Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals

There has been a recent increase in interest in the role of homocysteine (Hcy) in neurologic disease. High plasma levels of Hcy have been implicated in cerebrovascular disease, with evidence for both an increase in strokes as well as microangiopathy. More recently, it has been reported that high Hcy levels may lead to cognitive impairment independently of cerebrovascular disease. Hcy also has been reported as a risk factor for AD. Although severe hyperhomocysteinemia is rare, mild elevations of blood Hcy levels have been reported to occur in 5% to 7% of the general population.

We hypothesized that the high Hcy levels, combined with the increased risk for cerebrovascular disease and possibly neurodegeneration, should manifest in the brains of otherwise healthy elderly individuals in the form of brain atrophy and small vessel disease detectable on MRI.

Abstract—The authors examined the association of total plasma homocysteine (Hcy) levels with measures of atrophy and white matter disease on MRI scans in 36 healthy elderly individuals. Hcy had a significant positive relationship with lateral ventricle–brain ratios in the anterior (r = 0.49) and middle (r = 0.43) ventricular regions as measures of central atrophy, but not with cortical atrophy or white matter hyperintensities. In a logistic regression analysis, elevated Hcy was a significant determinant of increased anterior ventricle–brain ratio (≥0.34) after controlling for age, folate, B12, creatinine, and white matter disease (OR = 2.3; CI, 1.03–5.09).

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Methods. We examined 36 healthy community volunteer subjects (mean age, 71.6 years; range, 59–85 years; 18 men and 18 women), with a mean education of 12.0 years and a mean score of 113.1 on the National Adult Reading Test–Revised. Subjects had no history of neurologic or psychiatric disorder. No subject had a history of cerebrovascular disease, but the presence of cardiovascular disease was not exclusionary. Risk factors for cerebrovascular disease included hypertension in 12 subjects (33.3%), coronary artery disease in 5 (13.9%), and diabetes mellitus in 1 (2.8%). Subjects were not taking folate, B12, or B6 vitamins at the time of assessment. A fasting blood sample was obtained for total plasma Hcy and serum B12, folate, and creatinine levels. Total Hcy was measured using Abbott IMX-automated, fluorescence-based enzyme immunoassay (Abbott Laboratories) with a range of measurement 0.5 to 50 μmol/L, and demonstrated high repeatability. MRI was performed on a 1.5 T Signa GE magnet (GE, Milwaukee, WI) using the following protocol: 1.5-mm-thick T1-weighted contiguous coronal sections through whole brain using a FSPGR sequence and three-dimensional acquisition (repetition time = 14.3 milliseconds, echo time = 5.4 milliseconds) and 4-mm-thick T2-weighted FLAIR coronal slices through whole brain (repetition time = 8900, echo time = 145, inversion time = 2200, field of view = 25 cm, 256 × 192). Subjects underwent a detailed neuropsychological assessment using a predetermined battery of tests. MRI scans were rated by a trained rater for T2-weighted white matter hyperintensities (WMH) and atrophy. WMH were rated on a scale of 0 to 3 in three periventricular and four deep WM regions and these ratings were summed to give the total WMH score. The lateral ventricle–brain ratios (VBR) were measured on axially reconstructed T1-weighted slices in the anterior and middle ventricular regions using the following standardized images: through the putamen, choroid plexus, basal ganglia, and thalamus for frontal VBR; and through the lateral ventricles, anterior/posterior WM and cenrum...
cortical atrophy, with none of the other variables being significant.

Elevated Hcy level was a significant risk factor (OR 2.3; CI, 1.03–5.09) for high anterior VBR as a measure of central atrophy, with none of the other variables being significant.

Discussion. The significant correlation between Hcy levels and an index of brain atrophy in a healthy elderly population is an intriguing finding. Even though the sample size was small, the correlation was robust and was not explained by confounding factors such as vitamin deficiency or subcortical WM changes. The atrophy measure that best correlated with Hcy levels was ventricular dilatation and not cortical atrophy, suggesting that it possibly reflected loss of WM. On neuropsychological testing, we did not find an association between cognitive performance and Hcy or VBR measurements, which suggests that the ventricular dilation associated with high Hcy levels may not be cognitively deleterious as determined by standard neuropsychological tests. This may be related to the redundancy present in brain function such that a threshold must be reached before functional impairment becomes obvious. The small size of the sample may, however, account for the lack of correlation of these variables in this study.

