Copper Perturbation in 2 Monozygotic Twins Discordant for Degree of Cognitive Impairment

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Background: Recent evidence indicates that peripheral tissue markers can provide information regarding changes affecting cellular metabolism in Alzheimer disease (AD). We previously reported that serum copper levels can discriminate subjects with AD from normal control subjects (with 60% sensitivity and 95% specificity) and from patients with vascular dementia (with 63% sensitivity and 85% specificity).

Objective: To study the correlation between AD and serum levels of transition metals and markers of peripheral oxidative stress.

Design: Case study.

Setting: General hospital inpatient wards and outpatient clinics.

Patients: A pair of elderly monozygotic female twins discordant for AD.

Main Outcome Measures: Biochemical analyses of peripheral-blood transition metals and indicators of oxidative stress and neurologic and neuropsychological assessments of clinical status for presence of cognitive impairment and AD.

Results: Serum copper and total peroxide levels were both 44% higher in the twin with greater cognitive impairment and a diagnosis of AD.

Conclusions: The cases reported support the hypothesis of a major involvement of copper and oxidative abnormalities in AD.

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ALZHEIMER DISEASE (AD) IS a heterogeneous neurodegenerative condition, and biomarker research has focused on a variety of possible disease-related mechanisms. Among these, the depletion of vitamins with direct or indirect antioxidant effects and the increase in homocysteine and in compounds involved in lipid peroxidation are thought to reflect oxidative changes with a potential pathogenetic significance in AD, according to the oxidative stress hypothesis of this condition. Abnormalities of trace metal metabolism also seem to be a primary neurochemical event in the genesis and progression of the disease. We have previously reported that copper levels measured in the peripheral circulation discriminate between patients with AD and normal control subjects, as well as patients with vascular dementia.

In this study, we compare putative markers of oxidative stress in a pair of 73-year-old female monozygotic twins discordant for AD, and we discuss the potential role of copper abnormalities in the pathogenesis of AD in agreement with proposed models of biometal-related neurodegeneration.

METHODS

CLINICAL ASSESSMENTS

The subjects studied were twin sisters (twins A and B) who underwent clinical evaluations, including comprehensive personal medical and family histories, psychiatric and neurologic examinations, extensive neuropsychological testing, and neuroimaging (magnetic resonance [MR] imaging or computed tomography [CT]), as well as routine laboratory tests. The neuropsychological evaluation included the Mini-Mental State Examination, the Mental Deterioration Battery, the Digit Span, and the Corsi test. The Mental Deterioration Battery provides scores on several cognitive domains: verbal and visuospatial skills, memory, constructive praxis, language, and logical-deductive skills. The battery consists of
a number of subtests: 4 performance scores derived from verbally presented stimuli (the Rey Auditory Verbal Learning Test, 15-word immediate and delayed recall, verbal fluency, and sentence construction) and 3 from visuospatial processing tests (Raven Colored Progressive Matrices Test, Immediate Visual Memory, and Copying Drawings with and without landmarks). In addition, the Digit Span was administered to test immediate verbal memory, and the Corsi test for visuospatial short-term memory. All tests were corrected, where appropriate, for age, sex, and education.

Brain MR imaging was performed with a 1.5-T scanner (Philips Intera, Best, the Netherlands). Conventional dual-echo fast spin-echo images were obtained (T1-weighted and T2-weighted sequences), with and without contrast. The CT scans were obtained on a multislice CT scanner (Aquilon, MULTIT; Toshiba Corporation, Shimoishigami, Japan) without contrast administration.

