Biochemical and Biophysical Research Communications **280,** 1389–1392 (2001)
doi:10.1006/bbrc.2001.4280, available online at http://www.idealibrary.com on IDEAL®

# Polymorphisms of Drug-Metabolizing Enzymes in Healthy Nonagenarians and Centenarians: Difference at GSTT1 Locus

Emanuela Taioli,*[,1] Daniela Mari,† Claudio Franceschi,‡ Massimiliano Bonafè,§ Daniela Monti,[¶] Stefano Bertolini,‖ Daniela Marinelli,** and Seymour Garte**

**Laboratorio Epidemiologico, Ospedale Policlinico IRCCS, Milan, Italy; †Institute of Internal Medicine, University of Milan, Milan, Italy; ‡Italian National Research Centers on Aging, INRCA, Ancona, Italy; §Department of Experimental Pathology, University of Bologna, Bologna, Italy; ¶Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy; ‖Atherosclerosis Prevention Centre, Department of Internal Medicine, University of Genoa, Genoa, Italy; and **Genetics Research Insitute, Strada della Carità 10, 20135 Milan, Italy*

Received January 5, 2001

**Drug metabolizing enzymes are involved in the detoxification of several drugs, environmental substances, and carcinogenic compounds, and their polymorphisms have been associated with risk for a variety of cancer. In this paper, we compared the frequency of polymorphisms in cytochrome P450-1A1 gene (CYP1A1), a phase 1 gene (oxidation, activation), and of two polymorphisms of glutathione *S*-transferase enzymes (GSTM1, GSTT1), two phase 2 genes (conjugation, detoxification). Two groups were studied and compared, i.e., 94 nonagenarians and centenarians and 418 control subjects of younger age. A significant difference in the proportion of nonagenarians and centenarians homozygotes for a GSTT1 deletion (28%) was observed in comparison to control subjects (19%, $P = 0.03$). The distribution of the other gene polymorphisms did not differ in the two groups. These findings on phase 2 drug-metabolizing enzyme gene polymorphisms may help in disentangling gene–environmental interactions which can have a role in successful aging and longevity, as well as in cancer incidence in the oldest old.** 

***Key Words:*** **ageing; longevity; centenarians; genetics of ageing; genetics of longevity; cancer; cancer and ageing; environmental carcinogens.**

Human longevity can be regarded as a multifactorial trait, highly dependent on the interaction between genetic and environmental factors.

A significant proportion of centenarians are in good health (category A and B, as defined by Franceschi *et al.*, 2000) either from a physical or a cognitive point of view (1). They are the best example of successful aging, and represent a valuable model for studying the genetic factors impinging on survival (risk factors for cancer and other major age-related diseases) (2). In fact, some risk factors for age-related diseases are hyporepresented in centenarians (3), whilst others are unexpectedly present in healthy centenarians at equal or even increased frequency, in comparison to controls (4–7). Nevertheless, a number of studies revealed that in centenarians important changes occur in genetic variability of loci which are not risk factors for specific diseases, but are rather responsible for modulating the individual capacity to cope with stress, such as Tyrosine Hydroxilase and mitochondrial DNA (8, 9). In this scenario, enzymes which are involved in the metabolism of potentially dangerous endogenous and exogenous compounds, are likely to play a major role in human longevity. A variety of polymorphisms have been reported in these genes, and have been associated with susceptibility to cancer (10) and other age-related diseases such as Parkinson disease (11). Among these candidate genes, there are Phase 1 (oxidation, activation) and Phase 2 (conjugation, detoxification) drug metabolizing genes, such as cytochrome P450 genes and glutathione *S*-transferase enzymes (GST). Cytochrome P450 genes are involved in the first step of the metabolism of polycyclic aromatic hydrocarbons contained in tobacco smoke, whilst GST genes are involved in the detoxification of several drugs, environmental substances, and carcinogenic compounds. In particular, common polymorphisms leading to complete deletion of the gene cause a loss of GST functional activity. Individuals with such gene deletions exhibit decreased conjugation activity, and are consequently exposed to higher internal doses of potentially toxic intermediary

[1] To whom correspondence should be addressed at Laboratorio Epidemiologico, Ospedale Policlinico IRCCS, Via F. Sforza 28, 20122 Milano, Italy. Fax: +39 02 55038207. E-mail: sgen@iol.it.



