Gene polymorphisms of oxidative stress enzymes: prediction of elderly renutrition

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ABSTRACT

Background: The free radical theory of aging suggests that damage caused by oxidative stress leads to impaired physiologic functions. This damage is stemmed by an adequate antioxidant status, which minimizes the occurrence of infection, thus potentially playing a role in improving nutritional status. The role played by genetic factors remains unknown.

Objective: The aim of this study was to investigate whether a single nucleotide polymorphism (SNP) of a gene coding for endogenous antioxidant enzymes could influence either nutritional status or renutrition process in an elderly population.

Design: Nutritional and inflammatory status were studied in 77 elderly outpatients and in 99 malnourished elderly inpatients over 6 wk of health care treatment. Renutrition efficiency was evaluated with use of the ratio between initial transthyretinemia and 6-wk variation. A genetic study was performed on superoxide dismutase (Ala-9Val), glutathione peroxidase (Pro197Leu), and catalase (from promoter to the first intron).

Results: Among the SNPs studied, the G-844A, A-89T, and C-20T catalase SNPs could each be markers predicting renutrition efficiency. These catalase mutant alleles were associated with a lower efficiency of renutrition in malnourished elderly subjects, regardless of initial nutritional and inflammatory status. Genotyping one of these catalase SNPs could make it possible to identify a high-risk subpopulation of mutant allele carriers within the elderly polyphathological population.

Conclusion: In a malnutrition setting, this subpopulation should be given personalized health care, including a strengthened refeeding program. Thus, catalase genotyping could enable earlier recovery of satisfactory nutritional status and thus avoid the consequences of malnutrition, which are especially deleterious in the elderly. Am J Clin Nutr 2008;87:1504–12.

INTRODUCTION

Protein-energy malnutrition affects about 4% of community-dwelling and 30–50% of hospitalized elderly subjects (1). This high prevalence is due to age-related diseases together with aging processes (1). The free radical theory of aging (2, 3) suggests that alterations in physiologic functions may be caused by an accumulation of oxidative stress (OS) damage occurring during aging. The accumulation of free radicals activates transcription factors such as NFκB (4), and the subsequent release of proinflammatory cytokines contributes to chronic wasting disease (5) and sarcopenia (6). Furthermore, antioxidant supplementation has been shown to improve the aging-induced dysregulation of immune function and reduce the risk of infection in hospitalized elderly patients (7–9). Because infectious diseases have a detrimental influence on nutritional status, the lower incidence of infection promoted by antioxidants may help improve nutritional status. The antioxidants used in supplementation, such as vitamin E, are free radical scavengers. These antioxidants minimize OS damage by cooperating with the endogenous antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT).

Certain single nucleotide polymorphisms (SNPs) located within the SOD, GPx, or CAT genes may lead to decreased or impaired regulation of their enzymatic activity, thereby promoting OS. The Ala-9Val SNP located in the manganese SOD (Mn-SOD) gene and the Pro197Leu SNP of the GPx1 (GPx) gene have previously been associated with OS-related diseases (10–13). The majority of known SNPs located within the translated region of the CAT gene appear to be silent (14–16), except for the SNP responsible for Japanese acatalasemia (17). A promising route of research would be to study the SNPs of the CAT gene 5′-untranslated region because they may be responsible for modulating transcription rate and regulation and, therefore, response to OS. The CAT C-262T SNP, which is the most widely studied to date, has been associated with a decrease in CAT activity (18) and an increased or decreased risk of developing OS-related diseases (19, 20). The other SNPs located within this region (21, 22) include G-844A, which has been associated with hypertension (21). This prospective study was conducted in an elderly population to investigate whether the Ala-9Val SNP of Mn-SOD, the Pro197Leu SNP of GPx, and/or the SNPs of the CAT gene 5′-untranslated region can influence either nutritional status or the renutrition process because renutrition becomes progressively more difficult with age (23, 24).

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2 Supported by Danone.

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Received October 26, 2007.

Accepted for publication December 19, 2007.
SUBJECTS AND METHODS

Subjects

From December 2000 to December 2004, 99 malnourished white elderly inpatients were recruited from the Geriatric Nutritional Unit and 77 white elderly outpatients were recruited from the Gerontological Evaluation Center, both at Charles Foix Hospital, Ivry-sur-Seine, France, to form the malnourished and the control populations, respectively.

