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Research Article

Association Between Maternal C677T (rs180113) Polymorphism and Neural Tube Defects in Villa Clara, Cuba: A Population-Based Study

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Abstract

Objectives: To identify the most frequent clinical phenotype among cases with neural tube defects and to establish the association between maternal C677T polymorphism in MTHFR gene and the risk of appearing of neural tube defects in their offspring.

Patients and Methods: An analytical cases-control design and population-based study was conducted in the Villa Clara province, Cuba, between years 2013 and 2018. The prevalence rates were determined according to the different clinical phenotypes of the neural tube defects. The genotyping of the sequence variant C677T in the MTHFR gene was performed using PCR-RFLP method. The genotype and allelic frequencies were compared between cases and control groups according seven different models of genetic association.

Results: Spina bifida was the most frequent clinical phenotype among neural tube defects cases, with an adjusted prevalence rate of 0,63 per 1000 births. The frequency of homozygote TT genotype were 0,21 among mothers with affected offspring with neural tube defects and 0,06 in the control group, these differences were statistical (this word would be removed) significant (p = 0,015). The distribution of genotype and allelic frequencies among different clinical phenotypes was homogeneous. Significant associations between T allele and these congenital defects was identified in three genetic models of codominance TT vs CC [OR 4,87 (IC 95% 1,31 - 18,06), (TT vs CT) and (CC vs TT), besides recessive TT vs CT + CC [OR 3,88 (IC) 95% 1,22 - 12,35)] and additive models (T vs C). When the genetic association was analyzed for the codominant genetic model CC vs TT, OR: 0,21 (IC 95% 0,05 - 0,76) p = 0,011, a protective effect was found when maternal T allele was absent, with respect to the risk for neural tube defects in their offspring.

Conclusion: The current study provides evidence on the maternal MTHFR C677T polymorphism might be associated to the increased risk of neural tube defects in their offspring, which it's in fact related with diminished enzymatic activity reported in individuals with TT genotype.

Keywords: *Single Nucleotide Polymorphism; Neural Tube Defects; Anencephaly; Spina Bifida; Encephalocele; MTHFR Gene, Genotype Frequency, Allelic Frequency; Cuba*

Introduction

Neural tube defects (NTDs) constitute a heterogeneous group of congenital defects with great phenotypic variability, resulting from partial or complete failure of neural tube fusion at any level of the rostrocaudal axis between 23 and 27 days of embryonic life. They are classified into open and closed. The first are the most frequent and in them the nervous tissue is exposed on the body surface, among these are included anencephaly, encephalocele and spina bifida [1-3].

Although folic acid supplementation and fortification of foods have reduced the incidence of NTDs, they are still the most common type of major birth defect in many countries. An estimated 338,109 cases of spina bifida and anencephaly occur annually worldwide, with estimated prevalence rates between 2 and 6 per 1,000 births [1,4,5].

Failures in primary or secondary neurulation cause the appearance of a NTD, although detailed analysis has also revealed alterations in the gastrulation process and other cellular and molecular mechanisms. Most NTDs are isolates where genetic and environmental factors are involved. The most notable environmental factor is folic acid deficiency, while among genetic factors, sequence variants in genes involved in the metabolism of folic acid and homocysteine, such as methylenetetrahydrofolate reductase, are described [2,3,6,7].

The methylenetetrahydrofolate reductase (MTHFR) gene has a gene locus at 1p36.3, is 22 kb long, and results in a 7.5 - 9.5 kb transcript that codes for the MTHFR protein (OMIM #607093) made up of 656 amino acids. Recent studies describe that this gene consists of 12 exons (OMIM #005957), the first of which is non-coding [8].

The human MTHFR protein is a homodimer with a total molecular mass of 150 kD, each subunit consisting of an N-terminal catalytic domain (1-356 amino acids) and a C-terminal regulatory domain (363-656 amino acids) linked by a linker region (357-362 amino acids). The catalytic domain is sufficient to carry out all the enzymatic reactions that catalyze the physiological unidirectional and irreversible reduction of 5.10 MTHF (methyl tetrahydrofolate) to 5 MTHF, which is the predominantly circulating form of folic acid and is the donor co-substrate. of carbon groups, used for the remethylation of Homocysteine to Methionine, which is the precursor of a chain of methylation reactions of substances of biological interest, such as the synthesis of DNA, proteins, neurotransmitters and phospholipids [8-10].

