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A *GCH1* Haplotype and Risk of Neural Tube Defects in the National Birth Defects Prevention Study

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Abstract

Tetrahydrobiopterin (BH_4) is an essential cofactor and an important cellular antioxidant. BH_4 deficiency has been associated with diseases whose etiologies stem from excessive oxidative stress. GTP cyclohydrolase I (GCH1) catalyzes the first and rate-limiting step of *de novo* BH_4 synthesis. A 3-SNP haplotype in *GCH1* (rs8007267, rs3783641, and rs10483639) is known to modulate *GCH1* gene expression levels and has been suggested as a major determinant of plasma BH_4 bioavailability. As plasma BH_4 bioavailability has been suggested as a mechanism of neural tube defect (NTD) teratogenesis, we evaluated the association between this *GCH1* haplotype and the risk of NTDs. Samples were obtained from 760 NTD case-parent triads included in the National Birth Defects Prevention Study (NBDPS). The three SNPs were genotyped using TaqMan® SNP assays. An extension of the log-linear model was used to assess the association between NTDs and both offspring and maternal haplotypes. Offspring carrying two copies of haplotype C-T-C had a significantly increased NTD risk (risk ratio [RR] = 3.40, 95% confidence interval [CI]: 1.02–11.50), after adjusting for the effect of the maternal haplotype. Additionally, mothers carrying two copies of haplotype C-T-C had a significantly increased risk of having an NTD-affected offspring (RR = 3.46, 95% CI: 1.05–11.00), after adjusting for the effect of the offspring haplotype. These results suggest offspring and maternal variation in the *GCH1* gene and altered BH_4 biosynthesis may contribute to NTD risk.

Keywords

GCH1 gene; GTP cyclohydrolase I; haplotype; neural tube defects; tetrahydrobiopterin (BH_4)

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1. INTRODUCTION

Tetrahydrobiopterin (BH_4) is a naturally occurring nutrient and an essential cofactor for several important biological processes. A deficit in biosynthesis or regeneration of BH_4 may result in nitric oxide (NO) deficiency and oxidative stress. BH_4 is a cofactor for nitric oxide synthase, which regulates methionine synthase (MS) activity and neural tube closure. In chick embryos, the inhibition of BH_4 biosynthesis reduces MS activity and leads to defects in neural tube closure [1, 2].

Serum BH_4 concentrations are regulated by the enzymes GTP cyclohydrolase I (GCH1), 6-pyruvoyl-tetrahydropterin synthase (PTS), and sepiapterin reductase (SPR), respectively (Figure 1). GCH1 is the rate-limiting enzyme in this pathway and is a major determinant of BH_4 levels [3]. Recent studies have reported that a functional haplotype in the *GCH1* gene, A-T-G (rs8007267-rs3783641-rs10483639), is associated with lower *GCH1* gene expression, lower plasma and vascular BH_4 availability, and adverse health outcomes [4, 5]. The complete haplotype is comprised of 15 SNPs, but can be determined by three tagSNPs: rs8007267 in the promoter region; rs3783641 in intron 1 and rs10483639 in the 3' un-translated region.

As other potentially oxidative pathologic mechanisms (e.g., maternal pre-gestational diabetes, obesity, and folate deficiency) are associated with neural tube defects (NTDs) [6–10]; and as BH_4 deficiency may modulate neural tube closure, we hypothesized that BH_4 concentrations, as regulated by the *GCH1* haplotype, are associated with NTDs. Therefore, we evaluated the role of offspring and maternal *GCH1* haplotypes defined by these three tagSNPs on the risk of NTDs in the National Birth Defects Prevention Study (NBDPS).

2. MATERIALS AND METHODS

2.1 Subjects

The study population included NTD case-parent triads ($n = 760$) from the NBDPS, with estimated dates of delivery between January 1, 1999 and December 31, 2007. Details of the NBDPS have been published elsewhere [11]. In brief, the NBDPS is a population-based, case-control study of major structural birth defects. For the period 1999–2007, case infants with one or more congenital anomalies were ascertained through ten birth defects surveillance systems throughout the United States (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah) and included live births, stillbirths, and induced pregnancy terminations. NTDs included in the NBDPS had British Pediatric Association (BPA) codes for the diagnoses anencephaly (740.0), craniorachischisis (740.1), spina bifida (741.0), and encephalocele (742.0). Abstracted data for all NTD case infants were reviewed by clinical geneticists using specific criteria, including standardized case definitions and confirmatory diagnostic procedures [12]. Infants/fetuses with known single gene disorders or chromosomal abnormalities were excluded from the NBDPS. Mothers completed a one-hour computer assisted telephone interview (CATI) in English or Spanish between 6 weeks and 2 years after the estimated date of delivery. The interview included sections on maternal conditions and illnesses, lifestyle and behavioral factors, and multivitamin use.

