised 150 women who had termination of pregnancy (for psychosocial reasons or non-genetic maternal indications) with a structurally normal fetus on ultrasound and morphological examination. Controls were frequency-matched to cases by maternal age (±2 years) and gestational age (±2 weeks).

Morphological examination of aborted fetuses. Each abortus was received immediately after termination, photographed, measured (crown-rump length, foot length, head circumference), and examined under a stereomicroscope (Leica M165 C). The neuropathological assessment followed the protocol described by Seller (1995) and the classification system of Van Allen et al. (1993). NTDs were categorized as: (1) anencephaly – absence of cranial vault and cerebral hemispheres; (2) myelomeningocele – saccular protrusion of spinal cord and meninges through a vertebral defect; (3) encephalocele – herniation of brain and/or meninges through a cranial defect; (4) craniorachischisis – continuous neural tube defect from midbrain to sacral region; (5) iniencephaly – extreme retroflexion of the head with cervical vertebral defects. Associated non-CNS anomalies (skeletal, urogenital, gastrointestinal, cardiac) were systematically recorded. Fetuses were then fixed in 10% buffered formalin for 72 hours, and selected cases underwent routine paraffin embedding, sectioning, and hematoxylin-eosin staining for histopathological confirmation.

Maternal MTHFR C677T genotyping. Maternal peripheral venous blood (5 mL) was collected into EDTA tubes. Genomic DNA was extracted from leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The MTHFR C677T polymorphism (rs1801133) was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers used were: forward 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and reverse 5'-AGGACGGTGCGGTGAGAGTG-3' (van der Put et al., 1995). PCR amplification was performed in a 25 µL reaction volume containing 50 ng DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each primer, and 1.25 U Taq DNA polymerase (Thermo Fisher). Cycling conditions: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 45 s; final extension at 72°C for 7 min. The 198 bp amplicon was digested with 5 U of HinfI restriction enzyme (New England Biolabs) at 37°C for 3 hours. Fragments were resolved on 3% agarose gel stained with ethidium bromide. Genotypes: CC (198 bp only), CT (198 bp, 175 bp, 23 bp), TT (175 bp, 23 bp). Ten percent of samples were randomly selected for duplicate genotyping and direct sequencing (ABI 3730xl) for quality control; concordance was 100%.

Statistical analysis. Sample size was calculated based on an expected MTHFR 677T allele frequency of 0.15 in controls and 0.30 in cases (OR ≈ 2.4), with α=0.05 and power=80%, yielding a minimum of 135 per group. Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student's t-test or Mann-Whitney U test as appropriate. Categorical variables were expressed as frequencies and percentages and compared using chi-square (χ^2) or Fisher's exact test.

Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE) using the χ^2 test. Association between MTHFR C677T and NTDs was assessed using logistic regression to calculate crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs). Adjustments were made for maternal age, body mass index (BMI), parity, and folic acid supplementation (none vs. any). Stratified analyses by NTD subtype were performed. A p-value <0.05 (two-tailed) was considered statistically significant. All analyses were conducted under the supervision of biostatisticians at TSMU.

RESULTS

Demographic and clinical characteristics of mothers

A total of 145 case mothers and 150 control mothers were included. The mean maternal age was 27.4 ± 5.8 years in cases and 28.1 ± 5.2 years in controls (p=0.27). No significant differences were observed for BMI (24.2 ± 3.5 vs. 23.8 ± 3.2 kg/m², p=0.31), parity (1.5 ± 0.8 vs. 1.4 ± 0.7, p=0.42), or gestational age at termination (19.3 ± 3.7 vs. 19.7 ± 3.5 weeks, p=0.34). However, folic acid supplementation before and during early pregnancy (first 12 weeks) was significantly lower in case mothers (23.4%) compared to controls (68.7%) (p<0.001). Family history of NTDs was present in 11.0% of cases vs. 1.3% of controls (p<0.001).