The MTHFR gene plays a key role in the epigenetic regulation of the embryogenesis process [11].

The most studied single nucleotide polymorphism is C677T (OMIM: 607093.0003) located in exon 4 of the MTHFR gene, which consists of the substitution of Cytosine for Thymine at codon 222, which causes the substitution of Alanine for Valine at nucleotide 677.

The homozygous allelic combination CC represents the wild-type genotype of the C677T polymorphism, the heterozygous combination CT and the homozygous TT represent genotypes that cause enzymes with a 30% and 60% decrease in their reductase activity, respectively. It is described that the homozygous TT genotype increases the probability of presenting low concentrations of AF by 2.24 times (95% CI = 1.85 - 2.70 $p < 0.001$) [12-14].

The province of Villa Clara is located in the central region of the island of Cuba, it occupies the fifth place in extension among the 15 Cuban provinces, with 8,413.13 square kilometers, representing 7.7% of the total area of the Caribbean country [15].

To the best of our knowledge there is no evidence that the association between the maternal C677T polymorphism of the MTHFR gene and the presence of NTD in the offspring has been previously studied in Cuba.

Goals of the Study

To identify the most frequent clinical phenotype among cases with NTD and to establish the association between the C677T polymorphism of the MTHFR gene in mothers and the appearance of NTD in their offspring.

Methods

An analytical observational study of cases and population controls was carried out between 2013 and 2018 in the province of Villa Clara, Cuba.

The universe was made up of 46,473 births that occurred between 2013 and 2018. Of these, 45,692 live births (LB), 425 stillbirths (SB) and 356 elective termination of pregnancy (ETP) registered at the Provincial Genetics Center and in the Statistics Departments of the University Gynecology-Obstetric Hospital " Mariana Grajales" and the Villa Clara Provincial Health Directorate.

The population was made up of 59 mothers with offspring affected by NTD in the study period.

The cases with NTD were obtained from the Cuban Registry of Congenital Malformations and the Cuban Prenatal Registry of Congenital Malformations (RECUMAC and RECUPREMAC for its acronym in Spanish) of the Provincial Center of Medical Genetics of Villa Clara, which were entered into a database created for this purpose using the SPSS program version 22.0 for Windows, Armonk New York, IBM.

Mothers with affected offspring (live birth or stillbirth, as well as termination of pregnancy) with NTD, whose clinical phenotype was classified according to the international classification of diseases (ICD-10) [16], were included.

Clinical variables

NTD clinical phenotypes:

- Anencephaly: It was defined as the partial or total congenital absence of the skull (ICD-10 code: Q00).
- Open spina bifida: Failure in the fusion of the vertebral arches that also affects the skin and soft tissues. Cases with meningocele, myelomeningocele, lipomeningocele and rachischisis were included (ICD-10 code: Q01).
- Encephalocele: It was defined as a congenital bone defect of the skull with herniation of the intracranial mass through the defect. All cases were included regardless of anatomical location (frontoethmoidal, nasoethmoidal, nasofrontal, nasoorbital, or occipital) (ICD-10 code: Q05).

Prevalence rates were calculated according to the destination of the product among LB and SB, as well as the adjusted prevalence rate (AP) for each clinical phenotype in the study population. All prevalence rates were calculated based on 1000 births.

For cases with ETP, the percentage for each clinical phenotype was determined, with respect to the total number of cases with DTD.

The AP rate was calculated according to the universe of cases, determining the proportion in which the different clinical phenotypes appeared in the products of pregnancy born alive or stillborn with NTD, as well as the ETP with respect to the total number of cases born (alive and dead); as shown by the following formula:

$$AP = \frac{LB\ with\ NTD + SB\ with\ NTD + ETP}{Total\ of\ LB + SB}\ x\ 1000\ births$$

Given the impossibility of carrying out the molecular study on the entire study population, a sample of 24 mothers with offspring with NTD was selected, for which a non-pure sampling was used, which, although it was not probabilistic, random elements were introduced, given that the selection of the cases was done blindly and randomly by the analyst in the laboratory at the time of taking the sample only identified with a code for analysis; until completing a defined quota, which was distributed among the groups of case and control mothers; but not identified by whoever processed them.

