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The *SLC19A1* 80G>A polymorphism is not associated with male infertility

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Abstract

Previous studies have revealed that genetic factors may be involved in regulating folate turnover, e.g. methylenetetrahydrofolate reductase polymorphism in the development of male infertility. Folate transporter, encoded by the *SLC19A1* gene, commonly referred to as reduced folate carrier (RFC) is a transmembrane protein, which transfers hydrophilic folates across the cell membrane. It was hypothesized that common polymorphism within the *SLC19A1* gene (rs1051266:G>A, 80G>A) may alter RFC function. The aim of this study was to investigate a potential association between the *SLC19A1* 80G>A polymorphism and male infertility in a case–control study. The *SLC19A1* 80G>A polymorphism was determined by means of a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay in 213 infertile Caucasian men and 226 ethnically matched controls. The distribution of *SLC19A1* genotypes in the infertile men was as follows: GG 26.8%, GA 51.2%, AA 22.1% and in fertile men: GG 24.8%, GA 50.4%, AA 24.8%, and was comparable in the both the evaluated groups. Odds ratios (95% confidence interval, CI): 0.90 (0.59–1.38) and 0.88 (0.56–1.36) for dominant and recessive models remained non-significant, also after adjustment for age: 0.89 (0.57–1.37) and 0.80 (0.51–1.25), respectively. Our study demonstrated that polymorphism 80G>A of the *SLC19A1* gene is not associated with male infertility.

Keywords: *Male infertility; genetic polymorphism; reduced folate carrier;* SLC19A1

Introduction

Infertility is a common problem affecting one in six couples, and in 30% of infertile couples, the male factor, in the form defective sperm quality, is a major cause (Brugo-Olmedo et al. 2001). However, factors responsible for many cases of male infertility have not been fully explained. The pattern of familial aggregation of male infertility also suggests an important role of heritable factors (Meschede et al. 2000).

Folates are key factors for the maintenance of genome integrity due to their role in DNA synthesis, repair and methylation. They are required for purine and pyrimidine, and thus DNA synthesis, as well as for the remethylation of homocysteine into methionine which is further metabolized into *S*-adenosylmethionine, the universal methyl donor for transmethylation of DNA. In this way, folates play a key role in epigenetic regulation of gene expression. Folate deficiency, either by insufficient nutritional uptake or linked to some single nucleotide polymorphism (SNP), will lead to an impaired DNA synthesis and repair, a hypomethylation of DNA and other molecules, and homocysteine accumulation (Fowler 2005, Forges et al. 2007). Previous studies have revealed that genetic factors may be involved in regulating folate turnover in the development of male infertility (Lee et al. 2006, Paracchini et al. 2006). It was found that methylenetetrahydrofolate reductase (*MTHFR*) 677C>T, methionine synthase (*MS*) 2756A>G and methionine synthase reductase (*MTRR*) 66A>G genotypes are associated with male infertility. However contrasting data were also reported, i.e. no association of male infertility with *MTHFR* polymorphism (Stuppia et al. 2003, Ebisch et al. 2003). Discrepancy in the results observed (especially

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for *MTHFR* polymorphism) may suggest an influence of environmental factors that varies in different populations studied or involvement of other not yet defined genetic factors. In our previous study an association between *ABCB1* (*MDR1*) 3435C>T polymorphism with male infertility was revealed (Drozdzik et al. 2009). The *ABCB1* transporter is implicated in transmembrane transport of many endogenous and exogenous compounds (e.g. pesticides in the case of male infertility), and possibly folates (Yang et al. 2008). Also some pharmacological data may support the role of folates in male fertility. Ebisch et al. (2006) reported that folic acid supplementation produced a significant increase in sperm count which was *MTHFR* polymorphism dependent.

