Interaction between a Heme Oxygenase-1 Gene Promoter Polymorphism and Serum β-Carotene Levels on 8-Year Lung Function Decline in a General Population

The European Community Respiratory Health Survey (France)

Armelle Guenegou¹, Jorge Boczkowski², Michel Aubier², Françoise Neukirch¹, and Bénédicte Leynaert¹

¹ Épidémiologie des Maladies Respiratoires, INSERM Unit 700, Faculté Xavier Bichat, Paris, France.
² INSERM Unit 700, Faculté Xavier Bichat, Paris, France.

Oxidative stress is thought to play a major role in the pathogenesis of chronic obstructive pulmonary disease, characterized by impaired lung function. A large number (≥33) of GT repeats (L-allele) in the gene of the powerful antioxidant enzyme heme oxygenase-1 has been associated with susceptibility to accelerated lung function decline. In contrast, β-carotene may help to protect against accelerated decline. To determine whether high serum levels of β-carotene might counterbalance the greater susceptibility of L-allele carriers, the authors analyzed the annual decline in forced expiratory volume in 1 second (FEV₁) in a general population sample of 523 French subjects (20–44 years, 50% men) examined in 1992 and 2000 as part of the European Community Respiratory Health Survey. Analysis of covariance, adjusted for sex as well as baseline age, body mass index, smoking, and FEV₁ showed that, among subjects with low β-carotene levels, L-allele carriers experienced a steeper mean FEV₁ decline than did noncarriers (mean = −58.8, 95% confidence interval: −73.2, −44.5 vs. mean = −34.7, 95% confidence interval: −38.9, −29.8 ml/year) (p = 0.009), whereas among subjects with high β-carotene levels, the FEV₁ decline was not different in L-allele carriers and noncarriers (two-sided p = 0.9). The results suggest that high levels of β-carotene might counterbalance the effects on FEV₁ decline of a genetically determined deficiency in antioxidant response.

beta carotene; follow-up studies; France; heme oxygenase-1; polymorphism, genetic; pulmonary disease, chronic obstructive; serum; spirometry

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECRHS, European Community Respiratory Health Survey; FEV₁, forced expiratory volume in 1 second; HO-1, heme oxygenase-1; SD, standard deviation.

In the next 20 years, chronic obstructive pulmonary disease (COPD) is expected to become the third largest cause of mortality worldwide (1). Oxidative stress is thought to play a major role in the onset and progression of COPD. In particular, oxidative stress may contribute to airway remodeling, leading to impaired lung function (assessed by forced expiratory volume in 1 second (FEV₁)) and to its accelerated decline (2). The enzyme heme oxygenase is considered as one of the most potent antioxidants because it catalyzes the degradation of heme to bilirubin, which is an efficient local scavenger of oxidants. In the promoter of the gene (i.e., in the region regulating the gene’s transcription), coding for the

Correspondence to Dr. Armelle Guenegou, Épidémiologie des Maladies Respiratoires, INSERM Unit 700, Faculty of Medicine Xavier Bichat, 16 rue Henry Huchard, 75018 Paris, France (e-mail: armelleguenegou@yahoo.fr).

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The statistical methods and definitions of variables are described elsewhere (4, 6). Briefly, FEV\textsubscript{1} decline was calculated as the value in 2000 minus the value in 1992 divided by the length of follow-up. The number of (GT)\textsubscript{n} repeats in the HO-1 gene promoter ranged from 11 to 41 repeats and showed a trimodal distribution with peaks at 23, 30, and 38 repeats. Therefore, we classified the alleles into three subclasses according to the number of (GT)\textsubscript{n} repeats, as previously reported (3, 8–10): small (S) allele (≤26 GT repeats), medium (M) allele (27–32 GT repeats), and long (L) allele (≥33 GT repeats), focusing on the long allele (L) that has been shown to be associated with a greater susceptibility to lung function decline (4), emphysema (3), lung adenocarcinoma (10), and COPD (11). Subjects with one or two L alleles (group L+) were thus compared with those with no L allele (group L–). Serum β-carotene levels were classified into tertiles. Subjects in tertile I (lowest values) were compared with those in tertiles II and III combined (highest values). The results from these comparisons were estimated by analysis of covariance and expressed as adjusted means and their 95 percent confidence intervals. All statistical tests were two sided. Confounding factors were sex and baseline age, body mass index, smoking status, and FEV\textsubscript{1}. In the present analysis, smoking status was coded into four classes: never smokers, former smokers (stopped for ≥1 year), moderate smokers (<20 cigarettes/day), and heavy smokers (≥20 cigarettes/day). By definition, former smokers had stopped smoking for at least 1 year. Therefore, former smokers who had stopped for less than 1 year were classified as either moderate or heavy smokers according to their previous smoking habits. The mean number of pack-years at baseline for heavy smokers was almost three times higher than that for moderate smokers, with a mean of 29.3 (SD: 14.5) versus a mean of 10.2 (SD: 9.0) pack-years (Mann-Whitney test: p < 0.0001), and double that for former smokers, with a mean of 29.3 (SD: 14.5) versus a mean of 14.0 (SD: 14.2) pack-years (p < 0.0001), whereas the mean number of pack-years did not differ between former and moderate smokers (p = 0.22). Moreover, heavy smokers started smoking at a younger age than did either moderate smokers, with a mean of 16.9 (SD: 2.7) versus a mean of 18.3 (SD: 4.0) years (p = 0.004), or former smokers, with a mean of 16.9 (SD: 2.7) versus a mean of 17.6 (SD: 2.9) years (p = 0.05). In the following analyses, we adjusted for smoking in two ways: using the four categories of smokers and using the number of pack-years.