In the same way, a molecular study of the C677T polymorphism of the MTHFR gene was carried out on 45 mothers with offspring without any type of congenital defect, born alive in one of the four Villa Clara´s hospitals, which were considered as controls.

A sample of 5 ml of venous blood was taken from the sample of mothers of the cases and controls by antecubital puncture, which were transferred to sterile polypropylene tubes with 200 µL of ethylenediaminetetraacetic acid (EDTA) as anticoagulant, which were refrigerated. at -20 °C until DNA extraction.

Leukocyte DNA extraction was performed automatically using the QIA symphony SP modular system at the Molecular Biology Laboratory of the National Center for Medical Genetics in Havana.

The C677T polymorphism of the MTHFR gene was studied with PCR/RFLP using standardized protocols, in 33 samples in the Molecular Biology laboratory of the "Carlos J. Finlay" Center for Research and Rehabilitation of Hereditary Ataxias in Holguín city and in 102 samples in the of the National Center of Medical Genetics of Havana city. Blind genotyping was performed on two samples in both laboratories.

Polymorphism was demonstrated after PCR amplification using the following primers, described by Frost in 1995: 5'-3' sense sequence: TGA AGG AGA AGG TGT CTG CGG GA and 5'-3' antisense sequence: AGG ACG GTG CGG TGA GAG TG [17].

The PCR was performed in a final volume of 25 µl and 1 µL of a 1/10 solution of the purified DNA was used, in a reaction mixture containing 1x the buffer of the polymerase used, each deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP) at 0.2 µM final concentration, 1.5 µM MgCl, 0.2 µM each primer and 2 units of Taq polymerase.

Amplification was performed in a MJ Research GIANT model thermocycler, temperature cycles included an initial denaturation step at 95°C for 5 minutes, followed by 35 denaturation cycles at 94°C for one minute, primer annealing at 68°C for one minute, and cure at 72°C for one minute, followed by final cure at 72°C for 10 minutes.

The enzymatic digestion of the amplified fragment was performed in a final volume of 30 µl containing 15 µl of the amplified product in 1X CutSmart buffer from New England Biolabs and 2 units of HinfI enzyme from the same commercial firm for 3 to 5 hours. which presents the recognition nucleotide sequence: 5'-GANTC-3' and revealed a bi-allelic polymorphism that produced fragments of different sizes: one of 198 bp corresponding to the C allele (with absence of the cut site for the restriction enzyme) and two fragments of 175 bp and 23 bp corresponding to the T allele (with the presence of the restriction site).

The fragments corresponding to each sample were characterized by electrophoresis in 3% agarose gel with 0.5 µg/del of ethidium bromide at 9 V/cm for 55 minutes, incorporating the 25 bp molecular weight marker in each electrophoretic run. and were visualized in UV Transilluminator (Digi-doc).

The following molecular variables were defined:

- MTHFR C677T alleles: Each of the two alternative forms of the C677T polymorphism of the MTHFR gene, according to the electrophoretic run. Final values: C allele or T allele.
- MTHFR C677T genotype: Two by two combination of alleles for the C677T polymorphism, characteristic of each individual; according to the electrophoretic run. Final values: homozygous CC, heterozygous CT or homozygous TT.

Given the limited number of molecular studies that were carried out in the mothers of the control group (45 samples), both for the verification of the population balance and for the analysis of the different allelic association studies, prior authorization from the main investigator, the results of the genotyping of 130 controls from the only population study for which there is evidence of having analyzed this polymorphism in Cuba, carried out in Holguín, were used. This is a province that together with Villa Clara and Sancti Spíritus are the only three provinces in the country with more than 80% of its population with white skin color [15]. This practice is common in international multicenter studies.

Statistical analysis

The data obtained were entered into a database created for this purpose using the SPSS version 22.0 Windows program.