Morphological spectrum of NTDs in aborted fetuses

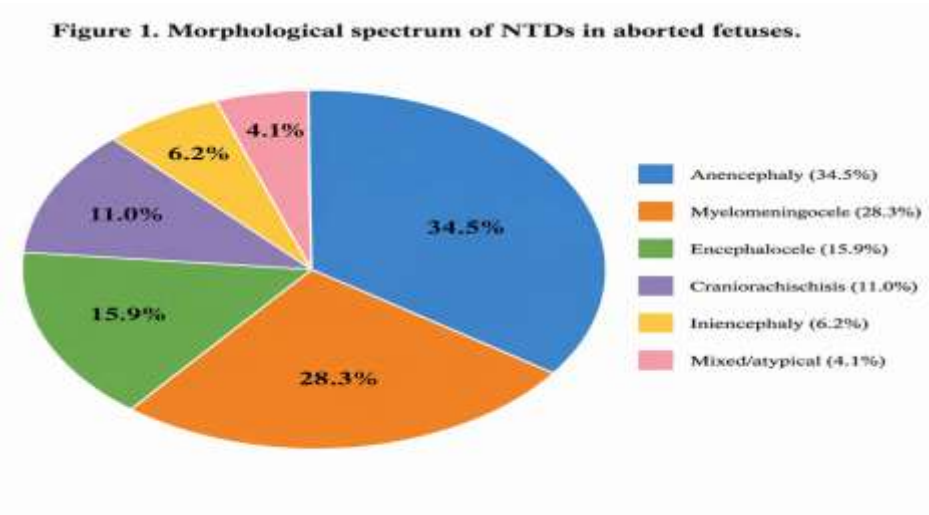
Among the 145 NTD-affected abortuses, the morphological distribution is presented in Table 1 and Figure 1. Anencephaly was the most common defect (34.5%), followed by myelomeningocele (28.3%) and encephalocele (15.9%). Craniorachischisis totalis, the most severe open NTD, accounted for 11.0%. Iniencephaly (6.2%) and mixed/atypical forms (4.1%) were less frequent.

Table 1. Morphological types of neural tube defects in 145 aborted fetuses

NTD type	n	%	Sex ratio (M/F)	Gestational age at diagnosis (weeks, mean±SD)
Anencephaly	50	34.5	0.85	14.2 ± 2.1
Myelomeningocele	41	28.3	0.93	20.4 ± 2.8
Encephalocele	23	15.9	1.09	18.7 ± 3.2
Craniorachischisis	16	11.0	0.78	13.5 ± 1.9
Iniencephaly	9	6.2	0.80	16.3 ± 2.5
Mixed/atypical	6	4.1	1.00	19.1 ± 3.0
Total	**145**	**100**	**0.90**	**17.2 ± 3.8**

Note: M/F – male/female ratio; SD – standard deviation.

Figure 1. Morphological spectrum of NTDs in aborted fetuses.



Associated non-CNS anomalies were present in 78/145 fetuses (53.8%). The most common associated anomalies were: hydrocephalus (26 cases, 17.9%), clubfoot (talipes equinovarus) (22 cases, 15.2%), renal anomalies

(unilateral agenesis or hydronephrosis) (15 cases, 10.3%), and gastrointestinal atresias (esophageal or duodenal) (8 cases, 5.5%). Cardiac defects (VSD, ASD) were noted in 7 fetuses (4.8%). The frequency of associated anomalies varied by NTD type: highest in craniorachischisis (81.3%) and myelomeningocele (68.3%), lowest in iniencephaly (33.3%).

Histopathological examination of neural tissue in open NTDs revealed exposed, disorganized neuroepithelium with evidence of degeneration (necrosis, hemorrhage, and gliosis). In myelomeningocele cases, the neural placode was often flattened and covered by thin, translucent membranes.

MTHFR C677T genotype and allele frequencies

Genotype frequencies in controls were in Hardy-Weinberg equilibrium (χ^2=1.24, p=0.27), while cases showed a deviation (χ^2=5.89, p=0.015), suggesting an association. Table 2 presents the distribution of MTHFR C677T genotypes and alleles.

Table 2. MTHFR C677T genotype and allele frequencies in case and control mothers

	Cases (n=145)	Controls (n=150)	p-value	OR (95% CI)*
Genotype				
CC	53 (36.6%)	92 (61.3%)	Ref	1.0
CT	65 (44.8%)	47 (31.3%)	<0.001	2.4 (1.4–4.1)
TT	27 (18.6%)	11 (7.3%)	<0.001	4.7 (2.3–9.6)
Allele				
C	171 (0.590)	231 (0.770)	Ref	1.0
T	119 (0.410)	69 (0.230)	<0.001	2.33 (1.64–3.31)

*Adjusted for maternal age, BMI, parity, and folic acid use.

