Research article

A Common Variant in MTHFD1L is Associated with Increased Risk for Spina Bifida

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Abstract

Genetic polymorphisms within folate pathway genes represent potentially meaningful risk factors for neural tube defects (NTDs). Here we investigated the association of three previously identified polymorphisms in the folate pathway gene MTHFD1L with spina bifida in a U.S. population consisting primarily of Hispanic and non-Hispanic Caucasians. MTHFD1L allele 1 was found to significantly reduce maternal risk of having a baby with spina bifida, while allele 3 significantly increased maternal risk to have a spina bifida baby. This study confirms that rs3832406 is associated with an increased risk of spina bifida.

Keywords

Neural tube defects; Genomic variants; One carbon metabolism; MTHFD1L

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1. Introduction

Neural Tube Defects (NTDs) are clinically significant congenital malformations affecting the development of the central nervous system. The prevalence of these defects is approximately one in every 2,000 live births per year in the U.S. and 0.5 to 2 per 10,000 live births worldwide [1]. Neural tube closure (NTC) is the morphological process occurring in early embryogenesis that ultimately creates the adult central nervous system including the brain and spinal cord. Initiation of NTC begins with the elevation of neural folds from the neural plate which ultimately fuse at the dorsal midline along the rostral-caudal embryonic axis [2]. NTDs arise secondary to a failure of neurulation and are phenotypically classified as anencephaly, myelomeningocele (spina bifida) and craniorachischisis [3]. Fetuses with anencephaly and craniorachischisis die prior to, or shortly after birth [4].

Low maternal folate plasma concentrations have been identified as an import ant risk factor for NTDs. Studies dating back over 30 years show that a significant portion of NTDs can be prevented by maternal folic acid supplementation [4–6]. Wald (2001) reported that folic acid (5 mg per day) administered prior to, and at the early stages of pregnancy, can decrease the risk of NTDs by about 85% in women that have average serum folate levels [7]. Our laboratory demonstrated in a murine model in which the primary folate receptor gene (*Folr1*) which is crucial for folate transport during central nervous system development was inactivated, hence compromising maternal-to-fetal folate transport during development resulted in nullizygous embryos with NTDs. Furthermore, supplementation with folic acid restored the normal phenotype in a significant percentage of the nullizygous *Folr1* embryos [8]. Thus, maintaining sufficient maternal folate stores is essential for normal NTC and prevention of NTDs.

Based on strong human epidemiological data and a significant experimental mouse literature as well, it is now clear that genes involved in the metabolism and transport of folate represent excellent candidate genes for evaluating specific contributions to the genetic risk of NTDs. Two genes that code for enzymes that are involved in cytoplasmic metabolism of folate, Methylenetetrahydrofolate Reductase (*MTHFR*; MIM# 607093) and C_1-Tetrahydrofolate Synthase (*MTHFD1*; MIM# 172460), have previously been shown to be associated with an increased NTD risk [9–11]. Mutations in genes encoding the glycine cleavage system predispose to neural tube defects, as observed in $Amt^{-/-}$ mice and supported by *AMT* mutations identified in human NTD patients [12]. In our previous study, we found that $Mthfd1l^{-/-}$ mice presented 100% NTDs [13].

A study performed in an Irish NTD population found that a gene encoding the mitochondrially localized C_1-Tetrahydrofol-ate Synthase (MTHFD1L) enzyme influences NTD disease risk [14]. A polymorphism of the *MTHFD1L* gene, rs3832406, was found to affect the alternative splicing efficiency of the MTHFD1L mRNA transcripts [14]. Three alleles of rs3832406 are recognized that consist of a varying number of short tandem repeats of the sequence ATT. In the Irish population, the presence of allele 1 (ATT_7) in children showed an increased NTD risk, allele 2 (ATT_8) was associated with a decreased NTD risk, and allele 3 (ATT_9) did not influence NTD risk [14]. In this study, we investigate MTHFD1L as a candidate gene for NTD risk association in a US NTD population consisting primarily of Hispanic and non-Hispanic Caucasians. Unlike the Irish population study, no association was found between MTHFD1L alleles in children with spina bifida. However, when examining the mothers' genotype, alleles 1 and 3 were found to have a significant

association with spina bifida risk.