Our study is cross-sectional, and we cannot conclude that high Hcy levels caused brain atrophy. For this to be determined, a longitudinal design would be more appropriate. However, the results are interesting enough to warrant speculation on possible mechanisms. The lack of association between Hcy levels and WMH make it unlikely that MRI-visible microangiopathy is a mediating factor for the above association. Because vascular injury is a major mechanism by which Hcy causes neurologic dysfunction, we cannot rule out the possibility that MRIinvisible microangiopathy was related to ventricular dilation seen in our subjects, an issue that requires longitudinal examinations and neuropathologic evaluation. It also is possible that Hcy has a neurotoxic effect, which has been suggested, either directly or through its metabolite homocysteic acid. Alternatively, Hcy may promote neuronal apoptosis either directly or through increased intracellular S-adenosylhomocysteine. The association of hyperhomocysteinemia with cerebral atrophy is a possible explanation for its proposal as a risk factor for AD.

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References


Results. Subjects had a mean total Hcy level of 13.3 μmol/L (SD 3.9; range, 7.3–24.4) and this was not significantly correlated with folate (r = 0.15) or B12 (r = 0.12) levels, but was correlated with creatinine levels (r = 0.35). Subjects did not demonstrate deficits in the cognitive domains of attention, executive function, memory, and language when compared with age-related averages, and Hcy did not have a significant correlation with these functions. Although all subjects had WMH on T2-weighted MRI (average whole brain WMH score = 5.7; range, 0.5–13.0; maximum possible, 21), neither the total WMH score (r = 0.01), nor the periventricular WMH or deep WMH scores separately, correlated significantly with total Hcy levels. However, after controlling for creatinine, B12, and folate in a multiple regression model, total Hcy had a significant correlation with both VBR measurements (partial correlations, r = 0.49, p = 0.008, for anterior VBR; r = 0.43, p = 0.02 for midsection VBR) (figure), but not with cortical atrophy (Spearman ρ = 0.225, p = 0.14). A logistic regression analysis was performed with anterior VBR as the dependent variable, dichotomized by a split-half procedure (<0.34 and ≥0.34), and Hcy, age, folate, B12, creatinine, and WM disease (all continuous) as independent variables. Elevated Hcy level was a significant risk factor (OR = 2.3; CI, 1.03–5.09) for high anterior VBR as a measure of central atrophy, with none of the other variables being significant.
Alexander disease is a pathologically defined condition characterized by the presence of innumerable Rosenthal fibers throughout the CNS. There is a range of clinical severity and presentation in patients with Alexander disease—categorized by a more common early-onset (infantile) form and rare, late-onset (juvenile and adult) forms. Rosenthal fibers are cytoplasmic inclusions in astrocytes, which contain B-crystallin, a member of the small heat shock protein (HSP) family functionally described as molecular chaperones, capable of preventing the heat-induced aggregation of proteins. The chaperone protein was demonstrated to interact directly with glial fibrillary acidic protein (GFAP), an intermediate filament (IF) protein specific for matured astrocytes. Analysis of the B-crystallin gene failed to detect abnormalities, but a recent study in patients with Alexander disease revealed de novo heterozygous mutations of the GFAP gene. Subsequently, genotype–phenotype correlation among patients with infantile Alexander disease has been shown. In the juvenile form, molecular genetic explanation remains unclear, in part because of the rarity of the disease. We investigated the GFAP gene in the previously reported patient with juvenile Alexander disease and found a novel mutation, which could possibly explain molecular pathogenesis of the late-onset and milder clinical course.

**Case report.** Previously, we reported a 13-year-old boy diagnosed with juvenile Alexander disease. He presented with slowly progressive motor and mental deterioration, slurred nasal voice, an elevation of B-crystallin and HSP27 in the CSF, and characteristic neuroimaging findings: frontal dominant leukodystrophy with cyst formation, prominent involvement of arcuate fibers, diffuse symmetric lesions of basal nuclei, and subependymal stripes around the lateral ventricles. At age 8 years, the patient had a generalized febrile convulsion with bilateral frontal spikes. Thereafter, his school performance declined and motor function worsened. At age 13, the patient walked in a peculiar stooped posture and had mild cognitive deficiency. At age 21, he can walk short distances independently, speak slowly in simple sentences, and play video games.

**Methods.** Blood samples were obtained from the patient, his brother, and his parents after informed consent. Genomic DNA was isolated from leukocytes by the standard phenol/chloroform extraction method. On the basis of the DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 2000;20:6920–6926.
Results. Direct DNA sequencing of the patient revealed a heterozygous G to C transition at nucleotide 1,100 (1,100G→C) of GFAP gene in exon 6 (figure 1), resulting in an amino acid replacement of glutamic acid by aspartic acid at codon 362 (E362D). The nucleotide transition was recognized in both strands. An analysis of the other coding regions including exon–intron boundaries showed normal sequences in the patient. Because the mutation leads to loss of a unique MboI restriction site, we designed PCR primers around the mutated site to amplify a 228-base pair (bp) fragment, which normally leads to cleavage into 124-bp and 104-bp fragments by MboI digestion. Restriction-digestion in the family members did not reveal the mutation (figure 2), indicating a de novo mutation in the patient. By the same method, 75 normal Japanese control samples (150 alleles) were analyzed for the mutation and all revealed the normal pattern, suggesting that it is not a common DNA polymorphism.

