Gene 510 (2012) 180–184

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Reduced folate carrier A80G polymorphism and susceptibility to neural tube defects: A meta-analysis

Hai-Gang Wang [a,b], Jia-Li Wang [c], Jian Zhang [b], Li-Xia Zhao [a,b], Guang-Xi Zhai [b], Yu-zhu Xiang [d], Ping Chang [a,*]

[a] Department of Pharmacy, Qilu Hospital, Shandong University, Jinan, China
[b] School of Pharmacy, Shandong University, Jinan, China
[c] Department of Emergency, Qilu Hospital of Shandong University, Jinan, China
[d] Minimally Invasive Urology Center, Provincial Hospital Affiliated to Shandong University, Jinan, China

ARTICLE INFO

Article history:
Accepted 16 February 2012
Available online 10 September 2012

Keywords:
Birth defects
Polymorphism
Meta-analysis
Neural tube defects
Reduced folate carrier

ABSTRACT

The reduced folate carrier (RFC1) plays a crucial role in mediating folate delivery into a variety of cells. *RFC1* polymorphism (A80G) has been reported to be associated with increased risk of neural tube defects (NTDs). However, results derived from individually underpowered studies are conflicting. We performed a systematic search of MEDLINE and EMBASE databases and carried out a meta-analysis on the association between *RFC1* polymorphism (A80G) and NTDs risk. Overall, a significant correlation between *RFC1* A80G polymorphism and NTDs risk was found neither in infants nor in maternal (allele contrast in infants: $OR_{RE} = 1.15$, 95% CI: 0.92–1.45; allele contrast in mothers: $OR_{RE} = 1.24$, 95% CI: 0.98–1.56). The present meta-analysis failed to support a positive association between *RFC1* A80G polymorphism and susceptibility to NTDs. It is important to realize, however, that socio-economic factors, and gene–environment and gene–gene interactions, could have influenced the outcome of our meta-analysis. For this reason, a relationship between the A80G polymorphism and NTD risk cannot be entirely discounted.

1. Introduction

Neural tube defects (NTDs) are common birth defect caused by partial or complete failure of neural tube closure during early embryologic development (Kondo et al., 2009). NTDs can range from mild to severe symptoms depending on the location and severity of the defects. Three main defects are anencephaly, encephalocele and spina bifida. Anencephaly is usually associated with death within days of birth. The majority of individuals affected by spina bifida can survive with appropriate medical care, but they could not avoid a high degree of disability and the limited life expectancy. The incidence of NTDs is about 0.5–2 per 1000 births worldwide, which is higher in China with approximately 2.74 per 1000 births(Botto et al., 1999; Chen et al., 2010; Greene et al., 2009).

The exact mechanism underlying NTDs remains poorly understood, but is believed to involve a complex interaction between genetic and environmental factors. A multitude of epidemiological studies have reported that periconceptional folic acid supplementation substantially reduce the risk of NTD occurrence and recurrence by up to 75% (Berry et al., 1999; Blencowe et al., 2010; Blom & Smulders, 2010). These observations have resulted in considerable interest in the study of genetic polymorphisms in enzymes of folate metabolism that might modify risk for such malformations, e.g., C677T in the methylenetetrahydrofolate reductase gene (*MTHFR*). The enzyme MTHFR plays an important role in catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. MTHFR enzyme activity in individuals homozygous for the C677T polymorphism decreases approximately 70% *in vitro* (Frosst et al., 1995). In non-Latin European populations, the TT genotype was found to be associated with a statistically significant increase of risk of NTDs (Amorim et al., 2007).