2. Materials and Methods

2.1. Study Population

Spina bifida cases along with unaffected controls were obtained from the Dell Children's Medical Center of Central Texas (DCMCCT). The case samples include 219 infants with isolated spina bifida who are not affected by any other major birth defects. Among the 219 case infants, 94 are Non-Hispanic Caucasians, 63 are foreign-born Hispanics, and 62 are U.S.-born Hispanics. The control samples are from 173 non-malformed infants selected at random from all live births within the same timeframe as the cases. Among the 173 control infants, 74 are Non-Hispanic Caucasians, 50 are foreign-born Hispanics, and 49 are U.S.-born Hispanics. Samples from 262 mothers of the non-malformed children and 178 mothers of infants affected by spina bifida were also used in this study. The approval process includes a detailed review by the IRB of The University of Texas at Austin. For the patients enrolled at the DCMCCT, saliva samples were collected using a DNA collection kit (Oragene · DNA (OG-500), DNAgenoTek).

2.2. DNA Genotyping

Genomic DNA was extracted from all samples using the Gentra Puregene Kit (Qiagen). The polymerase chain reaction amplified fragment length polymorphism (PCR-AFLP) technique was used to genotype rs3832406 (c.781-6823ATT [9–11]) in genomic DNA. Each PCR reaction (10 µL) contained: 1x Taq DNA polymerase buffer (R007A, TaKaRa), 0.2 mM dNTP mix (R007A, TaKaRa), 0.5 U Hotstart Taq DNA polymerase (R007A, TaKaRa), ∼10 ng DNA and 0.2M of each PCR primers (forward primer: 5'FAM-TTCTCTTTCTTAGCCCC-A CG-3'; reverse primer: 5'-gtttcttAGAGCTTGCAGTGAG-CCTAGA-3'). The PCR program was as follows: 95°C 2 min, 11 cycles of 94°C 20s, 65°C - 0.5°C/cycle 40s, 72°C 1 min and 24 cycles of 94°C 20s, 59°C 30s, 72°C 1 min, followed by 72°C 2 min and hold at 4°C. 1 µL of the PCR products was diluted 20-fold in double distilled H2O. 1 µL of diluted PCR product was added to 8.9 µL of Hi-Di^{TM}formamide (4311320, Thermo Fisher) and 0.1 µL 600 LIZ^{TM}dye size standard (4408399, Thermo Fisher), denatured at 95°C for 5 min and run in capillary electrophoresis (ABI3730XL, Thermo Fisher). Genemapper 3.7 software was used for allele calling (Fig. 1).

2.3. Statistical Analysis

Frequencies of the MTHFD1L rs3832406 allele were compared between NTD cases and healthy individuals, as well as samples from mothers of cases and mothers of controls, using a chi-square test (RStudio and SHEsis). The Chi-Square Test of Independence was also used to test the significance of NTD risk dependency on genotype. Associations between rs3832406 alleles and Spina bifida were tested separately in mother case/control and infant case/control samples using a logistical regression in RStudio.

3. Results

3.1. The Number of ATT Repeats in Mothers is Associated with Case Outcomes

Previous genotyping of the rs3832406 variant revealed three alleles that differ in the length of a repeated ATT nucleotide sequence, with allele 1 having 7 ATT repeats, allele 2 having 8, and allele 3 having 9 ATT repeats [14]. We collected DNA samples from spina bifida infants and their mothers, as well as from unaffected infants and their mothers to serve as controls from a general North American population. The Chi-Square Test of Independence was run in mother/control samples and separately in case/control samples to test for associations. No association between rs3832406 alleles and spina bifida risk was observed in the infant samples (Table 2, Table 3). However, in the maternal samples there was a significant association between rs3832406 alleles and the risk of having a spina bifida affected offspring ($p = 0.005$) (Table 4).

Table 1. Genotype and Allele Frequencies of rs3832406 in NTD and Control Groups

Genotypes	Cases	Mothers (Case)	Mothers (Control)	Control
1–1	75	80	74	58
1–2	54	56	40	46
1–3	42	59	34	33
2–2	9	16	12	8
2–3	24	27	10	20
3–3	15	24	8	8
Total	219	262	178	173

Table 2. SHEsis single site test in infants

rs3832406 Allele	155 (freq)	158 (freq)	161 (freq)	164 (freq)	p-value
Case	245 (0.559)	96 (0.219)	96 (0.219)	1 (0.002)	0.767899
Control	196 (0.560)	82 (0.234)	70 (0.200)	2 (0.006)	