Percentage was used as a frequency statistician. The test of the hypothesis of independence or homogeneity was used, through the estimation of the Pearson Chi-square (X^2) statistic or Fisher's exact test to compare the frequency of the different genotypes between cases and controls and between the different phenotypes. clinical.

To verify if the C677T polymorphism was in Hardy-Weinberg gene equilibrium in the total group of 173 controls, the Chi-square goodness-of-fit was calculated, for which the allele frequencies were determined assuming the existence of Hardy-Weinberg equilibrium (HWE). The usual formulas were used: allele C = 2CC+CT/2n, allele T = 2TT+CT/2n. From these frequencies, the expected genotypic frequencies were obtained (CC = p^2, CT = 2pq and TT = q^2) [18].

If the observed and expected genotypic frequencies did not show significant differences and the Chi value calculated was lower than the tabulated for a significance level α = 0.05 and two degrees of freedom = 3.84, the null hypothesis was accepted, which expresses that there is HWE [17,18].

Prior to incorporating the samples into the control group, the existence of HWE was assessed in the controls from each province separately. In addition, an analysis of the genotypic frequencies observed between the control samples from both provinces was carried out, to verify that there were no significant differences between them, as a requirement to be used in the analysis of gene balance and in allelic association studies.

The distribution of gene and genotypic frequencies between the mothers of the cases and those of the controls was compared using the Chi-square test. An analysis of the homogeneity of the distribution of allelic and genotypic frequencies was performed in all the mothers of the cases and controls.

The gene and genotypic frequencies for each of the clinical phenotypes were determined and compared with respect to the frequencies in mothers of the control group, using the Chi-square test.

Once the genotypes corresponding to the polymorphism studied in mothers of cases and controls were available and the existence of gene balance was verified, we proceeded to verify using different allelic association designs and under the null hypothesis that neither of the two alleles of the polymorphism was more associated with cases than with controls than would be expected by chance. Since it is a non-functional polymorphism, models that establish hypothetical associations were tested under seven different genetic association studies: four codominance models and a dominance, recessiveness and additive models. For the allelic association studies under the different models, the Microstat statistical package was used, the Pearson Chi-square value for two degrees of freedom, the associated p, as well as the Odds Ratio and the 95% confidence interval were determined.

For all studies, a significance level of α = 0.05 was used for significant differences and α = 0.001 for highly significant differences.

Ethical considerations

The research was carried out in accordance with the regulations established in the Declaration of Helsinki on medical research involving human beings [19]. The study was approved by the ethics committee of the Biomedical Research Unit of the Villa Clara University of Medical Sciences, as part of the project entitled: "Interrelation of genetic and environmental factors in congenital defects with probable association with maternal deficiency of acid folic acid and other micronutrients in Villa Clara". All the mothers included in the study expressed their consent to participate in the research, as well as to take biological samples and extract DNA.

Results

Spina bifida was the most common clinical phenotype among NTDs, with an adjusted prevalence rate of 0.63 per 1,000 births. In 81.4% of the cases (48/59) with NTD, the couple requested the termination of the pregnancy, while 10 cases were diagnosed among live births (prevalence of 0.22 per 1000 live births) and prevalence of 2. 35 per 1000 stillborns (1/59) (Table 1).

Table 2 shows the analysis of the genotypic and allelic frequencies of the C677T polymorphism between cases and controls, it was found that the frequency of the homozygous TT genotype was 0.21 in mothers with offspring affected by NTD and 0.06. in the control group, differences that were significant (p = 0.015). The T allele had a frequency of 0.46 in the mothers of the cases and 0.32 in the controls. The allele frequencies between cases and controls were not different for either of the two alleles.