The study was approved by the Pitie-Salpetriere CCPRPB, Paris (French biomedical research volunteers protection committee) under research project no. 101–00. Written informed consent was obtained from all patients.

Inclusion and exclusion criteria

Based on French national recommendations for the diagnosis of elderly malnutrition, we established an albuminemia cutoff for inclusion of 35 g/L (25). The exclusion criteria for both populations were age <70 y, gastrointestinal disorder, progressive cancer (with metastasis and/or local or node satellite extension), poorly controlled diabetes (glycemia >11 mmol/L), severe renal failure (creatinine clearance <20 mL/min, as estimated by the Cockcroft and Gault formula), severe hepatic insufficiency (prothrombin levels <50%), heavy smoking (>5 cigarettes/d), alcohol consumption higher than one glass of wine/d, and antioxidant food supplementation at inclusion.

The main characteristics of the 2 populations studied are summarized in Table 1.

Study period

Nutritional status was measured in both populations at inclusion (D0) and reassessed at 3 and 6 wk postinclusion in the malnourished population.

TABLE 1

<table>
<thead>
<tr>
<th>Main descriptive characteristics of the malnourished and control populations at inclusion</th>
<th>Malnourished population</th>
<th>Control population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>74.7</td>
<td>67.5</td>
</tr>
<tr>
<td>Age (y)</td>
<td>85 ± 1</td>
<td>82 ± 1</td>
</tr>
<tr>
<td>Main disorder (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Orthopedic/rheumatological</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Neuropsychological</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Hematological</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Number of comorbidities</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Very light smokers (&lt;5 cigarettes/d) (%)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Alcohol consumers (1 glass of wine/d) (%)</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Sex, age, lifestyles, main etiology of hospitalization or consult, and associated comorbidities of the 2 populations at inclusion.

2 The malnourished population included 99 elderly (older than 70 y) inpatients characterized by an albuminemia lower than 35 g/L.

3 The control population included 77 elderly (older than 70 y) outpatients with an albuminemia higher than 35 g/L.

4 ± SD.

Medical care of the malnourished population

Medical care included treatment of the cause of inflammation and refeeding. As recommended (25, 26), energy intake was maintained at 30 ± 2 kcal·kg⁻¹·d⁻¹ on inclusion to 39 ± 2 kcal·kg⁻¹·d⁻¹ 6 wk later. Oral feeding was sufficient to reach the target values of energy intake for most of the malnourished inpatients, although 9%, 16%, and 19% of them required enteral feeding D0, 3 wk later, and 6 wk later, respectively.

Anthropometric measurements

Body mass index (BMI) was calculated through use of weight measurements and estimated height with measurement of knee height (27). Middle arm circumference (MAC) was measured.

Biochemical assays

Plasma vitamin E

Plasma vitamin E was assayed by HPLC (C18 column, Lichrosart 125–4; Merck, Darmstadt, Germany) (28). Vitamin E was quantified as μmol/mmol of total cholesterol. Total cholesterol was assayed using an enzymatic method (Vitros 250 analyzer, Johnson & Johnson, Raritan, NJ).

Serum-specific protein assay

Albumin (Alb), transthyretin (TTR), orosomucoid (ORO), transferrin (TRF), and C-reactive protein (CRP) serum concentrations were determined by immunonephelometry (Immage analyzer; Beckman Coulter, Fullerton, CA).

The Prognostic Inflammatory and Nutritional Index (PINI) was calculated using Alb, TTR, CRP, and ORO concentrations (29).

The variation in nutritional status from time D0 to 6 wk postinclusion was estimated using the TTRD0 percentage of TTRΔ. This calculation, named PcTTR, used the following formula (Equation 1):

\[
PcTTR = \frac{[\text{TTR}_{\text{6 weeks}} - \text{TTR}_{\text{D0}}]}{\text{TTR}_{\text{D0}}} \times 100
\]

CAT, SOD, and GPx enzymatic activities

Erythrocyte enzyme activity assays were performed using an EDTA blood sample. CAT activity was assayed using the Aebi method (30). Cu/Zn-SOD and GPx1 (GPx) activity assays were performed on a Hitachi 911 analyzer (Roche Diagnostics, Basel, Switzerland) using a Ransod Superoxide Dismutase SD125 kit (Randox, Crumlin, United Kingdom) for Cu/Zn-SOD and a Ransel Glutathione Peroxidase SC692 kit (Randox) for GPx1 (GPx). Enzyme activity was quantified as U/g of total hemoglobin (Hb) measured using an Advia 120 hematology System (Bayer, Leverkusen, Germany).