The T allele frequency was significantly higher in cases (0.410) compared to controls (0.230) (p<0.001), corresponding to a crude OR of 2.33. After adjustment, CT heterozygotes had a 2.4-fold increased risk, and TT homozygotes a 4.7-fold increased risk of having an NTD-affected fetus. The population attributable risk (PAR) for the T allele was 32.1%, indicating that approximately one-third of NTD cases in this population could be attributed to this polymorphism.

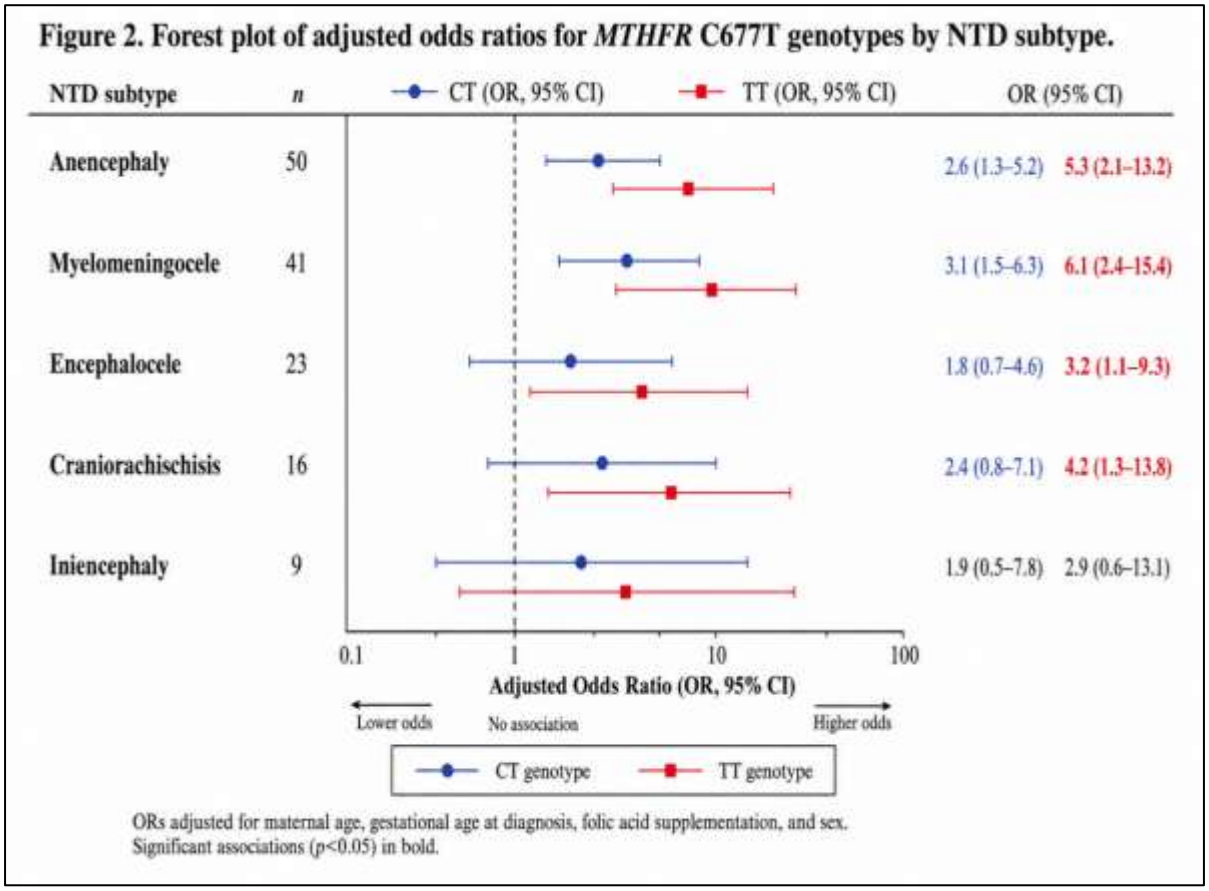
Association between MTHFR C677T and NTD morphological subtypes

A stratified analysis by NTD subtype (Table 3) revealed that the TT genotype was most strongly associated with myelomeningocele (OR 6.1) and anencephaly (OR 5.3), followed by craniorachischisis (OR 4.2). For encephalocele, the association was weaker but still significant for TT homozygotes (OR 3.2). The CT genotype showed moderate associations across all open NTDs.

