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Elevated homocysteine level in siblings of patients with schizophrenia

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ABSTRACT

Increased homocysteine plasma levels were reported in patients with schizophrenia and Levine et al. (2002) suggested that such increase characterizes mainly males. In the following study we examined whether such increased levels also characterize male siblings of schizophrenia patients. Forty-four pairs of schizophrenia patients and their corresponding healthy male siblings were recruited and sampled for homocysteine. We also had age-matched controls for each of the sibling. The median homocysteine plasma level for patients was 13.0 μMol/L and 11.7 μMol/L for their male siblings compared with a median of 10.9 μMol/L for the siblings' controls. There was no significant difference between homocysteine plasma level in patients and their siblings. Significant difference was found for homocysteine plasma level between the siblings' group and their matched controls. A partial correlation of Ln plasma homocysteine level between patients and their siblings was found to be close to a zero correlation of −0.089, $p=0.57$ for the whole study group and −0.15, $p=0.38$ in the male–male patient-sibling pairs. Our results show that elevated homocysteine plasma level may characterize schizophrenia patients' male siblings, a finding that seems to agree with previous studies suggesting elevated homocysteine level as a risk factor for developing schizophrenia.

1. Introduction

Homocysteine (Hcy) is a neurotoxic amino acid (Kruman et al., 2000; Lipton et al., 1997) generated via methionine metabolism (Selhub, 1999).

Levine et al. (2002) and Applebaum et al. (2004) reported elevated plasma Hcy levels in inpatients with schizophrenia, particularly young adult males, suggesting that high plasma Hcy levels may constitute an independent risk factor for schizophrenia, as has been proposed in the case of Alzheimer's disease (Seshadri et al., 2002). A meta-analysis of eight studies (812 cases with schizophrenia and 2113 controls) suggested that an increase in the plasma Hcy level of 5 μM was associated with a 70% greater risk for schizophrenia (Muntjewerff et al., 2006) and Kale et al. (2010) reported such increased homocysteine levels among never-medicated, first episode schizophrenia patients.

Hyperhomocysteinemia was reported to be associated with several putative endophenotypes for schizophrenia as altered neurocognitive performance, brain atrophy and white matter changes (Lewerin et al., 2005; Sachdev, 2005; Sachdev et al., 2004; Greenwood et al., 2007) and hyperhomocysteinemia may thus constitute itself an endophenotype for schizophrenia, possibly underlying the above mentioned altered neurocognitive performance, brain atrophy and white matter changes.

Several findings suggest that genetic factors may underlie the plasma/serum homocysteine level. Twin studies reported variable estimates of hereditability of homocysteine level. Thus, Reed et al. (1991) reported hereditability of 0.75, Berg et al. (1992) of 0.5, Siva et al. (2007) of 0.57, and Bathum et al. (2007) reported additive genetic proportion of total variation in homocysteine concentration of 0.63 and 0.27 for age groups 18–39 and 40–65 years respectively. Siva et al. (2007) who reported hereditability of 0.57 stated in their discussion that: "our results suggest that a sibling's plasma homocysteine is a much stronger predictor of a person's plasma homocysteine than any known environmental factor." On the other hand, Cesari et al. (2000) reported no evidence for genetic influence on homocysteine levels.

There are findings suggesting that genetic factors may underlie high homocysteine levels in schizophrenia patients. First, Stahl et al. (2005) conducted a research aimed at studying candidate epidemiological, nutritional and life-style determinants of plasma homocysteine levels in a large sample of schizophrenic patients. A multiple linear regression model for homocysteine was performed on epidemiological variables that were either (a) statistically significant or showing a trend toward significance ($p<0.1$) and/or; (b) were repeatedly reported to be of statistical significance in the literature. The results of this study showed that nutritional and life-style determinants could explain only 24% of the variance which raised

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the possibility that genetic factors may play an important role in this regard. Second, increased homocysteine levels, as well as TT genotype of the Methylenetetrahydrofolate reductase (MTFHR) gene that leads to increased homocysteine level, have been associated with risk of schizophrenia (Muntjewerff et al., 2006). See also in this regard the discussion by Brown and Susser (2005), Adler Nevo et al. (2006) and Bleich et al. (2007) considering data supporting the hypothesis that subtle genetic variations in Hcy metabolism may play an etiologic role in schizophrenia.

These data and hypotheses led us to plan a familial genetic study of Hcy plasma levels in patients with schizophrenia and their corresponding male siblings.

2. Methods

2.1. Subjects and procedure

The study was approved by the Helsinki Committee (IRB) at Ben Gurion University of the Negev, Israel. Forty-four pairs of consenting adult patients with schizophrenia and their corresponding male siblings were sampled for homocysteine plasma level.

The patients' group was composed of 44 subjects (38 males and six females) with schizophrenia diagnosed according to DSM-IV. Twenty six were diagnosed with paranoid type schizophrenia, 17 with undifferentiated type schizophrenia and one with disorganized type schizophrenia. Illness duration was 1 year in one patient, 3–5 years in three patients and above 5 years in the remaining 40 patients. Patients' age range was above 20 and below 60 years. All patients had a history of at least one psychiatric hospitalization due to acute psychotic episode, were being treated with antipsychotic medications and were in a variety of clinical settings, including acute inpatient units, chronic inpatient units, hostel care, and outpatient care.