The common carotid arteries, carotid bifurcations, internal carotid arteries, and vertebral arteries were studied with B-mode duplex sonography (7.5-MHz probe; Acuson, Aspen, Colo) according to standardized criteria for presence of atherosclerotic plaques and degree of stenosis. Intima and media thickness was also studied from the distal portion of the common carotid artery, 1 cm proximal to the carotid bulb, as the mean value of 3 repeated measures of the intima and media thickness at the far wall of each common carotid artery. Intracranial vessels were examined by transcranial Doppler (Multidop CV for copper was 2.45%, while interassay CV was 3%. Hydroperoxide trapping antioxidant capacity (TRAP) assay. Intra-assay CV of 1.95% and interassay CV of 3.1% were calculated in our laboratory. Serum iron level was determined by a colorimetric method (FerroZine; Roche Diagnostics, Mannheim, Germany). Transferrin was estimated by immunonutribidimetric assays (Roche Diagnostics) with the use of a goat anti–human transferrin antibody in Tris hydrochloride buffer.

All biochemical measures were performed on an automated analyzer (Hitachi 917; Roche Diagnostics). Total homocysteine was assayed by high-performance liquid chromatography on plasma samples in EDTA. All biochemical measurements were performed in triplicate, matched within the twin pair, and compared by z-score analysis, with reference values obtained from a healthy elderly population not affected by pathologic conditions able to alter copper metabolism or peripheral oxidative stress (eg, diabetes mellitus; inflammatory diseases; cardiac, respiratory, liver, or renal insufficiency; malignant tumors; and alcohol abuse). Apolipoprotein E (APOE) genotyping was performed according to established methods. For determination of zygosity, 10 highly polymorphic DNA sequences (3 located within genes TH01, FGA, and vWA and 7 in anonymous segments D2S1338, D3S1358, D8S1179, D16S539, D18S51, D19S433, and D21S11) were amplified by polymerase chain reaction with fluorescent-labeled primers and analyzed by capillary electrophoresis (ABI Prism Genetic Analyzer 310; PE Biosystems Foster City, Calif). Genotypes were determined by means of a software package (Genotyper 2.5; Applied Biosystems, Foster City). Concordance for all 10 loci predicts monozygosity with greater than 99.98% probability, regardless of the allelic pattern observed (calculations based on allele frequencies observed in 4 major racial groups, University of Utah DNA Diagnostic Laboratory, Salt Lake City).

REPORT OF CASES

Twin A

A 73-year-old woman was admitted to the hospital in June 2000 because of purposeless wandering and poor self-care in the context of progressive cognitive impairment. The insidious onset of slowly progressive memory impairment and cognitive decline dated back 5 years before hospitalization.

Medical history was pertinent for hypertension, chronic atrial fibrillation, and early menopause at age 45 years. The patient had never received estrogen therapy. Her medications included digoxin, enalapril maleate, verapamil hydrochloride, and aspirin. Family history was reportedly negative for dementing illnesses or strokes.

The patient underwent a comprehensive clinical evaluation including psychiatric and neurologic examinations. Results of laboratory tests were within normal limits. The patient refused to undergo brain MR imaging. A head CT scan showed enlarged cortical sulci and lateral ventricles (diffuse atrophy) and mild hypodensity of the white matter (Figure 1). Echo color duplex scanning of the cerebral vessels and intracranial blood flow velocities did not show any hemodynamically significant alteration. Electroencephalogram showed diffuse background slowing of alpha rhythms, most prevalent over the frontal regions. There was no clinical evidence of depression. Neuropsy-
and Stroke–Alzheimer’s Disease and Related Diseases Association met criteria of the National Institute of Neurological Diseases and Stroke of cerebrovascular origin. Twin B did not meet criteria for a diagnosis of dementia at any point during the 4 years of follow-up and actually showed cognitive improvement over time (Table 1).

Carotid duplex ultrasound scans showed a bilateral 50% stenosis of the internal carotid artery. The electroencephalogram was abnormal at the time of the stroke because of focal left temporal slowing, as is often observed in normal aging. Results of screening laboratory tests were normal.

Both twins had smoked approximately 23 cigarettes per day since youth, had completed 2 years of high school, had worked as office clerks, had never been married, and had lived together all their lives, therefore sharing a very similar lifestyle.