Table 3. Odds ratio of three alleles in infants

Case/controls	Odds ratio	CI	p-value
Allele 1	0.955163	0.5863033-1.546927	0.853
Allele 2	0.899570	0.6004607-1.348085	0.6076
Allele 3	1.069776	0.7077806-1.620753	0.749

Table 4. SHEsis single site test in mothers

rs3832406 Allele	155 (freq)	158 (freq)	161 (freq)	164 (freq)	p-value
Case	275 (0.525)	115 (0.219)	134 (0.256)	0 (0.000)	0.005188*
Control	223 (0.623)	74 (0.207)	60 (0.168)	1 (0.003)	

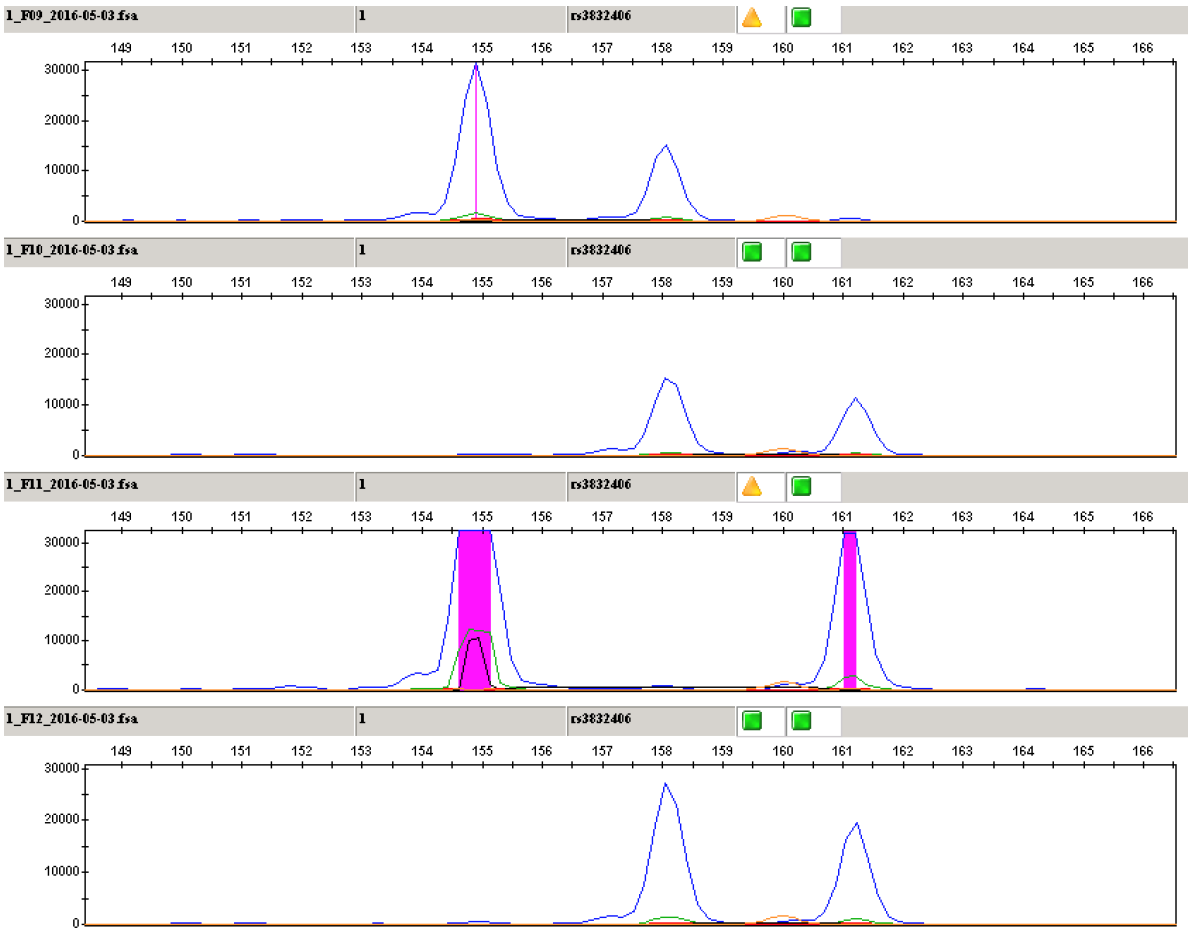

Fig. 1. Representative distribution of three alleles from GeneMapper. Allele1 size was 155, allele2 size was 158 and allele3 size was 161.