Other assays

Serum glucose and creatinine were measured on a Hitachi analyzer (Roche Diagnostics) using a glucose oxidase method and a modified Jaffé reaction, respectively. Ultrasensitive human serum TSH was determined by chemiluminescence immunoassay on an Access analyzer (Beckman Coulter).
DNA extraction

DNA extraction was performed with peripheral leukocytes with use of an EDTA blood sample. The classical phenol/chloroform extraction method was performed.

Sequencing

The first PCR amplified the CAT gene region located at (−998; +320), using Ampli taq DNA polymerase (Applied Biosystems, Foster City, CA), a GeneAmp 9700 thermocycler (Applied Biosystems) and the primers U−998 (5′-ct gcc gag ggt tag aaaa tc 3′) and L + 320 (5′ tgt ccc agt tgg cag aag 3′). The PCR runs were 95 °C, 5′; 30 × (95 °C, 1′; 60 °C, 40′; 72 °C, 1′15″); and 72 °C, 5′. PCR product was purified using a MinElute kit (Qiagen, Venlo, Netherlands).

Three PCR runs were performed using the first PCR product and Big Dye Terminator v3.0 and v3.1 kits (Applied Biosystems) and either each of the first PCR primers or primer L + 30(5′ ett cca cca ccc gag cct cat ctt 3′). Capillary electrophoresis was performed using POP-6 polymer and an AbiPrism 310 sequencing analyzer (Applied Biosystems).

After crosschecking the sequencing results from the 3 primers, the final sequence obtained was aligned with CAT reference sequence L13609 sourced through the GenBank website (31).

Single nucleotide amplified polymorphism reaction (SNaP)

The DNA fragment located at (−998; +287) was amplified under the same experimental conditions as for the first PCR and with the primers U−998, L + 287 (5′ cca cca ccc gag cct cat ctt 3′). The PCR product was purified using a MinElute kit (Qiagen), and 2 SNaP reactions were performed starting with this product. The first SNaP studied CAT gene SNPs located at positions −844, +143, and +150, whereas the second SNaP focused on SNPs at positions −330, −89, and −20 by use of a SNaP shot Multiplex kit (Applied Biosystems) on a GeneAmp 9700 thermocycler. The PCR product sorting was performed using POP 4 polymer and an Abiprism A310 sequencing analyzer (Applied Biosystems).

Mn-SOD and GPx1 genotyping

The genotyping studies on Mn-SOD Ala-9Val SNP and GPx1 (GPx) Pro197Leu SNP used an identical SNaP protocol.

Results

Nutritional, inflammatory, and antioxidant status at inclusion

The main nutritional and inflammatory characteristics at inclusion of both the malnourished and the control populations are reported in Table 2. The values for anthropometric parameters (weight, BMI, and MAC) and the serum values for Alb, TTR, and TRF were significantly lower in the malnourished population than in the control population. Moreover, CRP and ORO values indicated an inflammatory process on inclusion in the malnourished population but not in the control population.

Levels of vitamin E, an antioxidant vitamin, were measured in the malnourished and the control populations (Table 2). Antioxidant enzymatic status was evaluated in the malnourished population at inclusion based on the erythrocytic activities of CAT, GPx, and Cu/Zn-SOD (Table 3).

Evolution in nutritional and inflammatory status in the malnourished population during the renutrition process

Variations in Alb, TTR, CRP, ORO, and PINI between D0, 3 wk, and 6 wk postinclusion, highlighted in Figure 1, indicated malnutrition and inflammation at inclusion that improved significantly during hospitalization. In addition, these concomitant evolutions of nutritional and inflammatory status were strongly correlated (P < 0.0001). Weight, BMI, and MAC values were 55.8 ± 1.7 kg, 22.1 ± 0.7 kg/m², and 24.0 ± 0.6 cm at wk 3 postinclusion and 56.7 ± 1.9 kg, 22.5 ± 0.7 kg/m², and 25.0 ± 0.6 cm at wk 6 postinclusion, respectively. There was no significant change in anthropometric measurements over the study period.