2.2 Genotyping Analysis

Buccal brushes from mothers, fathers, and infants were collected as part of the NBDPS [13]. DNA was extracted from buccal cells. A standard quality control procedure was applied to each sample before they were submitted to the NBDPS sample repository [13]. To assure genotyping proficiency, high quality, and high concordance among all NBDPS laboratories, annual evaluations are conducted to confirm the performance of each laboratory (See

Supplemental Material). Our laboratory at the University of Texas Austin Dell Pediatric Research Institute has passed all of these evaluations with a score of 100%. SNPs were assayed using TaqMan method on the ABI PRISM® 7900HT Sequence Detection System (Life Technologies Corporation, Carlsbad, CA). Genotype calls were made by TaqMan® Genotyper Software v1.0.1. SNP allele designation used in our paper is consistent with the dbSNP (reference genome assembly, NCBI Build 37.3).

2.3 Statistical Analysis

The characteristics of cases and parents were summarized using counts and proportions for the following variables: phenotype (spina bifida, anencephaly, encephalocele); infant sex (male, female); maternal age (<20, 20–34, ≥35 years); race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, other); education (<12, 12, 13–15, >15 years); folic acid supplementation during three months before conception through the first month of pregnancy (no, yes); body mass index (underweight [<18.5 kg/m^2], average weight [18.5–24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obese [≥30.0 kg/m^2]; and pre-pregnancy diabetes (no, yes). For each analyzed polymorphism, samples for which a genotype could not be assigned and triads that had genotype combinations that were inconsistent with Mendelian inheritance were determined. For each subject, the number of genotyping failures (i.e., genotypes that could not be assigned) was determined. These analyses were performed using Intercooled Stata, version 12.1 (StataCorp LP, College Station, TX).

An extension of the log-linear model was used to assess the association between NTDs and both offspring and maternal haplotypes. This was done using HAPLIN version 4.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin/), running under R version 2.14.0 (R Foundation for Statistical Computing) [14]. HAPLIN estimates the relative risk (RR) of a single-or double-dose of each haplotype using a maximum likelihood approach. In instances where phase is unknown, it is constructed using available family information. Incomplete triads are included and missing information is imputed using the expectation maximization algorithm [14]. Haplotypes are estimated using information from all available subjects; therefore the derived haplotype frequencies are identical for mothers and offspring. A threshold of 2% for haplotype frequency was used to avoid the inclusion of rare haplotypes. For each haplotype, a RR and 95% confidence interval (95% CI) was estimated. The RR is a comparison of the specific haplotype compared to the reference group of the remaining haplotypes. The single-dose RR is the change for an individual carrying one copy (heterozygotes of the haplotype), and the double-dose RR compares homozygotes of the haplotype to the remaining subjects. In addition, a *P*-value for the overall effect of all haplotypes in the *GCH1* gene was determined using a likelihood ratio test to compare the log-linear model between a full model, which included terms for the effects of all haplotypes in each gene, with null models that included no effects. Additionally, analyses were conducted among those triads with spina bifida only to determine whether the results obtained using data from all triads were influenced by heterogeneity within the full group. We did not conduct analyses among those triads with only anencephaly or encephalocele due to small numbers.

3. RESULTS

Participation in the NBDPS for the period 1999–2007 was 74% among NTD case mothers, yielding 1,553 families available for analysis. Among those, 760 (49%) provided buccal brushes (1,791 individuals). Genotyping was performed on DNA samples derived from these 760 families. Based on quality control checks, one family was excluded for being inconsistent with Mendelian inheritance at more than one genotype. Additionally, 114 subjects were excluded for failure on more than one genotype, leaving a total of 740 case-parent triads (97% of the original sample). Of those, 296 were complete triads, 340 were

dyads, and 104 were monads with only one person in the family. After these quality control measures were applied, at least 96% of the samples for each variant were available for analyses; therefore the genotypes were considered of sufficiently high quality.

The distributions of key characteristics among NTD case-parent triads are presented in Table 1. Spina bifida was the most common phenotype among case subjects (n = 451, 61.0%). Furthermore, a majority of case mothers were non-Hispanic White (n = 443, 60.2%). The only characteristics presented in Table 1 that were significantly different between interviewed case mothers who provided buccal brushes and those who did not were race/ethnicity (the proportion of non-Hispanic White mothers was higher among those who provided buccal brushes) and education (the proportion of mothers with >12 years of education was higher among those who provided buccal brushes), data not shown.