0006-291X/01 $35.00

metabolites from xenobiotic agents. Data on the frequency of polymorphisms in some phase 1 genes (CYP2E1 (12), CYP2C19 (14), CYP2D6 (13, 14)) and phase 2 genes (NAT2 (13, 14), GSTM1 (13)), as well as other polymorphisms associated with cancer susceptibility, i.e., p53 (15, 16), did not show any difference between centenarians and the general population. In this study, we focussed our attention on other members of phase 1 gene family (CYP1A1), and phase 2 genes (GSTT1 and GSTP1) never studied before in centenarians, as well as on a gene which has been studied in French centenarians with negative results (GSTM1) (13). The hypothesis was that the frequency of the least favorable polymorphisms should be lower in centenarians than that observed in the general population.

## MATERIALS AND METHODS

Nonagenarians and centenarians in good health, i.e., belonging to category A and B according to Franceschi *et al.* (1) were identified in Northern Italy (Milan, Modena, Parma, Genoa), as part of an ongoing multicentric study in Italy with the aim of studying the mechanisms responsible for successful aging (1). A total of 94 subjects were included in the present study. The mean age of the group was 100.2 ± 2.1 years (range 95–105 years; median 100 years), 22% of whom were males and 88% females. The male to female ratio was in agreement with the recent data on the nation-wide study on Italian centenarians (1). None was a current smoker. In the rest of the present paper, for simplicity, these very old people are collectively indicated as "centenarians".

Control subjects were a sample of 418 healthy volunteers of the same ethnic group as the nonagenarians and centenarians, recruited in Northern Italy to study the geographic distribution of drug-metabolizing enzymes polymorphisms. They were recruited among blood donors ($n$ = 98), or health screening programs ($n$ = 320). All the subjects gave informed consent to participate in the study. The mean age of the control subjects was 46.0 ± 11.3 years (range 4–82 years; median 47 years), 46% males and 54% females. As by study design, sex ratio was significantly different in the two groups.

Genomic DNA was extracted from peripheral blood lymphocytes, and polymorphisms were assessed by PCR method, as previously described (17, 18). A multiplex PCR method was used to detect the presence or absence of the GSTM1 and GSTT1 genes in genomic DNA samples. This method uses both GST primer sets (GSTM1, 5′ GAACTCCCTGAAAAGCTAAAGC 5′ GTTGGGCTCAAATATACGGTGG; and GSTT1, 5′ TCCTTACTG GTCCTCACATCTC, 5′ TCACCGGATCATGGCCAGCA) in the same PCR and includes a third primer set for albumin (5′ GCCCTCTGCTAACAAGTCCTAC, 5′ GCCCTAAAAAGAAAATCGCCAATC), and uses 30 cycles with denaturing at 94°C for 1 min, annealing at 64°C for 1 min, and extension at 72°C for 1 min. For analysis of CYP1A1 the following primers were used: 5′ TTAGGAGTCTTGTCTCATGCCT and 3′ CAGTGAAGAGGTGTAGCCGCT for analysis of the Msp1 RFLP using PCR conditions as previously described (17).

Nomenclature used: for CYP1A1, the term "wild type" refers to the homozygotes for the common allele (absence of the restriction site), "heterozygous" refers to the presence of the polymorphism on one allele, "homozygous" refers to the presence of the restriction site on both alleles. For GSTM1 and GSTT1, "null" refers to homozygotes for deletion allele, while "present" refers to both homozygotes and heterozygotes (which cannot be distinguished by the experimental procedure).

**TABLE 1**

Genotype Frequency of Drug-Metabolizing Gene Polymorphisms in Centenarians and Younger Subjects

| | | Centenarians ($n$ = 94) | Control subjects ($n$ = 418) |
|---|---|---|---|
| Genes | Genotypes | $n$ (%) | $n$ (%) |
| CYP1A1 | Wild type[a] | 70 (77)[b] | 328 (79) |
| | Heterozygous | 20 (22) | 81 (19) |
| | Homozygous | 1 (1) | 9 (2) |
| GSTM1 | Null[c] | 47 (53) | 211 (51) |
| | Present | 41 (47) | 198 (49) |
| GSTT1 | Null[c] | 25 (28) | 76 (19) |
| | Present | 63 (72) | 330 (82) |

*Note.* Statistical analysis: CYP1A1, $\chi^2 = 0.94$, $P = 0.68$; GSTM1, $\chi^2 = 0.10$, $P = 0.74$; GSTT1, $\chi^2 = 4.18$, $P = 0.03$.