SNP selection for the GPx, Mn-SOD, and CAT gene study

Ala-9Val SNP from the Mn-SOD gene and Pro197Leu SNP from the GPx gene were studied in both the malnourished and the control populations.

The CAT gene study began by sequencing the (−932, +272) region from the 5′-untranslated region to the first intron, which was performed for approximately one-quarter of the malnourished population (26 patients). This study highlighted 7 SNPs: CAT G−844A, CAT C−379T, CAT C−330T, CAT A−89T, CAT T−20C, CAT T+143C, and CAT G+150A. Because this sequencing study only identified one mutant allele of the CAT C−379T SNP, this SNP study was not followed up. The 6 other SNPs were then studied in the entire malnourished and in the control populations.

Genotyping of Mn-SOD, GPx, and CAT SNPs

The Hardy-Weinberg equilibrium was maintained in both populations for every SNP studied. There was no between-group difference in genotype distribution of the SNPs studied (Figure 2). The GPx, Mn-SOD, and CAT gene study performed in both populations revealed an almost equal share of wild-type and mutant alleles of Mn-SOD SNP Ala-9Val. The wild-type alleles of CAT C−330T, CAT G+150A, and GPx Pro197Leu SNPs were the most prevalent. Frequency of the mutant allele was markedly higher than 0.5 in G−844A, A−89T, T−20C, and T+143C CAT SNPs.

Throughout the 2 populations, only one genotype of a total 174 was different between CAT A−89T and T−20C SNPs, and only 10
and 11 of 174 were different between CAT G-844A and CAT A-89T or T-20C SNPs, respectively.

**Nutritional, inflammatory, antioxidant status, and antioxidant enzyme SNP interactions at inclusion**

On admission, there was no correlation between Alb or TTR and vitamin E or the erythrocytic activities of CAT, Cu/Zn-SOD, or GPx in the malnourished population. Similarly, at inclusion, CAT, Mn-SOD, and GPx SNPs were not associated with Alb, TTR, CRP, ORO, PINI, or vitamin E in either population or with antioxidant enzyme activity in the malnourished population.

**Nutritional, inflammatory, antioxidant status, and antioxidant enzyme SNP interactions during the renutrition process**

To study the effects of OS on the renutrition process, we used PcTTR to assess variation in nutritional status within 6 wk. We investigated the links between PcTTR and nutritional parameters, inflammatory parameters, or antioxidant enzyme activities and SNPs at inclusion. As shown in **Table 4**, PcTTR was correlated to Alb, TTR, PINI, and total cholesterol values at inclusion and to G-844A, A-89T, and T-20C CAT SNPs. As shown in **Figure 3**, the mutant allele of each of these CAT gene SNPs appeared to be a bad prognostic factor for changes in nutritional status. Whereas a patient homozygous for the mutant allele would only show a 12% increase in TTR over 6 wk, the patient homozygous for the wild-type of these SNPs, inflammatory parameters, or antioxidant enzyme activities and SNPs at inclusion. As shown in **Table 4**, PcTTR was correlated to Alb, TTR, PINI, and total cholesterol values at inclusion and to G-844A, A-89T, and T-20C CAT SNPs. As shown in **Figure 3**, the mutant allele of each of these CAT gene SNPs appeared to be a bad prognostic factor for changes in nutritional status. Whereas a patient homozygous for the mutant allele would only show a 12% increase in TTR over 6 wk, the patient homozygous for the wild-type of these SNPs, inflammatory parameters, or antioxidant enzyme activities and SNPs at inclusion. As shown in **Table 4**, PcTTR was correlated to Alb, TTR, PINI, and total cholesterol values at inclusion and to G-844A, A-89T, and T-20C CAT SNPs. As shown in **Figure 3**, the mutant allele of each of these CAT gene SNPs appeared to be a bad prognostic factor for changes in nutritional status.