Clinical phenotype	LB	LB Prev rate	SB	SB Prev rate	ETP	%	Total	%	AP
Anencephaly	0	0.00	0	0.00	25	42.37	25	42.37	0.54
Spina bifida	10	0.22	1	2.35	18	30.51	29	49.15	0.63
Encephalocele	0	0.00	0	0.00	5	8.47	5	8.47	0.11
Total	10	0.22	1	2.35	48	81.36	59	100.0	1.28

Table 1: *Frequency and prevalence of the different clinical phenotypes of neural tube defects according to condition at birth. NTD: Neural Tube Defects; LB: Live Births; SB: Stillbirths; ETP: Elective Termination of Pregnancy; LB Prev Rate: Prevalence Rate in Live Births; SB Prev Rate: Prevalence Rate in Stillbirths; AP: Adjusted Prevalence.*

Groups	Genotypes (Genotypic Frequency)				Alleles (Allele Frequency)		
	DC	TC	TT	Nu.	C	T	Nu.
Cases	7 (0.29)	12 (0.50)	5 (0.21)	24	26 (0.54)	22 (0.46)	48
Controls	75 (0.43)	87 (0.50)	11 (0.06)	173	237 (0.68)	109 (0.32)	346
p	0.170	0.977	0.015		0.286	0.172	

Table 2: *Genotypic and allelic frequencies of the maternal C677T polymorphism of the MTHFR gene in cases and controls. Nu: Number.*

The highest genotype frequencies for the homozygous TT genotype were observed among mothers of cases with anencephaly, followed by spina bifida, whereas none of the mothers of encephalocele cases were found. The highest allele frequencies for the mutated T allele were found among mothers of children with anencephaly. The frequency distribution of genotypes and alleles in the sample of cases with different clinical phenotypes was homogeneous (Table 3).

Clinical Phenotype	Genotypes (Genotypic Frequency)				X^2		Alleles (Allele frequency)			X^2	
	CC	CT	TT	Nu.	Value	p	C	T	Nu.	Value	p
Anencephaly	3 (0.27)	5 (0.46)	3 (0.27)	11	1.75	0.781	11 (0.50)	11 (0.50)	22	2.61	0.24
Spina bifida	3 (0.33)	4 (0.44)	2 (0.22)	9			10 (0.56)	8 (0.44)	18		
Encephalocele	1 (0.25)	3 (0.75)	0 (0.00)	4			5 (0.62)	3 (0.38)	8		

Table 3: *Genotypic and allelic frequencies of the maternal C677T polymorphism of the MTHFR gene in the different clinical phenotypes. Nu: Number.*

Table 4 shows the genetic association studies for the dams of products with NTD. Significant associations were observed between the T allele and these birth defects in three codominance genetic models TT vs CC [OR 4.87 (95% CI 1.31 - 18.06), (TT vs CT) and (CC vs TT), as well as in the recessive models TT vs CT + CC [OR 3.88 (CI) 95% 1.22 - 12.35)] and in the additive model (T vs C). When the association for the CC vs TT model was analyzed, the OR: 0.21 (95% CI 0.05 - 0.76) p = 0.011, showed a protective effect for the absence of the T allele.

Discussion

The identification of spina bifida as the most frequent clinical phenotype observed among the cases in the present study is consistent with that observed in the northwestern region of Nigeria, although with a frequency 1.5 times higher (72.70%), whereas anencephaly and encephalocele frequency described in the aforementioned study had double the frequency that in Villa Clara province (22.40% and 4.60%, respectively) [20]. Also in the Batna region, in Algeria, spina bifida was the most frequent NTD (66.66%); followed by anencephaly (19.50%) and encephalocele (5.50%) [2].

Association models	Genotypes (%)			OR (95% CI)	X^2 -	
	TT	TC	CC		Value	P
Codominance Model TT vs CC						
Cases	5 (17.00)	12 (50.00)	7 (29.17)	4.87 (1.31 - 18.06)	6.36	0.011
Controls	11 (6.36)	87 (50.29)	75 (43.35)			
Codominance Model				**TT vs CT**		
				3.29 (0.97 - 11.13)	3.96	0.046
Codominance Model				**CT vs CC**		
				1.48 (0.55 - 3.95	0.61	0.434
Codominance Model				**CC vs TT**		
				0.20 (0.06 - 0.76)	6.36	0.011
Dominance Model						
	CT + TT	**CC**		**CT + TT vs CC**	1.73	0.187
Cases	17 (70.83)	7 (2.17)		1.86 (0.73 - 4.71)		
Controls	98 (56.65)	75 (43.35)				
Recessive Model						
	TT	**CT + CC**		**TT vs CT + CC**	5.89	0.015
Cases	5 (20.83)	19 (79.17)		3.88 (1.22 - 12.35)		
controls	11 (6.43))	162 (93.64)				
Additive Model						
	T	**C.**		**T vs C**	3.89	0.048
Cases	22 (45.83)	26 (54.17)		1.83 (0.99 - 3.39)		
Controls	109 (31.50)	237 (68.50)				

