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Further evidence that hyperhomocysteinemia and methylenetetrahydrofolate reductase C677T and A1289C polymorphisms are not risk factors for schizophrenia

Elisabet Vilella [a,c,*], Carmen Virgos [a], Michelle Murphy [b], Lourdes Martorell [a], Joaquín Valero [a], Josep Maria Simó [c], Jorge Joven [c], Joan Fernández-Ballart [b], Antonio Labad [a]

[a] *Psychiatric University Hospital Institut Pere Mata, Unitat de Psiquiatria, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C/Sant Llorenç 21, 43201 Reus, Spain*

[b] *Unitat de Medecina Preventiva i Salut Pública, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, 43201 Reus, Spain*

[c] *Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, 43201, Reus, Spain*

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Abstract

It has been suggested that total plasma homocysteine (tHcy) concentrations and methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms are risk factors for schizophrenia. We conducted a case-control study to investigate whether tHcy levels and *MTHFR* C677T and A1298C variants are associated with schizophrenia, giving special consideration to confounding factors. Logistic regression analysis showed that neither tHcy nor *MTHFR* polymorphisms were associated with schizophrenia. Homozygosity for *MTHFR* C677T was associated with higher tHcy concentrations in control and schizophrenia groups ($P < 0.01$), which was mainly driven by the male group. The A1298C variant did not show any association with tHcy concentrations. In conclusion, these results do not confirm an independent relationship of tHcy and *MTHFR* genotype with risk of schizophrenia.

Keywords: Homocysteine; Methylenetetrahydrofolate reductase; Schizophrenia

1. Introduction

Several lines of evidence support the theory of an association between methyl carbon metabolism, in which homocysteine is an intermediate metabolite, and schizophrenia. The first reports supporting this hypothesis emerged in the middle of last century when a higher incidence of schizophrenia patients was detected among homocysteinurics compared with the general population (Spiro et al., 1965). Furthermore, the administration of oral loads of methionine (the precursor of homocysteine) to patients with schizophrenia exacerbated their psychotic symptoms (Cohen et al., 1974). Also, folate status has been reported to be lower in schizophrenic patients than in controls, and interestingly, treatment of schizophrenic patients with folate improves their clinical and social recovery (Godfrey et al., 1990).

Methyltetrahydrofolate is the single carbon donor for the conversion of homocysteine to methionine. Finally, some authors have reported hyperhomocysteinemia in their schizophrenic patients (Regland et al., 1995; Levine et al., 2002) and recent reports also show an association between the 677T allele of the methylenetetrahydrafolate reductase (*MTHFR*) gene and schizophrenia (Arinami et al., 1997; Joober et al., 2000). However, there is conflicting evidence on the association between both the *MTHFR* gene and homocysteine concentrations in schizophrenia. We did not observe the association first reported by Regland et al. (1997) in our schizophrenic patients (Virgos et al., 1999). Although some controversies exist, the weight of the reported evidence in the literature suggests that both total

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; NMDA, *N*-methyl-D-aspartic acid; tHcy, total plasma homocysteine concentration.

* Corresponding author. Tel.: +34 977 759338; fax: +34 977 759322.
E-mail address: elisabet.vilella@urv.net (E. Vilella).

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plasma homocysteine (tHcy) levels and *MTHFR* gene polymorphisms are risk factors for schizophrenia.

However, the conclusions so far need further discussion in the light of recent findings. For instance, different reports indicate that schizophrenic patients do not have increased plasmatic homocysteine levels (Reif et al., 2003; Virgos et al., 1999; Goff et al., 2004). Goff et al. (2004) suggested that low folate status observed in schizophrenic patients can be attributed to a decreased activity of glutamate carboxypeptidase II (GCPII), an enzyme present in the intestinal brush border that facilitates the absorption of dietary folate. Interestingly, GCPII in brain is involved in *N*-methyl-D-aspartic acid (NMDA) metabolism and hypoactivity of NMDA receptors has been implicated with schizophrenia. This provides new insights into the possible relationship between homocysteine as a marker of folate status and schizophrenia. Furthermore, recent studies have demonstrated that alcohol and tobacco are very important confounding factors when evaluating plasma homocysteine (Refsum et al., 2004; Brown et al., 2004; Strandhagen et al., 2004). Such important confounding factors have not been considered in previous studies.