**RESULTS**

Measurements of copper, total peroxides, TRAP, iron, and transferrin in serum, and total plasma homocysteine levels, are reported in Table 2. For either twin, analysis of the z scores indicated no abnormalities with respect to reference values for TRAP, iron, and transferrin levels. For twin A’s copper and total peroxide levels, instead, the z-score units were above the reference range mean. Twin B’s serum copper level was borderline, while total peroxide levels were higher than reference values. Twin A had a 44% increment in both copper and peroxide concentrations compared with her twin sister. Total plasma homocysteine level did not differ between the twins, but it was higher than reference values. A correlation between degree of cerebral cortical atrophy and biochemical markers could not be performed because of the use of different imaging procedures (CT scan in twin A and MR imaging in twin B). The APOE genotype was €3/€3.

**COMMENT**

The cases presented in this study, with a clinical picture of 2 identical twins discordant for AD but with very similar habits and lifestyle, provide information about the role of copper and oxidative dysfunction. This is therefore a good opportunity to study environmental and lifestyle factors that could affect individual antioxidant efficiency and oxidative stress markers in AD. Differences in antioxidant capacity due to both epigenetic and genetic factors could in fact be responsible for the variations in serum concentrations of oxidative indicators observed in subgroups of patients with AD, or for the overlap that has been often noted between patients and normal controls. The main finding in the study of this twin pair is that differences in copper levels corresponded to a different clinical picture, in agreement with our previous reports. The patient who had greater serum levels of copper and oxidative stress indicators, twin A, had overall worse scores on all cognitive testing and met criteria for a diagnosis of AD. Her twin sister, whose levels were much lower, yet slightly increased compared with normal, remained free of dementia 4 years after her sister’s death and after the initial stroke that brought her to our attention. Only a longitudinal follow-up of the surviving twin will eventually determine whether she will also develop dementia. Currently, all evidence suggests that her minor cognitive deficits are on a vascular basis. Both women had a number of common vascular risk factors,
including heavy smoking and early menopause. Twin A also had atrial fibrillation, even though she had no clinical or neuroimaging evidence of any significant cerebrovascular impairment. Twin B, compared with her sister, had instead a significant vascular burden, which appeared to be the major determinant of her mild cognitive deficits of a focal cortical dysfunction consistent with the location of her infarcts. Interestingly, previous work from our group had shown that cerebrovascular dysfunction has little impact on serum copper variations, and the cases presented herein support this view.

We detected no differences in iron metabolism between the twins and in relation to normal reference values. Likewise, TRAP capacity, an index of oxidative stress susceptibility, did not differ within the twin pair. The twins were negative for the presence of the genetic susceptibility risk factor APOE ε4 allele. Both twins had elevation of their homocysteine levels, a known risk factor for cardiovascular and cerebrovascular disease. Homocysteine levels therefore appeared independent of cognitive status. Total peroxide levels, instead, were associated with the presence of cognitive impairment and, in particular, with presence of dementia, since they were more elevated in the twin with AD. This finding is consistent with published evidence of increments in F2-isoprostane, hydrogen peroxide, and lipoperoxidation products in brains of patients with AD. However, we have previously shown that elevation of peroxide levels, though present in dementia, is not specific for AD. Conversely, copper elevation, as also noted in this study, ap-

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<td>Corsi span forward</td>
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<td>4</td>
<td>3</td>
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<td>5 ± 2</td>
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Abbreviations: AVLT, Rey Auditory Verbal Learning Test; NT, not testable.

*Boldface type indicates values that were abnormal or below the cutoff.

Figure 2. Brain magnetic resonance images of twin B. A, T2-weighted axial image demonstrating T2 prolongation in the left occipital cortex and white matter. B, Axial T1-weighted image showing an area of linear enhancement of cortical and subcortical white matter after contrast administration. C, T2-weighted image showing small areas of prolonged T2 in the left frontal white matter consistent with chronic infarctions.

