CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians

José A. G. Agúndez, Inmaculada Rodríguez, Manuel Olvera, José María Ladero, María A. García, José M. Ribera, Julio Benítez

Department of Pharmacology, School of Medicine, University of Extremadura, Badajoz, Spain
Services of Geriatrics and Gastroenterology of the San Carlos University Hospital, Medical School, Complutense University, Madrid, Spain

Address correspondence to: J. M. Ladero.

Abstract

Background: enzymatic polymorphisms affecting the metabolic disposition of xenobiotics may modulate the rate of activation or deactivation of carcinogens and other toxic environmental chemicals. Hence, these polymorphisms may influence the risk of suffering some types of cancer and other degenerative diseases that are incompatible with extreme longevity.

Aims: to establish the distribution of three well known enzymatic polymorphisms that affect the CYP2D6, NAT-2 and CYP2E1 genes and the activity of their enzymatic gene products, involved in the disposition of many xenobiotics, in a group of nonagenarians and in much younger controls.

Patients: the three genotypes were determined in 41 nonagenarians (10 males, mean age 92.2 years, range 90-98) free of known malignancies or neurodegenerative diseases. The control groups comprised 217 healthy volunteers (128 males, mean age 36.3 years; SD, 12.7) for the CYP2D6 and NAT2 genotypes and 137 (116 males, mean age 32 years; SD, 18.8) for the CYP2E1 genotype.

Methods: after extraction of DNA from white blood cells, polymerase chain reaction and restriction fragment polymorphism methods were used to identify the allelic variants of the three genotypes.

Results: we found no qualitative or quantitative difference in the mutations underlying the three genetic polymorphisms studied, nor in the expected enzymatic phenotypes. Instead, a close parallelism exists between advanced age and younger groups.

Conclusion: longevity does not seem to be related to any special configuration of these three polymorphic traits. Comparisons with younger controls may be adequate when studying the distribution of these polymorphisms in diseases affecting old people. No genetically determined differences in the activation of drugs metabolized by these enzymes are to be expected in very old people.

Keywords: longevity, CYP2D6, CYP2E1, NAT2, cytochrome P450, acetylator, polymorphisms

Introduction

The ageing process is largely multifactorial and many of these factors are under genetic control. Cardiovascular consequences of atherosclerosis and cancer are the two main causes of death in the post-reproductive period. Genetic polymorphisms affecting the structure or function of lipoproteins have been linked to accelerated atherosclerosis and hence to a shorter life expectancy. Modifications in the apo-AI-CIII-AIV gene cluster [1] may have such an effect; moreover, carriers of the e4 allele of apo E rarely reach advanced ages [2, 3] and show a higher risk of developing Alzheimer's disease [4]. Genes determining a moderate level of Lp(a) lipoprotein may be related to increased longevity [5].

Another possible link between genetic polymorphisms and longevity is the association of several forms of cancer to genetic polymorphisms of enzymes that metabolize carcinogens [6]. There are several genetic polymorphisms, inherited as single Mendelian traits, that affect the function of enzymes involved in the activation or deactivation of environmental carcinogens. The three polymorphisms studied here have been linked to the risk of developing some common types of cancer [6-9] and CYP2D6 is also linked to the risk of early-onset Parkinson's disease [10]. We have studied the distribution of these enzymatic polymorphisms in a group of nonagenarians to see if there are any differences between this group and younger people that would suggest a role in determining human longevity.
Patients and methods

Forty-one patients over 90 (10 males, mean age 92.2 years, range 90–98) in good general health, who were free of known malignancies or neurodegenerative diseases, were recruited from the San Carlos University Hospital's Geriatric Unit.

The control group for NAT2 and CYP2D6 polymorphisms was 217 healthy subjects (128 males, mean age 36.3 years; SD, 12.7). The control group for CYP2E1 genotype included 137 healthy volunteers (116 males, mean age 32 years; SD, 18.4). All control subjects were of the same ethnic (white Spanish) and geographic (the central area of Spain) origin.

The study was approved by the ethics committee of the San Carlos University Hospital (Madrid), under the guidelines of the 1975 Declaration of Helsinki. Patients and controls gave informed consent to be included in the study.