Discussion. In common with other IF proteins, GFAP is composed of a N-terminal head domain, a central rod domain, and a C-terminal tail domain. The rod domain is subdivided into four segments of α-helix conformation, namely 1A, 1B, 2A, and 2B, each separated by non–α-helix L1, L12, and L2 linker regions. E362 is located in the vicinity of a consensus motif at the C-terminal end of 2B domain and is highly conserved both in other types of intermediate filaments (keratin 1, vimentin, peripherin, desmin, and the three neurofilament proteins) and in the GFAP gene across species (human, ox, mouse, (ABI, Foster City, CA) and analyzed on an ABI Prism 310 DNA analyzer.)

Figure 1. Sequencing data of GFAP genes obtained from a control subject (upper panel) and the patient (lower panel) demonstrate a heterozygous nucleotide change in the patient from G to C at position 1,100, which results in a replacement of glutamic acid by aspartic acid at codon 362.

Figure 2. Mbo I digestion analysis of PCR fragments from the patient (solid square), his brother, and his parents. The 228-base pair (bp) fragment corresponds to the undigested mutant PCR product. The patient showed both the wild-type and mutant patterns, indicating the heterozygous nature of the mutation. Other family members showed the normally digested pattern. Lane M indicates a size marker.

Figure 3. E362, located near a consensus motif at the C-terminal end of the 2B rod domain, is highly conserved both in other types of intermediate filaments; keratin 1 (Ker 1), vimentin (Vim), peripherin (Peri), desmin (Des), and low-, middle-, and high-molecular weight neurofilament proteins (NFL, NFm, and NFH), and in the GFAP gene across species—human, ox, mouse, rat, and goldfish (goldf). GenBank accession numbers, from top to bottom, are AF419299, AF304164, BC000163, L14565, M63391, S78296, Y00067, NM021076, Y08255, AF332062, NM017009, and L23876. E362 occurs at position “g” in the heptad repeat, where “a” and “d” are generally apolar residues.
rat, and goldfish) (figure 3). Together, these observations suggest that the present E362D is a novel disease-causing mutation in Alexander disease.

The first report on GFAP gene mutation associated with Alexander disease included 12 patients—11 with early-onset (infantile form) and one with onset at age 10 (probable juvenile form)—although limited clinical information was provided. Subsequently, reports of 16 patients with early-onset (infantile) Alexander disease showed the restricted localization of the mutations to exon 1 (coding for 1A rod region) and exon 4 (coding for 2A rod region) with only one in exon 8 (coding for tail region). R79 and R239 were two hot spots each including nine patients (32%) and 11 patients (39%) of the previously reported 28 patients with GFAP gene mutation. Within the clinical range of infantile form of Alexander disease, all of the patients with R79 mutation developed slightly milder symptoms with later onset than those with R239 mutation. The exceptional patient with onset at 10 years of age also had the common R79 mutation, obscuring the molecular explanation of the juvenile form. Moreover, a recent review reported that none of the previously identified mutations was found in either the additional juvenile or the single adult case screened. The patient described in this article is valuable in investigating genotype–phenotype correlation between the infantile and the juvenile forms of Alexander disease.

Most of the GFAP mutations that localize to highly conserved regions across intermediate filaments have similarities to disease-causing mutations in other intermediate filament genes. The E362D mutation in position 106 of the 2B domain found in our patient is homologous to an E477D mutation in keratin 1 equivalent to the position 106 of the 2B domain, reported to be responsible for a mild case of epidermolytic hyperkeratosis. In the report, mutagenic expression study demonstrated that heterozygous E106D substitution in 2B domain produced much shorter (2.1 ± 0.4 μm vs >20 μm in control) and thinner keratin chain assemblies, and that E106A substitution (abolition of a charged residue) formed only small subfilamentous structures (0.05 to 0.2 μm), corresponding in size to a single molecule. The authors speculated that the E106 residue is critically involved in the formation of salt or H-bonds with nearby molecules, or both, and is thereby essential for the stability of intermediate levels of molecular alignments. Possibly a similar molecular mechanism would apply for the pathogenesis of the juvenile Alexander disease with E106D substitution in α-helix 2B domain of GFAP. The observation provides a further clue to establish common genotype–phenotype correlation across many intermediate filament diseases.

References