Table 2 includes the RR estimates and 95% CIs for the association between offspring and maternal *GCH1* haplotypes (rs8007267-rs3783641-rs10483639). Four haplotypes (C-T-C, C-T-G, T-A-C and T-A-G, had allele frequencies above the threshold (0.02) ranging from 3.89 (95% CI: 2.47–6.12) for T-A-G to 73.27 (95% CI: 69.22–77.03) for C-T-G (allele frequencies for each SNP are included in Supplemental Table 1). The *P*-value for the overall effect of all haplotypes was 0.08. The following haplotypes were not significantly associated with NTD risk: C-T-G, T-A-C, and T-A-G. However, offspring with two copies of the C-T-C haplotype had a greater risk of developing an NTD compared to all other offspring (RR = 3.40, 95% CI: 1.02–11.50), after adjusting for the maternal genetic effect. Additionally, mothers with two copies of the C-T-C haplotype were more likely to have offspring with an NTD (RR = 3.46, 95% CI: 1.05–11.00), after adjusting for the offspring genetic effect. These results were similar (e.g., the estimated RRs were similar) when analyses were restricted to spina bifida cases only, therefore only results in the full group are presented.

4. DISCUSSION

In one of the largest studies of its kind using data from the NBDPS, we observed significant associations between offspring and maternal *GCH1* haplotypes and NTD risk. Specifically, case infants and mothers with two copies of the C-T-C haplotype had more than a 3-fold increase for NTDs. The C-T-G, T-A-C, and T-A-G haplotypes were not associated with NTD risk.

GCH1 and genes encoding other key enzymes in the BH_4 biosynthetic pathway are expressed during early embryonic stage and are known to play an important role in central nervous system development [15]. Although there is no direct evidence regarding the functional effect of the C-T-C haplotype, previous studies have demonstrated that haplotype variability in *GCH1* is associated with the enzymatic activity and BH_4 bioavailability [4]. The “functional” haplotype originally reported by Antoniades and colleagues [4] was observed in our study population with a haplotype frequency of 1.19% and could not be evaluated owing to its low frequency.

An important strength of our study is use of data from the NBDPS, the largest population-based study of birth defects, which provided a unique opportunity to examine both maternal and offspring genetic effects on NTD risk. Furthermore, we employed a case-parent triad design, which is immune to confounding by race/ethnicity (i.e., population stratification) in the assessment of offspring genotypes [16]. The log-linear modeling approach to analyses also allowed us to include data from incomplete triads (i.e., genotype data is missing for one or two individuals) [14, 17]. An additional strength of the NBDPS is the extensive and standardized case review employed by clinical geneticists, which maximizes homogeneity among case groups.

The main weakness of this study was the limited proportion of families with biologic samples available (49%). The low percentage of families on which the genetic findings are based could limit our ability to generalize these findings. However, the demographic differences between those who were included in this study and those who were not, is not expected to be associated with genotypes. Moreover, it is unlikely that those who chose to contribute samples were substantially different in genotypes to those who did not. Additionally, although we had a relatively large sample size, since the significant haplotypes were relatively uncommon (haplotype frequencies ~5%), we were not able to conduct additional stratified analyses (e.g., based on additional phenotypes or folic acid supplementation).

To our knowledge, this is the first study evaluating the *GCH1* haplotype in the BH_4 biosynthesis pathway as possible risk factors for NTDs. A significant association with the haplotype in offspring *GCH1* gene was observed. The results suggest that disrupted BH_4 biosynthesis may contribute to abnormal neural tube closure. Replication of these findings in other populations and further investigation on the BH_4 biosynthesis and regeneration pathways as potential mechanisms contributing to the population burden for NTDs is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the California Department of Public Health.

Abbreviations

BH4	tetrahydrobiopterin
DHPR	quinoid dihydropteridine reductase
GCH1	GTP cyclohydrolase I
NBDPS	National Birth Defects Prevention Study
NO	nitric oxide
NTDs	neural tube defects
PCD	pterin-4a-carbinolamine dehydratase
PTS	6-pyruvoyl-tetrahydropterin synthase
SPR	sepiapterin reductase

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Highlights

- Plasma BH4 may be associated with neural tube defects (NTDs)
- GCH1 haplotype affects BH4 bioavailability
- We evaluated the GCH1 haplotype in 760 NTD trios
- Offspring with two copies of haplotype C-T-C had a significantly increased NTD risk
- Mothers with the same haplotype had an increased risk of having an affected child
- These results suggest variation in the *GCH1* haplotype may contribute to NTD risk