Previous analyses of two French ECRHS subsamples showed that both the HO-1 promoter polymorphism and serum β-carotene levels were more strongly related to lung function decline in heavy smokers than in other smoking categories (4, 6); this is consistent with there being a stronger protective effect in subjects at greater risk of oxidative stress. Therefore, we first verified that the interaction between heavy smoking and each antioxidant system was found in this third ECRHS subsample; for this particular analysis, smoking status was coded as heavy smoking to be compared with any other status.

The interaction between the promoter polymorphism in the HO-1 gene and serum β-carotene levels was tested by adding the term HO-1 group × β-carotene tertile, both being coded as binary variables.

**MATERIALS AND METHODS**

Data were collected in the centers of Paris and Grenoble as part of the multicenter longitudinal European Community Respiratory Health Survey (ECRHS) (7). Of the 1,194 French subjects (20–44 years) examined in 1992 (ECRHS I), 88 refused to participate again in 2000 and 242 did not respond (because they had moved or were moving, had died, or for unknown reasons), leaving 864 who were followed up (because they had moved or were moving, had died, or for unknown reasons), leaving 864 who were followed up in 2000 (ECRHS II) (participation rate = 72.4 percent). Among the 864 subjects participating in 2000, information on FEV\textsubscript{1}, HO-1 genotype, and serum β-carotene levels for both ECRHS I and ECRHS II was not available for 192, 14, and 135 subjects, respectively. The analysis thus included the 523 subjects (272 in Grenoble and 251 in Paris) for whom complete data were available. We found no difference for sex (half of the subjects were men), baseline body mass index (mean = 22.7 (standard deviation: SD): 3.1) kg/m\textsuperscript{2}, FEV\textsubscript{1} (FEV\textsubscript{1} percent predicted = 105.3 (SD: 13.6)), and COPD status (presence of the FEV\textsubscript{1}/forced ventilatory capacity ratio lower than 70 percent = 4.4 percent) between the subjects included and those not included in analyses because of either nonparticipation in ECRHS II or missing data. The analyzed subjects were slightly older, with a mean of 36.9 (SD: 7.1) versus a mean of 35.1 (SD: 7.2) years (p < 0.0001), less likely to have been examined at the Paris center (48 vs. 61 percent) (p < 0.0001), and less likely to be heavy smokers (≥20 cigarettes/day), but they were more likely to be never smokers (12 vs. 18 percent and 42 vs. 37 percent, respectively) (χ\textsuperscript{2} test: p = 0.02). However, the lifetime number of cigarette pack-years was not significantly different between included and excluded subjects because the mean number of pack-years was higher for included than excluded heavy smokers, with a mean of 29.3 (SD: 14.0) versus a mean of 24.5 (SD: 15.7) pack-years (Mann-Whitney test: p = 0.009), but was not different between included and excluded former smokers (p = 0.22) and moderate smokers (p = 0.83). Neither COPD status nor COPD comorbid conditions that might have an effect on lung function, such as current asthma or chronic cough and/or chronic sputum, were differently distributed between included and excluded subjects whether nonsmokers or heavy smokers.
TABLE 1. Adjusted annual mean FEV₁* decline according to either the HO-1 microsatellite polymorphism or the serum β-carotene level at baseline (each risk factor analyzed separately), France, 1992 and 2000†