Table 4: *Genetic association studies of the C677T polymorphism of the MTHFR gene in mothers of cases with neural tube defects.*

In the period studied, a spina bifida-anencephaly ratio of 1.16:1 was identified in the province of Villa Clara; this differs from Gashaw [21], who observed a higher frequency of anencephaly (54.3% of cases) with an anencephaly to spina bifida ratio of 1,42 a1 and an incidence of 3.3 per 1000 pregnancies, in the Ethiopian region of Amhara.

The prevalence rate of anencephaly identified in this study was similar to that observed in a cohort study conducted in Morocco (0.50 per 1,000 births) [3], and lower than those found in Asian populations in India (1,04 per 1,000 births) and in the Iranian region of Gorgan (1.31 per 1,000 births) [22].

The fact that in the present study, more than half of the people in the control group were CT heterozygous and about six percent were TT homozygous and that the allele frequency of the mutated T allele was higher in the case group than in the controls, is similar to the finding found by Nauman [23], in a case-control study conducted in Pakistan, where the frequency of the TT and CT genotypes was higher among the 109 women with offspring affected by birth defects than among the 100 women in the control group, the frequency of the T allele was also higher than that of the C allele in the cases.

The MTHFR gene presents a great allelic genetic heterogeneity, approximately 90% of the European population presents some of the variants with a frequency greater than 1%. The most commonly studied polymorphic variant and the one that most influences the reduction of the biologically active form of folic acid is C677T (rs1801133) [13].

The frequency of TC heterozygotes observed in the control group is slightly lower than that identified in a study in a healthy population carried out in Colombia (0.46), while the frequency of TT homozygous individuals in the Colombian population doubles (0.13) to that found in the present study [24].

Persons with the heterozygous CT and homozygous TT genotype have approximately 30 - 35% and 60 - 70% reduction in MTHFR enzyme activity *in vitro*, respectively [6,17].

In the mothers of the cases with NTD, the highest genotype frequencies of the homozygous TT genotype were observed, coinciding with what was described by Cai [25], although this researcher also found that mothers with the heterozygous TC genotype also had a risk of having offspring. with NTD.

However, in a case-control study conducted in India, no individual with TT genotype was found in either the NTD case group or the control group, the authors attributed this to the small size of the studied sample [7].

A meta-analysis that included 19 case-control studies comprising 2,228 NTD cases and 4,220 controls found that the C677T polymorphism of the MTHFR gene had a highly significant correlation with the development of NTD, particularly spina bifida [11].

In the present study, it was observed that mothers with the T allele might have a higher risk of having NTD in their progeny than those with the wild-type C allele, consistent with what has been described by other researchers, who identified a higher frequency of the minor allele of the polymorphism C6677 in the mothers of NTD cases than in that of controls [11,23,26].

They exist, however, inconsistencies between the different studies, for some researchers the MTHFR C677T polymorphism constitutes a risk factor for NTDs and for others it does not, which may be related to different adjustments of the variables, in addition to the fact that much larger sample sizes are required to be able to obtain more robust results [27].

Fang [27] in a case-control study compared the genotypic and allele frequencies of 152 patients with NTDs and 169 healthy controls and observed that for the MTHFR C677T polymorphism the risk of presenting NTDs is significantly increased in patients with the T allele or with the TT genotype. Also in a meta-analysis carried out by Chinese researchers, which included 1500 cases and 1650 controls, they concluded that the maternal MTHFR C677T polymorphism was significantly associated with the risk of having offspring with NTD [28].

In contrast, a study conducted in western Mexico found no evidence that maternal homozygosity or heterozygosity status for the MTHFR C677T polymorphic variant was associated with the presence of NTD in offspring [29].