### Table 2

Main anthropometric and biological characteristics of the malnourished and control populations at inclusion.

<table>
<thead>
<tr>
<th>At inclusion</th>
<th>n</th>
<th>Malnourished</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>90</td>
<td>57 ± 2</td>
<td>67 ± 2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>76</td>
<td>23.2 ± 0.7</td>
<td>28.0 ± 0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAC (cm)</td>
<td>52</td>
<td>23.9 ± 0.6</td>
<td>27.3 [20.9; 39.7]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alb (g/L)</td>
<td>99</td>
<td>27.4 ± 0.5</td>
<td>39.4 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TTR (mg/L)</td>
<td>99</td>
<td>28.0 [19.0; 34.0]</td>
<td>39.0 [35.0; 44.0]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TRF (g/L)</td>
<td>99</td>
<td>166 ± 6</td>
<td>248 ± 5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>99</td>
<td>167 [71; 279]</td>
<td>247 [171; 330]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ORO (g/L)</td>
<td>99</td>
<td>1.82 ± 0.05</td>
<td>2.41 ± 0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PINI</td>
<td>99</td>
<td>35 ± 5</td>
<td>6 ± 1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tCHOL (mmol/L)</td>
<td>98</td>
<td>3.7 [0.3; 173.9]</td>
<td>0.6 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vit E (μmol/mmol tCHOL)</td>
<td>77</td>
<td>4.5 ± 0.1</td>
<td>0.3 [0.2; 1.9]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>99</td>
<td>5.7 ± 0.1</td>
<td>5.2 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Glycemia (mmol/L)</td>
<td>77</td>
<td>5.7 [3.3; 7.9]</td>
<td>5.1 [3.5; 7.8]</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>90</td>
<td>5.0 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Table 3**

Erythrocytic activities of the antioxidant enzymes in the malnourished population at inclusion.

<table>
<thead>
<tr>
<th>CAT</th>
<th>GPx</th>
<th>Cu/Zn SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 62)</td>
<td>(n = 27)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>U/g/Hb</td>
<td>81 ± 2</td>
<td>29 ± 2</td>
</tr>
<tr>
<td></td>
<td>1109 ± 54</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4**

Data are presented as median [5th percentile; 95th percentile]. Two-sample Wilcoxon tests were performed to compare one parameter value in the 2 populations.
SNPs or all 3 SNPs were roughly equivalent. This link between CAT SNPs and PcTTR was not found for PcPINI, PcCRP, or PcORO.

Because the CAT A-89T SNP was the most significant predictive marker of renutrition efficiency, we used this SNP to extend the study on prediction of renutrition efficiency. We first explored the relative risk of renutrition inefficiency, which was defined as a PcTTR value below 30%. Risk of the renutrition inefficiency was 5.04 for a homozygous mutant genotype and 3.37 for a heterozygous genotype compared with a homozygous wild genotype ($P < 0.005$). We then explored potential interactions between prediction of evolution in nutritional status via CAT SNPs which were not predictive and independent of erythrocytic CAT activity on admission. Thus, PcTTR prediction via CAT A-89T SNP was also independent of Mn-SOD and GPx SNPs, independent of other CAT SNPs which were not predictive and independent of erythrocytic CAT activity on admission. Thus, PcTTR prediction via CAT A-89T SNP was independent of both CAT activity and nutritional or inflammatory biochemical status at inclusion.

To further extend the study of the predictive ability of PcTTR, a multivariate regression analysis was run in our malnourished elderly population. This analysis led to the following equation:

$$\text{PcTTR} = 42.1 - 0.2 \times \text{TTR} + 0.1 \times \text{PINI} + 16.3 \times (\text{number of CAT A-89T wild-type alleles})$$

In Equation 2, PcTTR was dependent on TTR and PINI values at inclusion and strongly dependent on the presence of one or 2 wild-type alleles of CAT A-89T SNP, thus confirming the significance of this CAT A-89T SNP in the prediction of renutrition efficiency.

**DISCUSSION**

To explore the consequences of OS on malnutrition and renutrition care in the elderly, we studied nutritional, inflammatory, and antioxidant status together with the SNPs of SOD, GPx, and CAT genes in malnourished elderly and elderly control populations.