Venous blood samples (20 ml) were obtained by venepuncture and anticoagulated in sterile glass tubes containing sodium citrate or ethylendiaminetetraacetic acid. The blood was transferred to sterile plastic vials and kept at -80°C until use. Genomic DNA samples were isolated from leucocytes as described elsewhere [11]. The analysis of the acetylator genotype was performed by using allele-specific polymerase chain reaction (PCR) amplification [12]. The identification of the CYP2D6 genotype was performed by the combined use of mutation-specific PCR and XbaI and EcoR1 restriction fragment polymorphism (RFLP) analyses as described elsewhere [13]. The analysis for the RsaI polymorphism at the 5'-flanking region of CYP2E1 was carried out by an amplification-restriction procedure [14] with minor modifications.

Intergroup comparisons were made using two-tailed Fisher exact or Mantel-Haenszel tests, as appropriate. Calculations were made by using the Epilnfo6 statistical software. The null hypothesis was rejected when \( P < 0.05 \).

Results

The prevalence of allelic variants (Figure 1), as well as the distribution of acetylator genotypes (Table 1) and therefore the predicted acetylator phenotypes, were very similar in both groups. Figure 2 and Table 2 show the distribution of CYP2D6 allelic variants and genotypes, respectively. There were no poor metabolizers in the study group and only eight (3.7%) in the control group. Although these differences were non-significant, we have calculated the expected prevalence of poor metabolizers (homozygous for defective CYP2D6 alleles), according Hardy-Weinberg's equilibrium for the prevalence of defect alleles, in both groups. According to this, the expected prevalence for poor metabolizers is 2.5% for cases and 2% for controls. These data are not significantly different from each other and from the actual values shown in the Table 2.

Table 3 shows the distribution of the RsaI polymorphisms of the gene CYP2E1. Again, no significant differences, but a narrow parallelism, was found.

Discussion

These results discount any relevant influence of CYP2D6, NAT2 or CYP2E1 genotypes on the probability of reaching an advanced age, at least in the Spanish population. We have performed this study after detecting a significant excess of homozygote CYP2D6 extensive metabolizers (EM) and a sixfold excess of the allele CYP2D6(C) in lung cancer patients [15], a twofold excess of the CYP2D6(B) allele in women with breast cancer [16], a marked excess of

Table 1. Distribution of acetylator genotypes

<table>
<thead>
<tr>
<th>No. of functioning alleles</th>
<th>No. (and %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
</tr>
<tr>
<td>0 (slow acetylators)</td>
<td>22 (53.7)</td>
</tr>
<tr>
<td>1 (heterozygote rapid acetylators)</td>
<td>16 (39.0)</td>
</tr>
<tr>
<td>2 (homozygote rapid acetylators)</td>
<td>3 (7.3)</td>
</tr>
</tbody>
</table>
Genetic polymorphisms in nonagenarians

Table 2. Distribution of \textit{CYP2D6} genotypes and phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>22 (53.7)</td>
<td>138 (63.6)</td>
</tr>
<tr>
<td>wt/A</td>
<td>0</td>
<td>5 (2.3)</td>
</tr>
<tr>
<td>wt/B</td>
<td>10 (24.4)</td>
<td>37 (17.1)</td>
</tr>
<tr>
<td>wt/C</td>
<td>3 (7.3)</td>
<td>8 (3.7)</td>
</tr>
<tr>
<td>wt/D</td>
<td>1 (2.4)</td>
<td>5 (2.3)</td>
</tr>
<tr>
<td>wt/L</td>
<td>0</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>wt/L2</td>
<td>2 (4.9)</td>
<td>14 (6.5)</td>
</tr>
<tr>
<td>B/B</td>
<td>0</td>
<td>6 (2.8)</td>
</tr>
<tr>
<td>B/C</td>
<td>1 (2.4)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>B/D</td>
<td>2 (4.9)</td>
<td>6 (2.8)</td>
</tr>
<tr>
<td>C/C</td>
<td>0</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>D/L2*</td>
<td>2 (4.9)</td>
<td>14 (6.5)</td>
</tr>
</tbody>
</table>