The allelic associations found for the presence of the T allele, in the mothers of offspring with NTD, coincides with what is reported in the literature where this polymorphism is considered as a genetic risk for a greater susceptibility to the occurrence of congenital defects [14,30].

The evidence that the mothers of the cases with NTD showed significant differences in the codominance models TT vs CC and TT vs CT, as well as in the recessive and additive models, indicate an association between the presence of the T allele in its homozygous state and the appearance of NTD. The term codominant implies that the three genotypes (CC, CT, and TT) may have different associated phenotypic risks [18].

The identification of an association between the C allele in the homozygous state and the absence of NTD, in the CC vs TT codominance model, indicates that in the present study the absence of the T risk allele behaves as a protective factor for DTN.

In a meta-analysis that included 13 studies that included 1500 cases (mothers with offspring affected by NTD) and 1654 controls, where it was observed that the combined risk for NTD in the offspring of mothers in the recessive model (TT vs CT+ CC) and in the additive model (T vs C) it was 1.65 and 1.39; respectively [28].

In the additive model, unlike the rest of the genetic association models, the gene frequencies and not the genotypic ones are compared. In the present study, the presence of the T allele gives mothers a 1.83 times higher risk of presenting NTD in their offspring compared to those who do not present it.

Conclusion

The present study provides evidence that the maternal C677T polymorphism of the MTHFR gene is associated with an increased risk of neural tube defects in offspring in mothers from the province of Villa Clara, a fact potentially related to the decrease described for enzymatic activity of the MTHFR in the TT genotype.

Codominance and recessive allelic interaction models offer greater opportunities for the detection of associations of the C677T polymorphism with NTD in the central province of Villa Clara, Cuba.

Importance of the Study

The C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR) gene has been reported to play a critical role in the pathogenesis of neural tube defects (NTDs). The association of the C677T polymorphism in the MTHFR gene and NTD susceptibility has been widely demonstrated, but the results are inconclusive. To the best of our knowledge the present study represents the first work exploring the relation between the maternal genotypes of this polymorphism with the risk of neural tube defects in their offspring in the Cuban population.

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Contribution of the Authors

All authors contributed to data collection, analysis, and processing, as well as to the preparation, review, and final approval of the manuscript.

Conflict of Interest

The authors declare that there are no potential conflicts of interest with respect to the research or publication of this article.

Bibliography

1. Kancherla V and Black R. "Historical perspective on folic acid and challenges in estimating global prevalence of neural tube defects". *Annals of the New York Academy of Sciences* 1414 (2018): 20-30.

2. Bourouba R., *et al*. "Risk factors of neural tube defects: A reality of Batna region in Algeria". *Egyptian Journal of Medical Human Genetics* 19.3 (2018): 225-229.

3. Forci K., *et al*. "Incidence of neural tube defects and their risk factors within a cohort of Moroccan newborn infants". *BMC Pediatrics* 21 (2021): 124-134.

4. Martinez H., *et al*. "Improving maternal folate status to prevent infant neural tube defects: working group conclusions and a framework for action". *Annals of the New York Academy of Sciences* 1414 (2018): 5-19.

5. De Joung M., *et al*. "Contribution of voluntary fortified foods to micronutrient intake in The Netherlands". *European Journal of Nutrition* 1 (2022): 1-15.

6. Taboada N. "Implication of molecular and cellular mechanisms in congenital defects sensitive to folate deficiency". *EC Gynaecology* 10.6 (2021): 57-68.

7. Goyal A., *et al*. "Study of C677T methylene tetrahydrofolate reductase gene polymorphism as a risk factor for neural tube defects". *The Asian Journal of Neurosurgery* 16 (2021): 554-561.

8. 5,10-Methylenetetrahydrofolate Reductase; Mthfr. OMIM (2018).

9. Castrense S., *et al*. "A glance into MTHFR deficiency at a molecular level". *International Journal of Molecular Sciences* 23.1 (2022): 167-186.