<table>
<thead>
<tr>
<th>Group of HO-1 polymorphism</th>
<th>Tertiles of β-carotene</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group L+†‡ (64 subjects§)</td>
</tr>
<tr>
<td></td>
<td>Mean FEV₁ (ml/year)</td>
</tr>
<tr>
<td>All†‡</td>
<td>−34.5</td>
</tr>
<tr>
<td>Heavy smokers¶¶</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
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* FEV₁, forced expiratory volume in 1 second.
† French participants in the European Community Respiratory Health Survey (ECRHS) I (1992) and II (2000) were aged 20–44 years at baseline and recruited in the cities of Grenoble and Paris (n = 523).
‡ Group L+, (GT)ₙ ≥ 33 repeats, that is, genetic deficiency in antioxidant defense.
§ Group L+ subjects by smoking status—heavy smokers: no (n = 58); yes (n = 6).
¶ Group L− subjects by smoking status—heavy smokers: no (n = 403); yes (n = 56).
# p values are two sided.
** Tertile limits: 0.00–0.246, 0.247–0.440, and 0.441–3.298 μmol/liter.
†† Tertile I subjects by smoking status—heavy smokers: no (n = 141); yes (n = 33).
¶¶ The total analysis of covariance models included sex, as well as ECRHS I data for FEV₁, age, body mass index, smoking status (coded into four classes), and either the HO-1 polymorphism group or the ECRHS I β-carotene tertile.
§§ Tertile II + III subjects by smoking status—heavy smokers: no (n = 320); yes (n = 29).
### The total analysis of covariance models included sex, as well as ECRHS I data for FEV₁, age, body mass index, heavy smoking (coded into two classes), and either the HO-1 polymorphism group plus the interaction term (heavy smoking × group of HO-1 polymorphism) or the ECRHS I β-carotene tertile plus the interaction term (heavy smoking × ECRHS I β-carotene tertile).

Written, informed consent was obtained from each subject before inclusion, and the protocol of the ECRHS was approved by the French Ethics Committee for Human Research and also by the National Committee for Data Processing and Freedom.

RESULTS

Follow-up lasted on average for 8.5 (SD: 0.7) years. The mean annual FEV₁ decline was −29.8 (SD: 32.0) ml/year, the mean serum β-carotene level at baseline was 0.41 (SD: 0.32) μmol/liter, 6.5 percent of the 1,046 alleles were classified as long (L), and 64 subjects were in group L+ (12.2 percent).

As previously observed, heavy smokers in group L+ had a faster FEV₁ decline than did heavy smokers in group L− (table 1). Subjects with low β-carotene serum levels had an accelerated FEV₁ decline, compared with subjects with high β-carotene levels, especially for heavy smokers.

We next analyzed the combined effect of polymorphism in the promoter of the HO-1 gene and serum β-carotene levels on FEV₁ decline. Figure 1 shows that, for group L+, there was a faster FEV₁ decline in subjects with low β-carotene levels (tertile I) than in those with higher levels (tertiles II and III combined), with a mean of −58.8 (95 percent confidence interval [CI]: −73.2, −44.5) versus a mean of −25.4 (95 percent CI: −34.0, −16.7) ml/year (p = 0.0005). By contrast, in group L−, the difference in FEV₁ decline between those with low serum levels of β-carotene and those with higher levels was not significant, with a mean of −34.7 (95 percent CI: −38.9, −29.8) versus a mean of −28.7 (95 percent CI: −32.4, −24.9) ml/year (p = 0.21).

For subjects with high β-carotene levels, the FEV₁ decline was similar in group L+ and group L− (p = 0.9). In contrast, for subjects with low β-carotene levels (tertile I), the FEV₁ decline was significantly faster in group L+ than in group L− (p = 0.009).

Adjustment for pack-years in ECRH I (1992) or ECRHS II (2000) did not affect these findings (p = 0.003 for both). A similar pattern of results suggesting an interaction between the HO-1 polymorphism and the level of β-carotene was observed when heavy smokers were excluded from the analysis (pinteraction = 0.09). For subjects with low β-carotene levels, the FEV₁ decline was faster in group L+ than in group L−, with a mean of −43.4 (95 percent CI: −58.1, −28.7) versus a mean of −30.9 (95 percent CI: −35.8, −25.9) ml/year, although the difference did not reach significance (p = 0.38).

DISCUSSION

For the first time in a general population sample, the effects on FEV₁ decline of the interaction between two antioxidant systems (microsatellite polymorphism in the promoter of the HO-1 gene and serum β-carotene levels) have been investigated. We observed that a long allele in the HO-1 gene promoter, which may lead to weak protein expression and activity (3, 8), was associated with an accelerated decline of lung function; however, this relation was not detected in subjects with high serum β-carotene levels.
These results might be explained by β-carotene and bilirubin targeting the same type of oxidants (singlet oxygen or the superoxide anion, both of which are strongly oxidizing reactive oxygen species) (12, 13). When serum β-carotene levels are high enough to deal with oxidant burden, β-carotene may compensate for the lack of biliverdin/bilirubin due to low levels of HO-1 protein.