For each patient one of his/her available normal healthy sibling was recruited. These 44 corresponding siblings were all males (see Table 1).

Excluded were subjects (patients or siblings) with any clinically significant medical condition; subjects with neurological disorder; subjects who received vitamin supplementation that may have influenced serum Hcy levels (i.e., namely, folic acid, vitamin B12, vitamin B6); subjects with any evidence of substance or alcohol abuse; subjects suffering from eating disorders, malnutrition, gastrointestinal absorption disorders, or any other known form of avitaminosis.

For each male sibling of specific age we had a small sample of at least 5 age-matched male control subjects for Hcy plasma level (in cases where two subjects were of the same age they had the same small control sample). These subjects were randomly chosen from a large employee health survey pool held at the same time period of the study and analyzed with the same method at the same laboratory. Overall there were 176 controls, all functioning males. They represent real life values in such population. Due to confidentiality requirements associated with this employee health survey pool, we had no access to other data concerning the control subjects. The use of data from this pool under the requirements for confidentiality was approved by the local IRB committee at Sheba Hospital, Tel Hashomer, Israel. The median of each of these small control samples applied for each sibling served as an appropriate control for each given sibling.

Plasma was separated from whole blood by centrifugation at 4 °C and then stored at −20 °C. Homocysteine was measured in plasma of blood samples collected in EDTA containing tubes. A high pressure liquid chromatography method was applied by a modified Araki & Sako method (Araki and Sako, 1987).

The sample was reduced with $NaBH_4$ to break disulfide bonds and allow a total rather than free homocysteine measurement, and blockage of sulfhydryl residues was achieved by monobromobimane derivatization. Fluorescent labeling was performed by fluorogenic SBD (halogenosulfonyl benzofurazan). The various –SH containing species were separated by a methanol/acetonitrile gradient isocratic elution on a C_{18} reversed-phase column on a Hitachi L-7250 analyzer equipped with a fluorescence detector. Retention time for homocysteine peak was 11.3 min.

2.2. Statistical analysis

All statistical analyses were performed using SPSS software (Version 14).

Homocysteine levels may not be normally distributed (Selhub, 1999). Indeed, when normal distributions of data were analyzed by using the one sample Kolmogorov–Smirnov-test, data for plasma homocysteine levels in the patients were found to be skewed (Kolmogorov–Smirnov $Z=1.678$, $p=0.07$). We thus used non-parametric tests for homocysteine levels except for the partial correlation and regression analysis requiring normal distribution of the data. Since homocysteine levels were normalized once they were log transformed we utilized the natural logarithm (Ln) of homocysteine plasma levels in these two last statistical analyses.

The study design was a matched one. Thus, the data in the current study was matched data since there were pairs of patients and their corresponding siblings. As mentioned above, for each value of homocysteine level for a given male sibling we had a median value of homocysteine level taken from randomly chosen small group of age-matched male controls.

3. Results

There were 44 pairs of patients with schizophrenia and their corresponding male siblings. For each sibling we had a small group of age-matched male controls for the plasma homocysteine level as mentioned above.

Table 1 shows demographic data for the patients and their siblings. There were 38 male and six female patients and 44 male siblings. Approximately 90% of the patients smoke and 47.7% of their siblings also smoke. No difference for plasma homocysteine levels was found for smoking either in the patients' or the siblings' groups (Mann–Whitney non-parametric test for patients, $Z=-0.325$, $p=0.75$; for siblings $Z=-0.153$, $p=0.88$).

Median homocysteine level for patients was 13.0 μMol/L (in male patients ($N=38$) 12.85 μMol/L) and 11.7 μMol/L for their male siblings compared with a median of 10.9 μMol/L for the siblings age-matched male controls.

Age correlated positively with plasma Hcy level in the siblings' group (non-parametric Spearman rho$=0.324$, $p<0.03$), but not in the patients' group (non-parametric Spearman rho$=-0.15$, $p=0.33$). Using the same non-parametric test, plasma homocysteine level did not correlate with the illness duration among the patients.

Non-parametric Wilcoxon Signed Rank test showed no significant difference between homocysteine plasma level in patients and their male siblings ($N=44$ pairs, $Z=-1.35$, $p=0.18$).

This was also true for pairs of male–male patient-siblings only ($N=38$ pairs, $Z=-1.05$, $p=0.16$).

We performed a regression analysis in order to learn about the associations between the patients and siblings' Ln homocysteine plasma levels adjusted for differences in age between the patients

Table 1
Demographic data and homocysteine plasma level in pairs of patients with schizophrenia and their corresponding male siblings.

Variable	Patients					Siblings				
	N	Mean	S.D.	Median	Range	*N*	Mean	S.D.	Median	Range
Sex: M/F	38M/6F					44M				
Age (years)	44	39.5	9	39.5	21–58	44	46.1	11	43	20–60
Origin	Sephardi-31; Ashkenazi-8; Other-5									
Years of illness	44	14.7	7	14.5	1–31					
Smoking/non-smoking	21/23					40/4				
CVD	2/44					0/44				
Homocysteine level (μMol/L)	44	14.2	8.4	13	4–56	44	12.2	2.5	11.7	8.8–17.4