Besides metabolism, the absorption of folate and its uptake into the cell is also of great significance (Relton et al., 2004a). The reduced folate carrier (RFC1) is a cell surface transmembrane protein that mediates bidirectional movement of folate across the membrane (Freisheim et al., 1989; Goldman et al., 1968; Kamen et al., 1991; Matherly et al., 1991). It has a much higher affinity for reduced folates, including the physiological substrate 5-methyltetrahydrofolate. Thus, RFC1 must operate efficiently to ensure intracellular folate concentration. A common polymorphism is an A-to-G change at position 80 (A80G) in exon 2 of *RFC1* gene replacing a histidine with an arginine in the protein (H27R) (Chango et al., 2000). It is still inconclusive whether this polymorphism in mothers or infants would be causal in determining NTDs susceptibility, although several studies aimed to answer this question.

Abbreviations: HWE, Hardy–Weinberg equilibrium; MTHFR, methylenetetrahydrofolate reductase; NTDs, neural tube defects; PCR, polymerase chain reaction; RFC1, reduced folate carrier; RFLP, restriction fragment length polymorphism; VYS, visceral yolk sac.

* Corresponding author at: Department of Pharmacy, Qilu Hospital, Shandong University, 107 Wenhuaxi Road, Jinan, 250012, China. Tel.: +86 531 82169638; fax: +86 531 86927544.
E-mail address: qlyycp@126.com (P. Chang).

0378-1119/$ – see front matter
http://dx.doi.org/10.1016/j.gene.2012.02.020

Here, we evaluate the association between *RFC1* polymorphism (A80G) and NTDs in a meta-analysis, collecting all the appropriate studies.

2. Methods

2.1. Study identification

We carried out a systematic search for studies reporting the association between *RFC1* A80G polymorphism and risk of NTDs in MEDLINE and EMBASE databases. The following search terms were used: 'neural tube defects', 'NTDs', 'anencephaly', 'spina bifida', or 'encephalocele'; "RFC1" or "reduced folate carrier" and 'gene', 'polymorphism' or 'genetic variant'. The obtained articles were examined by a quick view at the titles and abstracts and inappropriate articles were rejected in the initial screening. The remaining articles were assessed by full text reading. A snowball searching was carried out to identify additional potential studies in the references of identified articles. For studies with overlapping cases/controls, the most recent ones with the largest number of participants were included in the meta-analysis. The latest search was performed on August 7, 2011 without any language limitation.

2.2. Data extraction

Information related to result explanation were extracted from each articles by two authors independently, including first author, year of publication, study design, sample size, demographics (race/ethnicity and location), genotyping method, NTDs characteristics, source of controls and blinding of laboratory workers to participant status. The frequencies of the alleles and the genotypic distributions were extracted or calculated for both the cases and the controls. Discrepancies were resolved by discussion until consensus was achieved.

2.3. Statistical analysis

The association of *RFC1* A80G polymorphism with NTDs risk were examined and evaluated by odds ratio (OR) with the corresponding 95% confidence intervals (CIs). A pooled OR was obtained based on the individual ORs. Cochran's Q statistic was used to assess the heterogeneity between studies, and $P<0.10$ indicated significant heterogeneity(Higgins & Thompson, 2002; Zintzaras & Ioannidis, 2005). The pooled estimation of OR was calculated by the fixed effects model (Mantel–Haenszel methods) if there was no significant heterogeneity across studies, otherwise, it was calculated by the random effects model (DerSimonian and Laird's method) (DerSimonian & Laird, 1986; Mantel & Haenszel, 1959).

The distribution of genotypes in controls for all studies was tested whether it departed from Hardy–Weinberg equilibrium (HWE) by Pearson's χ^2 test(Hardy, 2003). Sensitivity analysis was carried out to examine the effect of excluding specific studies with controls not in HWE or evaluated by estimating the pooled ORs in the absence of each study(Tobias, 1999; Trikalinos et al., 2006). Potential publication bias was evaluated using Egger's regression test and the Begg–Mazumdar test, $P<0.10$ indicated statistically significant publication bias(Macaskill et al., 2001).

Analyses were performed with Stata software (version 10.0; Stata Corporation, College Station, Texas, USA), using two-side *P*-values.