In order to clarify some of these doubts, we conducted a case-control study to investigate whether tHcy levels and *MTHFR* C677T and A1298C polymorphisms are associated with schizophrenia after adjusting for well-established confounding factors.

2. Methods

2.1. Study participants

The study was performed in accordance with the guidelines of the institutions involved and was approved by the Hospital Sant Joan de Reus and Jordi Gol Gorina Foundation ethics committees. All participants gave signed informed consent.

A total of 158 unrelated schizophrenic patients diagnosed according to the ICD9 criteria (World Health Organization, 1977) were selected from 370 patients from our schizophrenia collection recruited in the 1996–1998 period (Virgos et al., 1999). All of them were in-patients who had chronic schizophrenia (94 males and 64 females) with a mean illness time span of 34 years (range 0 and 64). Around 50% of them had had the disease for longer than 37 years. As in-patients, their diet was institutionalised and relatively homogeneous. No vitamin or folic acid supplements were administered. Some female patients used oral contraceptives but none of them were on hormone replacement therapy. Patients whose genetic background was not Mediterranean Caucasian or who had cardiovascular disease were excluded.

The control group consisted of 234 unrelated participants from our HOMFOL collection (Murphy et al., 2002; Ferré et al., 2003). Briefly, a randomly selected representative sample of 409 individuals from the town hall population registers of two Catalonian villages was obtained in the 1999–2000 period. Participants were ostensibly healthy with no evidence of renal insufficiency, severe hepatic damage, neoplasia, oligophrenia, or dementia and with a Mediterranean Caucasian genetic background. Medication intake was not an exclusion criterion except in the case of vitamins and drugs interfering with homocysteine metabolism (methotrexate, tuberculostatics, theophylline, and B complex vitamins). Locally produced food is not fortified with vitamins in Spain. Pregnant women were not included in the study. The Goldberg's General Health Questionnaire (GHQ-28) was used to screen for psychiatric symptoms (Goldberg, 1978; Lobo et al., 1986). Control participants with a personal history of psychiatric disease (clinically documented) or a Goldberg score >6 were excluded. The participants' current and former alcohol and smoking consumption was recorded as described (Ferré et al., 2003).

2.2. Study design

Retrospective case-control study.

2.3. Blood collection and plasma homocysteine measurement

A 10-ml fasting blood sample collected in EDTA containing Vacutainer tubes was obtained from all participants. Tubes were kept at 4 °C until processing (within 2 h). Plasma was stored in aliquots of 250 μl in cryotubes at −80 °C until required for analysis, and leukocytes were prepared from the remaining pellet, from which DNA was extracted. Total plasma homocysteine was measured using an IMx Analyzer with the IMx Homocysteine assay (Abbot Laboratories). Adequate control samples were used to discard inter-assay variability >5%.

2.4. MTHFR genotyping

The C677T polymorphism was analyzed using our modified version (Virgos et al., 1999) of the method reported by Frosst et al. (1995) with PCR amplification and restrictase digestion. The A1298C polymorphism was also analyzed by PCR-RFLP using the MboII restrictase as previously described (Weisberg et al., 1998).

Genetic stratification of the sample was assessed following the method of Pritchard and Rosenberg (1999), using 25 independent SNPs. Haplotypes were estimated using the Arlequin program in order to calculate whether the two variants were in linkage disequilibrium.

2.5. Data analysis

Standard methods (Kolmogorov–Smirnov) were used to test whether the variables were normally distributed.