[a] "Wild type" refers to the homozygotes for the common allele (absence of the restriction site polymorphism), heterozygous refers to the presence of the polymorphism on one allele, homozygous refers to the presence of the polymorphism on both alleles.

[b] Totals may vary due to missing values.

[c] "Null" refers to homozygotes for deletion allele, while "Present" refers to both homozygotes and heterozygotes which cannot be experimentally distinguished.

### *Statistical Analysis*

Chi-square was used to compare the frequency of each polymorphism and of the combined genotype, in centenarians and in control subjects. Monte Carlo simulation was used (19), to take into account for cells with small numbers. Data were adjusted for sex, in order to control for a possible confounding factor, by multiple logistic regression, using the SAS package version 6.12. Linear regression analysis was used to test the association between age and each genotype.

## RESULTS

The frequency of the polymorphisms analyzed in this study is reported in Table 1.

A significant difference in the proportion of centenarians homozygotes for GSTT1 deletion was observed, in comparison to younger subjects (28% vs 19%: $\chi^2 = 4.18$; $P = 0.03$). Linear regression analysis confirmed the independent association between age and GSTT1 deletion ($F = 4.61$, $P = 0.032$). When the analysis was restricted to control subjects below the age of 60 years ($n$ = 386), the results did not change (data not shown). The distribution of the other gene polymorphisms did not differ in the two groups (CYP1A1*2A: $\chi^2 = 0.94$, $P = 0.68$; GSTM1: $\chi^2 = 0.10$, $P = 0.74$). When the multiloci genotypes for CYP1A1, GSTM1, and GSTT1 were examined (Table 2), a statistical borderline difference was observed between centenarians and control subjects ($\chi^2 = 15.67$; $P = 0.079$). The difference was mainly due to the CYP1A1-GSTM1-GSTT1 combinations 1-0-2 and 1-2-2 (Table 2), both containing the combination of CYP1A1 heterozygotes and GSTT1 deletion genotypes (8% in centenarians vs

**TABLE 2**

Multilocus (CYP1A1-GSTM-GSTT1) Genotypes in 87 Centenarians and 401 Control Subjects

| CYP1A1* | GSTM | GSTT1 | Centenarians *n* (%) | Control subjects *n* (%) |
|---|---|---|---|---|
| 0 | 0 | 0 | 21 (24) | 116 (29) |
| 1 | 0 | 0 | 6 (7) | 34 (8) |
| 2 | 0 | 0 | 0 (0) | 2 (0.5) |
| 0 | 2 | 0 | 27 (31) | 136 (34) |
| 1 | 2 | 0 | 7 (8) | 35 (9) |
| 2 | 2 | 0 | 1 (1) | 6 (1) |
| 0 | 0 | 2 | 10 (12) | 39 (9) |
| 1 | 0 | 2 | 4 (5) | 3 (1) |
| 0 | 2 | 2 | 8 (9) | 28 (7) |
| 1 | 2 | 2 | 3 (3) | 2 (0.5) |

*Note.* Statistical analysis: centenarians vs control subjects, $\chi^2 = 15.67$; $P = 0.079$. Only subjects in whom all the genes were tested are reported.

* Coding: CYP1A1, 0 = homozygotes for the common allele; 1 = presence of the polymorphism on one allele, 2 = presence of the polymorphism on both alleles. GSTM1 and GSTT1, 0 = homozygotes and heterozygotes for the common allele (which cannot be distinguished by experimental procedures); 2 = homozygous deletion of the allele.

1.5% in control subjects). According to the strong interaction between CYP1A1 and GSTT1, a significant difference in GSTT1 distribution between centenarians and control subjects occurred in CYP1A1 heterozygotes ($\chi^2 = 11.278$; $P = 0.003$), but not in CYP1A1 wild type genotypes ($\chi^2 = 1.249$; $P = 0.26$).

The frequency of the GSTP1 polymorphism did not show any difference between centenarians and control subjects (data not shown).