Table 3. Distribution of \textit{CYP2E1-RsaI} restriction fragment length polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (and %)</th>
<th>Controls</th>
<th>No. (and %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c1c1</td>
<td>39 (95.1)</td>
<td>130 (94.6)</td>
<td></td>
</tr>
<tr>
<td>c1c2</td>
<td>2 (4.9)</td>
<td>7 (5.4)</td>
<td></td>
</tr>
</tbody>
</table>

The \textit{CYP2E1} gene, which codes the synthesis of P4502E1, is affected by several polymorphisms at non-coding regions of the gene locus [20]. However, one of these polymorphisms is located at a regulatory site in the 5' flanking region, in a putative binding region for the transcription factor HNF-1 [7] and probably influences the functional rate of the enzyme [7]. P4502E1 is the main isozyme of the P450 cytochrome oxidase system involved in the metabolism of ethanol and of many other xenobiotics, including some carcinogens [8]. At present, conclusive data on a relationship between \textit{CYP2E1-RsaI} polymorphism and risk of any severe disease have only been shown in patients with liver cancer who were ethanol-abusers [9]. It has been suggested that this polymorphism could modulate the risk of liver cirrhosis in ethanol abusers [21, 22], but this has not been confirmed by us [23].

Taken together, these data suggest that specific combinations of enzymatic polymorphisms could influence the risk of developing some forms of cancer. Nevertheless, patients in this study were not smokers and were not affected by chronic liver disease, that are usually necessary for the development of lung and liver cancers, respectively. Smoking and drinking habits or chronic liver disease are usually incompatible with extreme longevity due to the high risk of death at an earlier age caused by diseases not related to these polymorphisms (atherosclerosis, chronic bronchitis, extrahepatic organic sequelae of alcohol abuse, etc.). The findings obtained in this comparative study make the selection of subjects for control groups easier for studies on diseases affecting people older than 50, as young healthy people are easier to find and recruit.

Another point raised by this study is that age-related differences in drug response and toxicity cannot be attributed to a different prevalence of functional genes of the studied drug-metabolizing enzymes, namely \textit{CYP2D6}, \textit{CYP2E1} and \textit{NAT2}, in very aged people. The genes studied here encode enzymes which are responsible for the metabolism of several drugs. These include tricyclic antidepressants, neuroleptics, beta-blockers and other antiarrhythmics (\textit{CYP2D6}); paracetamol, chloroxazone and ethanol (\textit{CYP2E1}) and hydrazines, aryamines, pyrazolones, caffeine and...
nitrazepam (NAT2) among others. Any genetic selection in older people would determine differences in the metabolism of these drugs, as compared with younger subjects.

Our findings, showing identical prevalence of mutations and, more importantly, identical prevalence of functional genes in nonagenarians and in younger controls, indicate that, in the drugs metabolized by such enzymes, pharmacokinetic differences due to decreased metabolism should be attributed to decreased liver blood flow rather than a genetic selection in very old people. Pharmacodynamic differences between elderly people and younger subjects, due to differences in tissue drug metabolism, should not be expected with drugs that are metabolized by these enzymes.

Key points
- There are no specific combinations of CYP2D6, CYP2E1 and NAT2 genotypes associated with extreme longevity. The distribution of these genotypes is similar in healthy very old people to that in the general population.
- Other factors, not only genetic but also related to lifestyle, determine the chance of reaching an advanced age.
- It is reasonable to use younger control subjects when studying the distribution of these genetic polymorphisms in diseases affecting older age groups.

Acknowledgements
We thank Mr Luis Lozano for excellent technical assistance. Supported in part by Grants CICYT-SAF92-0333 from Comisión Interministerial de Ciencia y Tecnología (Madrid) and FLIs 93/0632 and 94/0326 from Fondo de Investigaciones sanitarias de la Seguridad Social (Madrid). This study was carried out in coordination with COST B1-phase III.

References
Genetic polymorphisms in nonagenarians


Received in revised form 2 October 1996