Table 5. Odds ratio of three alleles in mothers

Case/controls	Odds ratio	CI	p-value
Allele 1	0.5859962	0.3585593-0.9399636	0.029253*
Allele 2	1.146151	0.7721792-1.707832	0.50006
Allele 3	1.76746	1.182570-2.663079	0.00589*

3.2. *Alleles of rs3832406 Associated with NTD Risk When Pre- sent in the Mother*

The same type of logistical regression described above was used to find the odds ratios for mothers of case/control infants. When present in the mothers, allele 1 of rs3832406, was significantly associated ($p = 0.029$) with an odds ratio value of 0.586 (Table 5). Therefore, mothers that are carriers of allele 1 have a significantly lower chance of having a NTD child compared to mothers lacking allele 1 genotype. As shown in Table 5, allele 2 did not significantly affect the risk of mother's to have a NTD child. When mothers carried allele 3, they had a significantly higher chance ($p = 0.006$; odds ratio 1.767) of giving birth to an NTD affected child than mothers without the allele 3 genotype (Table 5).

4. Discussion

During the last step of the mitochondrial 1C pathway, MTHFD1L is responsible for encoding the monofunctional 10-formyl-THF synthetase to produce formate. Most cellular formate originates from the mitochondria and is transported into the cytoplasm. Disruption of mitochondrial formate production will affect the cytoplasmic formate supply [15]. The association between maternal folate status and risk for NTDs has led to the development of a number of folate-pathway knockout mice that have been used to study the impact of folate on mammalian embryonic development [16]. A knockout mouse model confirms that MTHFD1L plays a critical role in NTC, and deletion of *Mthfd1l* in mice produced 100% penetrant NTDs in nullizygous embryos [13]. The strong linkage between MTHFD1L and NTD prevalence prompted us to study variants in this gene in a human NTD cohort.

A previous study has shown that the *Mthfd1l* rs3832406 polymorphism was a risk factor for NTDs in the Irish population studied, and the presence of an additional ATT repeat in allele 2 affected alternative splicing efficiency [14]. In the Irish population, allele 1 increased infant NTD risk, while allele 2 decreased infant NTD risk, and allele 3 had no associated risks. In the current study, no association between infant NTD risk and MTHFD1L rs3832406 al-

leles 1, 2 and 3 was observed. Interestingly, we identified a maternal association between rs3832406 and NTD risks in the USA study population. Our data shows that mothers with allele 3 of rs3832406 had significantly increased maternal risk of having an NTD baby, while mothers with allele 1 of rs3832406 had significantly decreased risk of offspring with an NTD. Embryos rely on nutrition from their mothers during early development, so it is likely that the defective folate metabolism of a mother could increase the risk of having an NTD infant. Defective MTHFD1L function may impact maternal formate supply to the embryo. Supplementation of Mthfd1l heterozygous dams with formate rescues the growth and NTD phenotype of Mthfd1l nullizygous embryos, supporting the importance of formate transfer from mother to embryo [13].

The different association results between the Irish population and the American population on rs3832406 and NTD risk could be due to several different reasons. Clearly, the two populations have different genetic backgrounds, therefore it is highly likely that there are different modifying genes and different single nucleotide variant (SNV)-SNV interactions which may contribute to the differences in the US and Irish populations with respect to NTD risks. Apart from the genetic background, the different environmental factors encountered by these populations should be considered as a possible explanation for the different effect of the alleles. For example, the diets of the two populations vary, which can impact how much a polymorphism will affect the expression of various genes and ultimately the phenotype. The U.S. also has a mandatory program in place for food fortification with folic acid while Ireland has not implemented a mandatory folate fortification program. These key nutritional differences could be a potential cause of the discrepancies in the results obtained with the Irish and American populations.

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Conflict of Interest

All the authors declare no conflicts of interest.

Author Contributions

Paige McKenzie performed the experiments and conceptualized the study with Drs. Lei and Finnell and assisted in writing the manuscript; Yunping Lei conceptualized the study and performed the experiments with Ms. McKenzie and assisted in writing the manuscript; Dean Appling provided scientific input into the study and assisted in writing the manuscript; Jessica Momb provided scientific input into the study and assisted in writing the manuscript; Richard Finnell conceptualized the study, wrote the manuscript and obtained the samples and funding for the study.

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