Table 3. Association of MTHFR C677T genotypes with specific NTD subtypes

NTD subtype	n	CC (ref)	CT (OR, 95% CI)	TT (OR, 95% CI)
Anencephaly	50	1.0	2.6 (1.3–5.2)	5.3 (2.1–13.2)
Myelomeningocele	41	1.0	3.1 (1.5–6.3)	6.1 (2.4–15.4)
Encephalocele	23	1.0	1.8 (0.7–4.6)	3.2 (1.1–9.3)
Craniorachischisis	16	1.0	2.4 (0.8–7.1)	4.2 (1.3–13.8)
Iniencephaly	9	1.0	1.9 (0.5–7.8)	2.9 (0.6–13.1)

*ORs adjusted as above. Significant associations (p<0.05) in bold.



Interaction with folic acid supplementation

Among case mothers with the TT genotype (n=27), only 3 (11.1%) reported any folic acid supplementation before or during early pregnancy, compared to 14/53 (26.4%) of CC carriers and 17/65 (26.2%) of CT carriers (p=0.048). In contrast, among control mothers with TT genotype (n=11), 5 (45.5%) used folic acid. The protective effect of folic acid was most pronounced in TT homozygotes: among non-supplemented women, TT genotype conferred an OR of 6.8 (95% CI: 2.9–15.9); among supplemented women, the OR was reduced to 2.1 (95% CI: 0.6–7.4), although statistical power was limited.

Additional analyses

No significant differences were found in MTHFR C677T distribution by fetal sex or gestational age at termination. Maternal age did not modify the association. In a multivariate logistic regression model including MTHFR genotype (CT + TT vs. CC), folic acid use (yes/no), and family history of NTDs, the adjusted OR for the T allele carrier status was 2.9 (95% CI: 1.7–4.9, p<0.001). Family history remained an independent risk factor (OR 4.3, 95% CI: 1.2–15.8), while folic acid use was strongly protective (OR 0.21, 95% CI: 0.12–0.37).

DISCUSSION

This study provides the first comprehensive morphological and genetic characterization of neural tube defects in aborted fetuses from Uzbekistan, specifically from the clinics of Tashkent State Medical University. Our findings demonstrate a diverse morphological spectrum of NTDs, with anencephaly and myelomeningocele predominating, and a significant association between the maternal MTHFR C677T polymorphism and the occurrence of these defects, particularly the open NTD phenotypes.

Morphological spectrum in context

The predominance of anencephaly (34.5%) in our series is consistent with reports from other predominantly Caucasian or Middle Eastern populations (Copp et al., 2015; Zaganjor et al., 2016). However, it contrasts with some European studies where myelomeningocele is more frequent due to prenatal screening and termination policies (Morris et al., 2012). The relatively high proportion of craniorachischisis (11.0%) is notable; this severe defect is often considered rare (1–2% of NTDs) (Seller, 1995). Its elevated frequency in our abortus series likely reflects early spontaneous or elective termination, as these fetuses are not viable and are rarely seen in liveborn registries. Our finding of associated anomalies in 53.8% of cases aligns with the work of **Kallen et al. (1999)** who reported a 49% association rate in a Swedish registry, and **Adilbekova et al. (2018)** who described a 51% rate in a preliminary Uzbek series.

The sex ratio (overall 0.90 male/female) with female predominance in anencephaly (M/F 0.85) is a well-known epidemiological feature, possibly related to differences in X-linked gene expression during neurulation (Copp & Greene, 2013).

MTHFR C677T association

The T allele frequency in our control mothers (0.230) is similar to that reported in other Central Asian and European populations (0.20–0.25) but lower than in some Middle Eastern groups (0.35–0.40) (Wilcken et al., 2003; Guo et al., 2010). The significant association we observed (OR for TT homozygotes 4.7) is stronger than in many Western studies but comparable to findings from China (OR 3.5–5.0) (Li et al., 2004) and Ireland (OR 3.2) (Kirke et al., 1996). This variability may reflect differences in folate nutritional status, environmental factors, or gene-gene interactions. Our population attributable risk of 32.1% suggests that MTHFR C677T is a major contributor to NTDs in Uzbekistan, highlighting a potential target for preventive interventions.