Based on Alb at inclusion, the hospitalized elderly population was considered severely malnourished according to French guidelines on elderly malnutrition (25). Mean TTR and Alb values in this population were even far lower than the values reported in a previous study on elderly malnutrition (32). Mean BMI at inclusion was lower than in our control population and lower than reported values for community-dwelling elderly subjects (33) but nevertheless remained within the range of normal values for the age bracket considered (34). However, because elderly subjects present age-related changes in body composition (35), a normal BMI value does not rule out malnutrition as it may be associated with weight loss, sarcopenia, and, possibly, edema.

As the malnourished patients’ vitamin E values on admission were equivalent to our controls, there was no vitamin E deficiency at inclusion. One limitation to this research was the number of missing data for CAT, SOD, and GPx activities in our malnourished population, which made the corresponding study less accurate. Nevertheless, although CAT activity remained unchanged, SOD activity was lower and GPx activity was higher compared with previous studies (36–38). Although similar changes in antioxidant enzyme activities have previously been reported with active smokers (39), these changes could not be imputed to the few very light tobacco consumers in the malnourished population considered here. These changes in SOD and GPx erythrocyte activities seemed to highlight evidence of OS processes at inclusion in our malnourished elderly patients.

The CAT genotyping study on one-quarter of our malnourished elderly population did not reveal any mutant allele for the known C-262T SNP. Thus, in our malnourished elderly population, the estimated frequency of CAT C-262T mutant allele, like that of the C-379T SNP, did not seem relevant enough to be involved in the pathophysiology of elderly malnutrition and renutrition. Consequently, we did not extend the study of these SNPs to the entire malnourished population. In contrast, the sequencing of the CAT gene 5’-untranslated region highlighted 6 other SNPs that were potentially of interest that were then studied.

**FIGURE 1.** Evolution of nutritional and inflammatory markers during the 6-wk health care period in the malnourished elderly population. Changes in serum concentrations of albumin (Alb), transthyretin (TTR), C-reactive protein (CRP), and orosomucoid (ORO) and changes in the Prognostic Inflammatory and Nutritional Index (PINI) between inclusion and 6 wk postinclusion. The patient numbers were 99, 77, and 63 at inclusion (D0) and 3 wk after and 6 wk after inclusion, respectively. Data are presented as means ± SEM. For each variable, means between the 3 times of D0, 3 wk, and 6 wk were globally compared using repeated-measures ANOVA. The $P$ value after repeated-measures ANOVA was 0.0001 for Alb, 0.0004 for TTR, 0.0108 for CRP, 0.0323 for PINI, and 0.0001 for ORO.
in every patient. Although to the best of our knowledge, the T+143C and G+150A SNPs have not yet been associated with any particular disease, the G-844A, C-330T, A-89T, and T-20C SNPs have previously been described in various diseases, including hypertension and vitiligo (21, 22, 40).

The mutant allele frequencies of CAT G-844A, SOD Ala-9Val, and GPx Pro197Leu SNPs were within reported ranges for white populations (11, 40–42). In contrast, the mutant allele frequencies of CAT C-330T, CAT A-89T, and CAT T-20C SNPs were markedly higher than the figures reported, to the point that there was even a frequency inversion for CAT A-89T and T-20C SNPs between our white population and the Asian population studied by Jiang et al (21). This may be due to an ethnic-origin impact on allelic frequencies, as previously discussed for SNPs of CAT or SOD gene (40, 42). Furthermore, in accordance with Jiang et al (21) and Oh et al (43), we found CAT A-89T, CAT T-20C, and CAT G-844A SNPs to be equally distributed.

To explore the impact of OS on the onset of malnutrition, we studied potential links with nutritional, inflammatory, and antioxidant status at inclusion in the 2 populations. There was no correlation between antioxidant enzyme activities, SNPs, vitamin E levels, and Alb, TTR, ORO, CRP, or PINI values at inclusion. However, there was no association between the 6 CAT SNPs, GPx Pro197Leu, Mn-SOD Ala-9Val, and erythrocytic activities of CAT, Cu/Zn-SOD, or GPx at inclusion. This result is to be compared with previous results demonstrating that CAT G-844A and GPx Pro197Leu SNPs did not lead to any activity change in the corresponding enzymes (18, 44). Therefore, the genotypes of the CAT, Mn-SOD, and GPx SNPs studied here were not predictive of nutritional, inflammatory, and antioxidant status at inclusion in our elderly patients.