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The copper abnormalities described herein seem to further support the proposed model of a major role of biometals in the pathogenesis of AD. According to this hypothesis, stochastic neurochemical events, such as the oxidation of β-amyloid (Aβ) peptide or a rise in copper or iron level, may cause a small population of Aβ to convert to a rogue form with adverse redox reactivity, responsible for hydrogen peroxide production and Aβ aggregation in plaques. The Aβ combines with ionic copper to form a catalytic complex that generates hydrogen peroxide. Hydrogen peroxide is freely permeable, and its generation from the brain amyloid mass into the blood of the cerebral blood vessels might elevate blood peroxide levels.

It is noteworthy that the twins in this study, despite their identical genetic makeup and exceedingly similar life experiences, differed in estrogen exposure. In fact, Twin B, unlike her twin sister, had had a full-term pregnancy as well as estrogen therapy after menopause. Although no conclusions can be drawn from a single twin pair, this observation is in line with the yet controversial body of literature on the protective role of estrogens against dementia. Discordance for presence of AD in identical twin pairs differing in estrogen exposure has already been reported. If estrogen exposure does indeed modify the risk of AD by delaying its onset, the discordance in AD onset and copper levels in the twin pair herein described could be at least partly explained. To determine whether copper could be considered a suitable “marker” for AD, as suggested by this and other previous reports, more extensive studies are needed.

Table 2. Comparison of Trace Metals and Oxidative Stress Species Assessed in June 2000 in Both Twinsa

<table>
<thead>
<tr>
<th>Biological Variables of Trace Metals and Oxidative Stress</th>
<th>Twin A</th>
<th>Twin B</th>
<th>Normal Reference</th>
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<td>Copper, µg/dL‡</td>
<td>145.9</td>
<td>101.9</td>
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<td>Copper, mg/L</td>
<td>2.47</td>
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<td>Total peroxides, U CARR</td>
<td>543</td>
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<td>Homocysteine, mg/L</td>
<td>3.63</td>
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<td>TRAP, mmol/L</td>
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<td>Iron, µg/dL</td>
<td>46</td>
<td>70</td>
<td>30-126</td>
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<tr>
<td>Transferrin, g/L</td>
<td>2.76</td>
<td>2.78</td>
<td>1.9-3.5</td>
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</table>

Abbreviation: TRAP, total radical trapping antioxidant capacity.

SI conversion factor: To convert copper from micrograms per deciliter to micromoles per liter, multiply by 0.157; homocysteine from milligrams per liter, multiply by 7.397; iron from micrograms per deciliter to micromoles per liter, multiply by 0.179.

*Boldface type indicates values that were abnormal or below the cutoff.
†Normal range as established on our normal elderly population (mean ± 2 SDs). (See Squitti et al for details.)
‡Copper assay according to the Abe method.
§Copper assay according to the atomic absorption spectrophotometer method.
<table>
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<th>Score Absolute Value</th>
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CONCLUSIONS

The present study shows that differences in copper levels corresponded to a different clinical picture in a monzygotic twin pair. The case reported, along with previous descriptions of twin pairs in the literature, is useful not to prove a putative cause or risk factor but, instead, to generate discussion about hypotheses of environmental, lifestyle, and in general stochastic neurochemical events causative of or protective from disease.

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Author contributions: Study concept and design (Drs Squitti, Dal Forno, and Rossini); acquisition of data (Drs Squitti, Cassetta, Lupoi, Lippolis, Pauri, Vernieri, and Cappa); analysis and interpretation of data (Drs Squitti, Dal Forno, and Rossini); drafting of the manuscript (Drs Squitti, Dal Forno, Lupoi, Vernieri, and Rossini); critical revision of the manuscript for important intellectual content (Drs Cassetta, Dal Forno, Lippolis, Pauri, Vernieri, Cappa, and Rossini); obtained funding (Drs Squitti, Cassetta, Pauri, and Rossini); administrative, technical, and material support (Drs Squitti, Pauri, and Rossini); study supervision (Drs Squitti, Dal Forno, and Rossini); radiological expertise (Dr Lupoi); neuropsychological expertise (Drs Lippolis and Cappa); technical expertise (Dr Vernieri).

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REFERENCES


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