Notably, the association was strongest for myelomeningocele (OR 6.1) and anencephaly (OR 5.3), the two most common open NTDs. This is biologically plausible because open defects are more dependent on folate-mediated one-carbon metabolism during primary neurulation (Greene & Copp, 2014). Encephalocele, which can arise from defects in secondary neurulation or later closure, showed a weaker but still positive association, consistent with the meta-analysis by Yan et al. (2012).

Interaction with folic acid

The marked difference in folic acid supplementation rates between cases (23.4%) and controls (68.7%) underscores the protective effect of periconceptional folate, even in the presence of genetic risk. Our observation that TT homozygotes among cases were least likely to have taken folic acid (11.1%) suggests that these women may be either less compliant or less aware of recommendations, or that the MTHFR variant itself might indirectly affect health behaviors—an area requiring further study. Importantly, the reduction of OR from 6.8 to 2.1 with supplementation indicates that folic acid can partially overcome the genetic predisposition, a finding that supports universal folic acid fortification policies in Uzbekistan, where no mandatory fortification currently exists.

Comparison with previous studies in Central Asia

Very few studies have addressed NTD genetics in Central Asia. Turaeva et al. (2015) in a small study from Tashkent reported a higher prevalence of MTHFR 677T in mothers of children with hydrocephalus (n=30), but no NTD-specific data. Our results fill this gap and are broadly concordant with the only neighboring study from Kazakhstan by Nurgalieva et al. (2019) who found an OR of 3.8 for TT genotype in 55 NTD cases. Thus, the MTHFR C677T appears to be a relevant risk factor across the region.

Clinical and public health implications

From a clinical perspective, our findings suggest that screening for MTHFR C677T in pregnant women, particularly those with a family history of NTDs or prior affected pregnancy, could identify high-risk individuals who may benefit from high-dose folic acid supplementation (5 mg/day) rather than the standard 0.4 mg. The Tashkent State Medical University clinics have now implemented a pilot program offering such testing. From a public health standpoint, given the high PAR (32%), mandatory flour fortification with folic acid in Uzbekistan could prevent a substantial number of NTD cases annually. Current estimates from our department indicate approximately 600 NTD-affected pregnancies per year nationwide; fortification could potentially reduce this by 30–50%.

Limitations

Several limitations must be acknowledged. First, the study was conducted in a single tertiary referral center, which may introduce selection bias (more severe NTDs referred for termination). Second, we did not measure maternal folate or homocysteine levels, so the functional impact of the MTHFR variant on metabolite status could not be directly assessed. Third, other potentially interacting polymorphisms (e.g., MTRR A66G, RFC1 G80A) were not analyzed. Fourth, the control group, while well-matched, consisted of women undergoing termination for non-genetic reasons; some residual confounding by socioeconomic status cannot be excluded. Finally, the sample size for rare NTD subtypes (e.g., iniencephaly) was limited, reducing statistical power for subtype-specific analyses.

Strengths

Despite these limitations, this study has major strengths: (1) It is the largest morphological NTD series from Central Asia to date. (2) The detailed neuropathological examination followed international standards. (3) Genotyping was robust with quality controls. (4) The inclusion of a well-matched control group and adjustment for confounders strengthens causal inference. (5) The stratification by morphological subtype provides novel insights into phenotype-genotype correlations.

CONCLUSION

The morphological spectrum of neural tube defects in aborted human fetuses from Tashkent State Medical University clinics is dominated by anencephaly and myelomeningocele, with a high frequency of associated

anomalies. The maternal MTHFR C677T polymorphism is significantly associated with NTD occurrence, especially with open defects (myelomeningocele and anencephaly), and this association is modified by periconceptional folic acid use. Our results support the integration of MTHFR genotyping into prenatal risk assessment protocols in Uzbekistan and underscore the urgent need for national folic acid fortification programs. Future multicenter studies should include homocysteine measurements and examine gene-nutrient interactions.

CONFLICT OF INTERESTS

All authors declare that they have no competing financial, personal, or professional interests that could inappropriately influence this work. No funding was received from commercial entities. The research was conducted independently as part of the scientific program of Tashkent State Medical University.

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