To analyze renutrition efficiency, we studied whether PcTTR was related to nutritional, inflammatory, and antioxidant status or to antioxidant enzyme SNPs. TTR, which has a short half-life

**FIGURE 2.** Genotypes for glutathione peroxidase (GPx), manganese superoxide dismutase (Mn-SOD), and catalase (CAT) single nucleotide polymorphisms (SNPs) in the malnourished population (97 white inpatients) and the control population (77 white outpatients). Distribution of genotypes for SNPs of GPx, Mn-SOD, and CAT genes studied among the malnourished (M) and the control (C) elderly white populations. These 2 populations were characterized by an age older than 70 y and defined with an albuminemia cutoff of 35 g/L. WW, homozygous wild-type; WM, heterozygous wild and mutant types; MM, homozygous mutant type.
We then sought to characterize the ability of CAT A-89T SNP genotype to predict improvement in patient malnutrition by exploring several factors that could potentially influence this relation. We found that patient CAT genotype for A-89T SNP, despite not modifying CAT activity, nevertheless remained a marker of nutritional therapy efficiency, regardless of the initial intensity of malnutrition and inflammation, and this predictive property was maintained in the multivariate regression model. However, our work did not make it possible to determine whether CAT A-89T SNP was itself responsible for affecting the renutrition process or whether it reflected a linkage disequilibrium with variation in the CAT gene or a nearby flanking gene.

In this elderly population presenting malnutrition, inflammation, and OS, the wild-type allele of G-844A, A-89T, or T-20C CAT SNPs seems to lead to a better body response against these stresses. This may be due to loss or creation of transcription factor binding sites, including one of these 3 SNPs. Because these 3 SNPs are strongly associated, the sequence analysis with use of MATINSPECTOR (Genomatrix, Munich, Germany) (46) software revealed that the wild-type allele of all 3 could be associated with the binding sites of transcription factors corresponding to PLAG1 (Pleomorphic Adenoma Gene 1), THAP1 (THanatos Associated Protein 1), and/or DICE (Downstream Immunoglobulin Control Element), about which little is known (47–49). These binding sites are lost when the mutant allele is present, but in turn the PAX6 (Paired Box 6) binding site is created. Because the PAX6 has been implicated in glucose metabolism (50) and associated with an increase in OS in glioma cells (51), it could

at only 48 h, has been shown to be a useful marker in early monitoring of malnourished patients. Indeed, TTR concentrations are closely related to early changes in nutritional status and to response to nutritional support (45). Therefore, nutritional improvement at 6 wk postinclusion could accurately be evaluated using PcTTR. Renutrition efficiency was linked to nutritional status at inclusion. As expected, lower Alb, TTR, and total cholesterol concentrations correlated to a more efficient renutrition program. Because the SNPs studied had no effect on nutritional status at inclusion, CAT G-844A, CAT A-89T, and CAT T-20C SNPs were all predictive markers of improved nutritional status as estimated using PcTTR. More specifically, the number of wild-type alleles of each of these SNPs seemed to be a positive prognostic factor for improvements in malnutrition status but did not seem to predict the observed inflammation improvement. The predicted improvement in a patient’s nutritional status would be more relevant with higher numbers of the wild-type allele. Furthermore, because these 3 SNPs are associated, we could not conclude that any single one or any combination of the 3 corresponded to a positive factor for nutritional status recovery, although it should be noted that A-89T SNP showed the strongest statistical significance. Moreover, working with A-89T SNP, the risk of the inefficient renutrition was 5-fold and 3-fold higher compared with homozygous wild genotype for homozygous mutant and heterozygous genotypes, respectively. However, this result has to be confirmed in a larger malnourished population because of the small homozygous wild genotype count in this study, although, of the 63 patients considered, 32 were heterozygous and 25 were homozygous mutant.