Therefore it was decided to study a polymorphism of another factor modulating cellular folate status, i.e. folate transporter, *SLC19A1* encoding reduced folate carrier (RFC). RFC, a bidirectional anion transporter ubiquitously expressed in mammalian cells and tissues, is a 65-kDa transmembrane protein comprising 591 amino acid residues, which transfers hydrophilic folates across the cell membrane using negatively charged glutamate residue of folate. In humans it exists in a highly glycosylated form with a mass of approximately 92 kDa. RFC is the major uptake route of reduced folates essential for a wide spectrum of biochemical reactions, including cellular proliferation. In addition to its generalized role as a folate transporter, RFC provides specialized tissue functions including absorption across intestinal/colonic epithelia, transport across the basolateral membrane of renal proximal tubules, transplacental transport of folates, and folate transport across the blood–brain barrier. Its expression was also found in human testis (Moscow et al. 1995). The gene coding for the human RFC protein (*SLC19A1*) is located on the long arm of chromosome 21, specifically the 21q22.2-q22.3 region (Drori et al. 2000). Tolner et al. studied cDNA from 16 genomic clones and obtained two overlapping sequences covering the *SLC19A1* gene (Tolner et al. 1998). The first standard, labelled λhRFC-1-1 is a 19-kb segment containing exons 1, 2, 3 and 4. The second standard, λhRFC-1-2, is slightly shorter at 17 kb and contains exons 5 and 6. The 5′ end of λhRFC-1-2 overlaps 1.5 kb of the 3′ end of λhRFC-1-1 and thus together they cover 34.5 kb. The full-length cDNA was confirmed as the *SLC19A1* gene by transfection into methotrexate-resistant cells. These cells became resistant to this folate analogue by producing a mutation in the protein, and no longer took up methotrexate.

One common mismatch polymorphism was identified at exon 2 of the folate transporter gene(*SLC19A1*), resulting in replacement of histidine to arginine (His 27Arg , c.80A>G). That SNP has been associated with lower plasma folate, and arguably higher homocysteine levels (Chango et al. 2000), and an association of 80A>G with neural tube defects was demonstrated in some studies (De Marco et al. 2001, Morin et al. 2003, Shaw et al. 2002).

Materials and methods

Subjects and study protocol

The study was carried out on 213 consecutive, otherwise healthy male patients (aged 22–56 years, mean 34.6 ± 6.1) undergoing semen analysis due to infertility workup. The inclusion criteria were: age 18–56 years; no children from current or previous relations with a history of at least a year of regular (2–3-weekly), unprotected sexual activity without conception; female partners aged up to 35 years with regular menstrual bleeding and/or progesterone levels in the luteal phase of the cycle >10 ng ml^{-1}, normal transvaginal ultrasound examination, negative testing for *Chlamydia trachomatis* infection, without history of pelvic inflammatory disease or abdominal operations.

The subjects were excluded from the study if semen analysis and clinical picture suggested obturatory azoospermia or testicular, epididymal or accessory gland infection. Also the subjects with known systemic disease, varicocele, history of mumps, testicular torsion or maldescent as well as trauma were not taken into consideration.

Semen samples were collected by masturbation after 2–7 days of abstinence from sexual activity. Sperm parameters were evaluated manually within 1 h of the sample collection. Sperm concentration, motility (percentage motility) and morphology were assessed according to the World Health Organization guidelines and criteria set in 1999 (World Health Organization 1999). Sperm concentration and motility were established in the Makler counting chamber (Sefi, Israel) under a phase-contrast microscope (Eclipse 200; Nikon, Tokyo, Japan).

Semen smears were stained according to the Papanicolaou method. Sperm morphology was assessed by a single technician: 200 sperm were analysed per slide using a 100x magnification oil-immersion lens. Sperm were classified as having normal or abnormal morphological features according to strict Kruger criteria (World Health Organization 1999).

The control group consisted of 226 healthy men (aged 21–56 years, mean 34.6 ± 9.5) recruited among consecutive men accompanying their female partners at term labour in the University Department of Feto-Maternal Medicine. Paternity was confirmed by the women; however the possible paternal discrepancy was additionally checked based on blood group verification.

Both the men undergoing infertility workup as well as the fertile controls were of Polish origin, recruited within the same geographical region.

The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from 450 µl of whole blood samples using a non-organic and non-enzymatic extraction method. The *SLC19A1* rs1051266:G>A polymorphism (80G>A, His27Arg) was determined using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay as previously described (Chango et al. 2000). Digestion with *Hin6*I endonuclease (Fermentas, Vilnius, Lithuania) was applied to discriminate between studied alleles.