10. Nasri K., *et al*. "Association of MTHFR C677T, MTHFR A1298C and MTRR A66G polymorphisms with neural tube defects in Tunisian parents". *Pathobiology* 86 (2019): 190-200.

11. Sadat R., *et al*. "Association of fetal MTHFR C677T polymorphism with susceptibility to neural tube defects: A systematic Review and Update Meta- Analysis". *Fetal and Pediatric Pathology* 41.2 (2022): 225-241.

12. Talita C., *et al*. "Association between MTHFR C677T and A1298C gene polymorphisms and maternal risk for Down syndrome: A protocol for systematic review and meta-analysis". *Medicine* 101 (2022): 1-6.

13. Vidmar G., *et al*. "Folate insufficiency due to MTHFR deficiency is bypassed by 5-Methyltetrahydrofolate". *Journal of Clinical Medicine* 9 (2020): 2836-2854.

14. Liu P., *et al*. "Association between MTHFR C677T polymorphism and congenital heart disease". *International Heart Journal* 61 (2020): 553-561.

15. ONEI. National Office of Statistics and Information. Republic of Cuba (2022).

16. International Classification of Diseases-10th Revision. National Center for Health Statistics (2021).

17. Taboada LN. "Genetic and environmental risk factors in mothers with offspring affected by folate-sensitive birth defects in Villa Clara". [doctoral thesis] University of Medical Sciences of Villa Clara (2022).

18. Lardoeyt R., *et al*. "Fundamentos de Genética Médica Poblacional". La Habana: ECIMED (2016): 366.

19. Shrestha B and Dunn L. "The declaration of Helsinki on medical research involving human subjects: a review of seven revision". *Journal of Nepal Health Research Council* 17.4 (2020): 548-552.

20. Oluwafemi RO and Abiodun MT. "Incidence, Spectrum and Outcome of Congenital Anomalies Seen in a Neonatal Intensive Care Unit in Southern Nigeria". *The Nigerian Postgraduate Medical Journal* 26 (2019): 239-243.

21. Gashaw A., *et al*. "Risk factors associated to neural tube defects among mothers who gave birth in North Shoa Zone Hospitals, Amhara Region, Ethiopia 2020: Case control study". *PLoS One* 16.4 (2021): 1-12.

22. Golalipour M., *et al*. "Prevalence of anencephaly in Gorgan, northern Iran". *Archives of Iranian Medicine* 13.1 (2010): 34-37.

23. Nauman N., *et al*. "Low maternal folate concentrations and maternal MTHFR C677T polymorphism are associated with an increased risk for neural tube defects in offspring: a case-control study among Pakistani case and control mothers". *Asia Pacific Journal of Clinical Nutrition* 27.1 (2018): 253-260.

24. Romero C., *et al*. "C677T (rs1801133) MTHFR gene-polyporphism frequency in a colombian population". *Colombia Médica* 4.2 (2015): 75-79.

25. Cai C., *et al*. "Association of neural tube defects with maternal alterations and genetic polymorphisms in one-carbon metabolic pathway". *Italian Journal of Pediatrics* 45 (2019): 1-7.

26. Shi H., *et al*. "Study on maternal SNPs of MTHFR gene and HCY level related to congenital heart diseases". *Pediatric Cardiology* 42.1 (2021): 42-46.

27. Fang Y., *et al*. "Association of main folate metabolic pathway gene polymorphisms with neural tube defects in Han population of Northern China". *Child's Nervous System* 34 (2018): 725-729.

28. Zhang C., *et al*. "Meta-analysis on relationship between the Chinese maternal MTHFR gene polymorphism(C677T) and neural tube defects in offspring". *Wei Sheng Yan Jiu* 47.2 (2018): 312-317.

29. Aranda S., *et al*. "MTHFR C677T and A1298C variants in Mexican mestizo infants with neural tube defects from Western Mexico". *Congenital Anomalies* 61.5 (2021): 188-192.

30. Amooee A., *et al*. "Association of fetal MTHFR 677C > T polymorphism with non-syndromic cleft lip with or without palate risk: A systematic review and meta-analysis". *Fetal and Pediatric Pathology* 40.4 (2021): 337-353.

Volume 12 Issue 2 February 2023