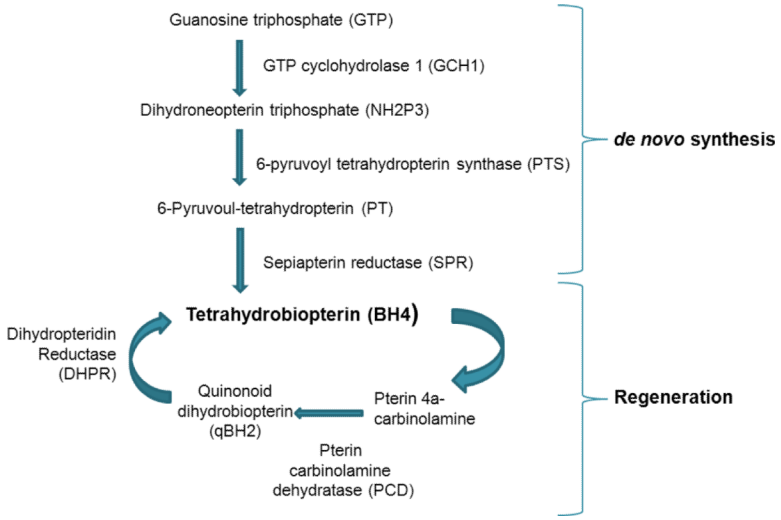

Figure 1. *De novo* biosynthesis and regeneration of tetrohydrobiopterin (BH_4)

The pathway for the *de novo* biosynthesis of BH_4 from GTP involves GTP cyclohydrolase I (GCH1), 6-pyruvoyl-tetrahydropterin synthase (PTS) and sepiapterin reductase (SPR). GCH1 catalyzes the rate-limiting step. The regeneration requires pterin-4a-carbinolamine dehydratase (PCD) and quinoid dihydropteridine (qBH2) and dihydropteridin reductase (DHPR).

Table 1

Characteristics of Neural Tube Defect Case-Parent Triads (n = 740), National Birth Defects Prevention Study, 1999–2007

Characteristic	No.	%
Phenotype		
Spina bifida	451	61.0
Anencephaly	215	29.1
Encephalocele	73	9.9
Infant sex		
Male	336	47.7
Female	368	52.3
Maternal age (years)		
<20	82	11.1
20–34	558	75.5
≥35	99	13.4
Race/ethnicity		
Non-Hispanic White	443	60.2
Non-Hispanic Black	34	4.6
Hispanic	219	29.8
Other	40	5.4
Education (years)		
<12	137	18.5
12	187	25.3
13–15	227	30.7
>15	188	25.5
Folic acid supplementation[a]		
No	346	46.8
Yes	393	53.2
Body mass index (kg/m^2)		
Underweight (<18.5)	28	4.0
Normal (18.5–24.9)	340	48.9
Overweight (25.0–29.9)	151	21.7
Obese (≥30.0)	176	25.3
Pre-pregnancy diabetes		
No	725	98.1
Yes	14	1.9

[a]Three months before conception through the first month of pregnancy

Table 2

Offspring and Maternal *GCH1* Haplotype and the Risk of Neural Tube Defects

Haplotype[a]	Haplotype frequency (95% CI)	Haplotype copies	RR[b] (95%CI)	*P*-value
Offspring				
C-T-C	4.88 (3.36–7.12)	Single	0.98 (0.56–1.74)	0.94
		Double	3.40 (1.02–11.50)	0.04
C-T-G	73.27 (69.22–77.03)	Single	0.91 (0.47–1.74)	0.77
		Double	0.98 (0.47–2.07)	0.96
T-A-C	17.71 (14.54–21.29)	Single	1.49 (0.89–2.50)	0.14
		Double	1.46 (0.60–3.47)	0.39
T-A-G	3.89 (2.47–6.12)	Single	0.59 (0.27–1.26)	0.17
		Double	2.72 (0.38–19.80)	0.32
Maternal				
C-T-C	4.88 (3.36–7.12)	Single	1.32 (0.84–2.04)	0.22
		Double	3.46 (1.05–11.00)	0.03
C-T-G	73.27 (69.22–77.03)	Single	1.00 (0.57–1.78)	0.99
		Double	1.24 (0.68–2.34)	0.50
T-A-C	17.71 (14.54–21.29)	Single	1.01 (0.67–1.51)	0.98
		Double	1.30 (0.70–2.45)	0.41
T-A-G	3.89 (2.47–6.12)	Single	0.68 (0.37–1.24)	0.20
		Double	3.47 (0.70–18.00)	0.13

[a]Haplotypes are named using the reference alleles on the forward strand, Genome Build 37.3.

[b]Comparison of the specific haplotype compared to the reference group of the remaining haplotypes