Statistical analysis

Allele and genotype frequencies were determined by direct counting of *SLC19A1* alleles. Concordance of genotype distribution with Hardy–Weinberg equilibrium was calculated using the χ^2 test. Based on *SLC19A1* 80G>A allele frequency in the control group (50.0%), we calculated that our study with 226 controls and 213 infertile patients has over 80% power to detect differences in allele frequency between the groups over 9% and differences in genotype frequencies over 13%. The effect of *SLC19A1* polymorphism was tested for a dominant and recessive model, as recommended by Zintzaras and Lau (2008). Associations between categorical variables were assessed by Fisher's exact test, using Statistica 8.0 (Statsoft, Warsaw, Poland). Odds ratios (OR) and confidence intervals (CI) were calculated using the Newcombe–Wilson method without continuity correction. Multivariate analysis was performed to calculate odds ratios adjusted for age.

Results and discussion

Folate deficiency leads to mild hyperhomocysteinemia, which has been associated with various pathologies. Molecular mechanisms of homocysteine-induced cellular dysfunction include increased inflammatory cytokine expression, altered nitric oxide bioavailability, induction of oxidative stress, activation of apoptosis and defective methylation. Although the exact mechanism underlying these phenomena is still not clear, there is a growing body of evidence demonstrating a relationship between folate and other B vitamin deficiencies, hyperhomocysteinemia and altered spermatogenesis, as well as male infertility (Forges et al. 2007).

Data in the published literature indicated that polymorphisms in genes involved in intracellular folate metabolism may be a possible cause of male infertility. However, the results reported are controversial as some studies supported associations between *MTHFR*, *MS*, *MTRR* genotypes with male infertility, whereas others did not (especially for *MTHFR* polymorphism) (Lee et al. 2006, Paracchini et al. 2006, Stuppia et al. 2003, Ebisch et al. 2003). In a large meta-analysis investigating gene polymorphisms in male infertility, the *MTHFR* 677C>T variant was indicated as the only significant genetic infertility risk factor among common SNPs in genes related to the folate pathway (Tüttelmann et al. 2007). The *SLC19A1* 80G>A SNP, considered to have an impact on folate/homocysteine metabolism, was extensively studied in other diseases associated with folate insufficiency, i.e. cardiovascular disease, as well as in relation to antifolate methotrexate treatment outcome. Therefore we decided to study an association between folate transporter, i.e. *SLC19A1* (RFC1) polymorphism and male infertility. The distribution of *SLC19A1* genotypes in the infertile men was as follows: GG 26.8%, GA 51.2%, AA 22.1% and in fertile men: GG 24.8%, GA 50.4%, AA 24.8%, and was comparable in both evaluated groups (Table 1). Similarly the risk of infertility was not increased in any analysed genotype, i.e. in 80 GA carriers ($p = 0.817$, OR 0.94, 95% CI 0.59–1.47) as well as 80 AA carriers ($p = 0.498$, OR 0.82, 95% CI 0.48–1.40). The genotype frequency distribution did not show a significant deviation from the Hardy–Weinberg equilibrium. Even though folate deficiency was associated with male infertility, the effect of the studied polymorphism is probably too mild to be regarded as a genetic risk factor. As the folate level is strongly dependent on dietary factors, the possibility that the effect of 80G>A may be revealed only in cases of folate deficiency cannot be excluded. Our study demonstrates that polymorphism 80G>A of the *SLC19A1* (RFC1) gene is not associated with male infertility.

Table 1. *SLC19A1* rs1051266:G>A genotype and allele frequencies in infertile and fertile men.

	Cases (infertile) ($n = 213$)		Controls (fertile) ($n = 226$)				
Genotype	*n*	%	*n*	%	*p*-Value	OR (95% CI)	OR (95% CI) adjusted for age
GG	57	26.8	56	24.8	0.663*	0.90 (0.59–1.38)*	0.89 (0.57–1.37)*
GA	109	51.2	114	50.4	0.574**	0.88 (0.56–1.36)**	0.80 (0.51–1.25)**
AA	47	22.1	56	24.8			
Allele							
G	223	52.3	226	50.0	–	–	–
A	203	47.7	226	50.0	0.499	–	–

Calculated for *dominant (AA+GA vs GG) and **recessive (AA vs GG+GA) models.
OR, odds ratio; CI, confidence interval.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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