3. Results

3.1. Characteristics of the included studies

A total of 28 potentially relevant articles were retrieved in the listed databases using our search strategy. After an initial screening of the title and abstract, 20 articles studying the relationship between *RFC1* A80G polymorphism and risk of NTDs were retained for a full text evaluation. Five articles were excluded for providing irrelevant data and 8 were later excluded for overlapping data published by the same investigators. Finally, 7 studies were included in the meta-analysis, provided data on 4281 individuals totally (1550 cases and 2731 controls). Fig. 1 presents a flowchart for the process of articles inclusion/exclusion.

Among these articles, 6 studies investigated the influence of *RFC1* A80G polymorphism in mothers of NTDs offspring on NTDs risk (De Marco et al., 2001; Morin et al., 2003; O'leary et al., 2006; Pei et al., 2009; Relton et al., 2004b; Shang et al., 2008), 5 studies investigated the association of *RFC1* A80G polymorphism in infants with NTDs (De Marco et al., 2001; O'leary et al., 2006; Pei et al., 2009; Relton et al., 2004b; Shaw et al., 2002). Table 1 summarizes the general characteristics of the studies included in the meta-analysis. All the studies were described as case–control in design. The control groups were either hospital outpatients or community controls. NTD cases with a wide range of severity (e.g. anencephaly, encephalocele and spina bifida) were included in most studies, except that by Morin et al.(Morin et al., 2003) and Shaw et al.(Shaw et al., 2002) selected spina bifida cases only. Two studies investigated the influence of the maternal periconceptional use of folic acid (Pei et al., 2009; Shaw et al., 2002). The identified studies were undertaken in different ethnicities: 4(De Marco et al., 2001; Morin et al., 2003; O'leary et al., 2006; Relton et al., 2004b) providing data on Caucasians, 2(Pei et al., 2009; Shang et al., 2008) on East Asians (Chinese), and one(Shaw et al., 2002) on mixed ethnic origins. In all studies, polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis was used for genotyping analysis. The distribution of genotypes in the control group deviated from HWE in one study(Shang et al., 2008). Two studies(Pei et al., 2009; Shaw et al., 2002) mentioned genotyping was performed under blind conditions. The studies were published from 1999 through 2009.

3.2. The association between infant RFC1 A80G polymorphism and NTDs risk

Table 2 shows the meta-analysis results for the association of infant *RFC1* A80G polymorphism with risk of NTDs. Overall, significant heterogeneity between studies was found in all genetic contrasts except recessive model. No significant association was observed in any genetic model test when all the eligible studies were pooled together (allele contrast: $OR_{RE}=1.15$, 95% CI: 0.92–1.45; dominant model: $OR_{RE}=1.22$, 95% CI: 0.87–1.69; recessive model: $OR_{FE}=1.11$, 95% CI: 0.92–1.35; additive model: $OR_{RE}=1.31$, 95% CI: 0.84-2.04; Fig. 2). Removal of any one study did not result in movement of the point estimate outside the 95% CIs, suggesting no single study exhibited excessive influence (Supplement Fig. 1). No significant publication bias was detected by formal statistics (Egger's test, $P=0.16$; Begg's test, $P=0.46$).

3.3. The association between mother RFC1 A80G variant and NTDs risk

There were 706 cases and 1387 controls included for the association between mother *RFC1* A80G variant and NTDs. Overall, no genetic model showed significant association (allele contrast: $OR_{RE}=1.24$, 95% CI: 0.98–1.56; Fig. 3; Table 2). Heterogeneity among studies was significant in all genetic contrasts with the exception of recessive model (allele contrast: $P_{Q\text{-}Test}=0.031$). Exclusion of one study with controls deviating from HWE(Shang et al., 2008) did not alter the pattern of results(allele contrast: $OR_{RE}=1.16$, 95% CI: 0.93–1.43). None of the single study exhibited excessive influence on the pooled results (Supplement Fig. 2). Significant publication bias was detected in meta-analysis (Egger's test, $P=0.05$; Begg's test, $P=0.06$), indicating there is differential magnitude of effect in large vs small studies.