The high quality of the data has been established in our previous publications (4, 6). It is not possible to compare the long allele frequency in our population with those in other populations because, to our knowledge, the HO-1 microsatellite polymorphism has been described only in case-control studies. Levels of β-carotene in the four subgroups of subjects defined according to smoking status were similar to those reported in studies exploring relations between serum levels of antioxidants and either lung function (14–16) or smoking status (17). We tested baseline serum β-carotene levels and lung function at the same time. The validity of our findings depends in part on the assumption that the β-carotene levels determined represent usual, long-term, average levels. Diet is the sole source of serum β-carotene (18–20) and, indeed, serum β-carotene and more generally carotenoids may be considered as biomarkers of fruit and vegetable intakes (21). Serum concentration assays are likely to be better markers than dietary intakes assessed by questionnaires (22). Reported correlations between dietary intake and serum level ranged between 0.15 and 0.4 (23) and might appear modest. However, β-carotene is a precursor of vitamin A and, as a consequence, a part of the dietary intake cannot be found in the serum levels. Furthermore, serum β-carotene has been shown to decrease with increased smoking intensity (17). The average body mass index in analyzed ECRHS subjects was lower than the mean body mass index found in other French general population cohorts (24, 25). However, in these cohorts, subjects were older, and it is well known that body mass index rises with age (26). The percentage of the subjects included in our analyses who were smokers was similar to the World Health Organization estimations for adults aged ≥18 years (27) for men and slightly higher in the ECRHS for women. The mean annual FEV₁ decline in the ECRHS smokers was lower than the annual FEV₁ decline observed in the studies describing the natural history of lung function decline (28, 29). However, ECRHS smokers, and ECRHS heavy smokers in particular, were younger than the subjects in previous studies; this apparent discrepancy is thus consistent with the observation that the rate of loss seems to accelerate slightly with aging (28). Although a baseline FEV₁ percent predicted was

![FIGURE 1. Adjusted annual mean decline in forced expiratory volume in 1 second (FEV₁), with 95% confidence intervals, according to the HO-1 microsatellite polymorphism and serum β-carotene level, France, 1992 and 2000. Participants in the European Community Respiratory Health Survey (ECRHS) I (1992) and II (2000) were aged 20–44 years at baseline and recruited in the cities of Grenoble and Paris (n = 523). Results are shown for subjects with one or two long (L) alleles (≥33 GT repeats; group L⁺) (part A) and for those with no L allele (group L⁻) (part B). The total analysis of covariance model included sex, as well as baseline FEV₁, age, body mass index, smoking status (coded in four classes), tertile of β-carotene, group of HO-1 polymorphism, and the interaction term (tertile of β-carotene × group of HO-1 polymorphism) (pInteraction = 0.002). p values were two sided. group L⁺ indicates a genetic deficiency in antioxidant defense.](https://academic.oup.com/aje/article-abstract/167/2/139/127658)
higher than 100 percent, the mean annual FEV1 decline in L-allele carriers with low serum β-carotene levels may be clinically significant. Their mean annual FEV1 decline is double that reported in nonsmokers from very large studies (generally between −20 and −30 ml/year) (30–33). This is a substantial difference. Moreover, the Working Group on Action to Control Chemicals (WATCH) in the United Kingdom reported on the health significance of occupationally induced declines in FEV1 and indicated that a decline is clinically significant when greater than 50 ml (34).

Tobacco smoking is a major risk factor for FEV1 decline, which may be a confounder as well as an effect modifier. Its possible additional role in the interaction requires further consideration. When we first investigated differences in decline in FEV1 between carriers of long or short alleles in the promoter of the HO-1 gene (4), an association between an accelerated decline and the long allele in the HO-1 gene promoter was observed only in heavy smokers, who are most at risk of oxidative stress and, thus, of COPD. It therefore seemed likely that the interaction would be strengthened in the subjects of this smoking category. Unfortunately, the population analyzed in the present paper was not large enough to test for interaction between HO-1 and β-carotene levels in heavy smokers separately. However, in our first investigation of the differences in decline in FEV1 between subjects with low and high serum β-carotene levels (6), an association between an accelerated decline and low serum β-carotene levels was also observed in never smokers. Likewise, even after excluding heavy smokers or when considering only never smokers to discard any residual confounding by smoking (results not shown), our results suggested that, in subjects with low levels of β-carotene, carriers of long allele(s) had a steeper FEV1 decline than did noncarriers (exceeding −50 ml/year). These various results, although very preliminary, suggest that β-carotene might counterbalance a genetically determined deficiency in the HO-1 antioxidant response against “normal” levels of environmental or endogenous oxidants. It nevertheless remains unclear whether this counterbalance is exerted in cases of high oxidative stress (heavy smoking).

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Conflict of interest: none declared.

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