### TABLE 4

<table>
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<th>Univariate correlation</th>
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<td>NS</td>
</tr>
<tr>
<td>CRP</td>
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</tr>
<tr>
<td>CAT T-20C</td>
<td></td>
<td>0.398</td>
<td>&lt;0.005</td>
</tr>
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</table>

1 For 63 malnourished inpatients, the PcTTR was calculated (TTRD0 percentage of TTR delta) to estimate the variation of nutritional status from time D0 to 6 wk postinclusion.

2 Data presented included weight; BMI; middle arm circumference (MAC); serum concentrations of albumin (Alb), transferrin (TTR), transferrin (TRF), CRP, and orosomucoid (ORO); Prognostic Inflammatory and Nutritional Index (PINI); serum concentration of total cholesterol (tCHOL); and catalase gene polymorphisms (CAT G-844A, CAT A-89T, or CAT T-20C).

3 Spearman’s correlation coefficient tests were performed, and P values are presented.

4 Taking into account the number of wild-type alleles.

**FIGURE 3.** Evolution in nutritional status of the malnourished elderly population according to G-844A, A-89T, and T-20C catalase (CAT) genotypes. Prealbumin variation in percentage transthyretin (PcTTR) was calculated (TTRD0 percentage of TTR ∆) to estimate the variation of nutritional status from time at inclusion (D0) to 6 wk postinclusion. Total patient numbers were 61 for glutathione peroxidase (GPx) and superoxide dismutase (SOD) single nucleotide polymorphisms (SNPs), 63 for each CAT SNP, and 55 for the association of all 3 SNPs. Number of homozygous wild-type (WW), heterozygous wild and mutant types (WM), and homozygous mutant-type (MM) patients were 26, 27, and 8, respectively, for GPx Pro197Leu; 12, 29, and 20 for SOD Ala-9Val; 32, 30, and 1 for CAT G+150A; 1, 13, and 49 for CAT T+143C; 6, 37, and 20 for CAT G-844A; 34, 24, and 5 for CAT C-330T; 6, 32, and 25 for CAT A-89T; 6, 31, and 26 for CAT T-20C; 96, 30, and 19 for the association of G-844A, A-89T, and T-20C CAT SNPs, respectively. PcTTR values are presented as means ± SEM. For each SNP or association of SNPs, Kruskal-Wallis tests were performed to compare the PcTTR values of the 3 genotypes. *P < 0.05, **P < 0.01, ***P < 0.005.
play a role in the effects of these 3 SNPs on malnutrition improvement.

Malnutrition is frequently observed in hospitalized elderly patients presenting acute or chronic diseases associated with hypercatabolic state and depleted visceral protein concentrations. Moreover, elderly malnutrition increases morbidity and mortality. Elderly malnutrition therefore needs to be diagnosed and treated quickly to avoid these negative consequences. As the CAT mutant alleles of G-844A, A-89T, and T-20C SNPs are associated with the efficiency of elderly malnutrition health care, patient genotyping could make it possible to identify a high-risk subpopulation of mutant allele-carriers within the elderly polypathologic population. In the malnourishment setting, this subpopulation should be given personalized health care, including a strengthened refeeding program. In conclusion, catalase genotyping could enable earlier recovery of satisfactory nutritional status and preclude the consequences of malnutrition, which are especially deleterious in the elderly.

We thank Luc Cyrober (Nutrition Department, University of Paris Descartes) for critical reading of the manuscript, Christine Forassassi (Gerontological Evaluation Center, Charles Foix Hospital, Ivry-sur-Seine) for the work on patient recruitment, and Amina Lahlou (Geriatric Rehabilitation Unit, Charles Foix Hospital, Ivry-sur-Seine) for support on using the medical software. We would also like to acknowledge Danone for their financial support.

The authors’ responsibilities were as follows—VNA, ARS, and JM: designed the global research project; ARS: recruited the inpatients; ARS, JM, and EEF: collected the data; VNA and EEF: drafted the genetic part of the study; EEF, VNA, MH, and XD: performed and oriented the genetic study; MS: managed the assays of oxidative stress enzymes; JLG: performed the statistical analysis; VNA and EEF: analyzed the data and drafted the manuscript; and ARS, JLG, and DD: advised on data analysis and manuscript drafting. None of the authors had a conflict of interest.

REFERENCES