## DISCUSSION

In this study we confirm the lack of association between GSTM1 and longevity, as previously described in a French population of centenarians (3), and we report new data for two polymorphisms in drug metabolizing enzymes which have never been tested in centenarians, namely CYP1A1 and GSTT1. We did not find any difference in the frequency of CYP1A1 in centenarians, as compared to control subjects of younger age. A significant increase in the GSTT1 homozygous deletion in centenarians was observed, and this is the first report of a significant difference regarding drug metabolizing enzyme gene polymorphisms in centenarians. This finding is rather unexpected, as the deletion of the GST genes is considered a risk factor for cancer, causing the lack of conjugation of toxic compounds of environmental origin. Accordingly, a decrease in frequency of the GSTT1 deletion in centenarians would be expected. However, this finding is not totally surprising and it is not the first description of an increased risk factor in centenarians. Indeed, an increased frequency of alleles and genotypes conferring susceptibility to cardiovascular diseases and thrombosis, such as PAI-1 and ACE, have been reported in centenarians (4, 7, 20). In order to explain this paradox, which emerged from the research on centenarians, we have proposed a mathematical model suggesting that the over-crossing of the mortality curves at certain ages can explain these phenomena (21, 22). This model assumes that a genetic risk factor can confer an increased risk to die until a certain age, above which its impact is nullified or reversed. This phenomenon may occur because those people who survived at very advanced ages have been selected by mortality forces, and thus could benefit from those genetic variants which are detrimental for younger people. For example, a profile predisposing to hypercoagulability could be beneficial at very advanced ages, but could predispose to cardiovascular diseases in the middle aged people (4, 20). Accordingly, the lack of GSTT1 (or other phase 2 genes) could be related to deleterious effects in young age (high risk of cancer) but it could exert long term beneficial effects on survival, by avoiding the catabolism of important substances, which have chemopreventive effect on cancer and other diseases. Indeed, a recent study, shows that among subjects consuming a diet rich in isothiocyanates (a lung carcinogen inhibitor), those with deletion of GSTM1 and/or GSTT1 have higher levels of this compound in the urine than subjects with the wild type genotype (23). Another possibility is that the association between GSTT1 locus and longevity regards indeed a proximate locus of unknown nature whose variants are in linkage disequilibrium with the one we studied. Further studies are needed to clarify this point.

In conclusion, GSTT1 can be added to the list of candidate longevity genes which have been identified so far, and it can open a new perspective on a possible "pharmacogenetics of longevity".

## ACKNOWLEDGMENTS

This work was supported by grants from AIRC "Healthy centenarians as a model to study genetic and cellular factors involved in cancer susceptibility", University of Bologna ex-quota 60%, M.U.R.S.T. Projects "Genetic determinants of human longevity" and "Genetic factors involved in human aging and longevity", Ministry of Health Project "Marcatori genetici e biologici di invecchiamento normale e patologico" to C.F., and M.U.R.S.T. Project "Immunogenetics of Longevity" to D. Monti.

## REFERENCES

1. Franceschi, C., Motta, L., Valensin, S., Rapisarda, R., Franzone, A., Berardelli, M., Motta, M., Monti, D., Bonafé, M., Ferrucci, L., Deiana, L., Pes, G., Carru, C., Desole, S., Barbi, C., Gemelli, C., Lescai, F., Olivieri, F., Marchegiani, F., Cardelli, M., Cavallone, L., Gueresi, P., Cossarizza, A., Troiano, L., Pini. G., Sansoni, P., Passeri, G., Lisa, R., Spazzafumo, L., Amadio, L., Giunta, S., Stecconi, R., Morresi, R., Viticchi, C., Mattace, R., De Benedictis,

G., Baggio, G., and the Italian Multicenter Study on Centenarians (IMUSCE). (2000) Do men and women follow different trajectories to reach extreme longevity? *Aging Clin. Exp. Res.* **12,** 77–84,

2. Heijmans, B. T., Westendorp, R. G., and Slagboom, P. E. (2000) Common gene variants, mortality and extreme longevity in humans. *Exp. Gerontol.* **35,** 865–877
3. Gerdes, L. U., Jeune, B., Ranberg, K. A., Nybo, H., and Vaupel, J. W. (2000) Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: Apolipoprotein E gene is a "frailty gene," not a "longevity gene". *Genet. Epidemiol.* **19,** 202–210.
4. Mannucci, P. M., Mari. D., Merati, G., Peyvandi, F., Tagliabue, L., Sacchi, E., Taioli, E., Sansoni, P., Bertolini, S., and Franceschi, C. (1997) Gene polymorphisms predicting high plasma levels of coagulation and fibrinolysis proteins. A study in centenarians. *Arterioscler. Thromb. Vasc. Biol.* **17,** 755–759.
5. Mari, D., Mannucci, P. M., Duca, F., Bertolini, S., and Franceschi, C. (1996) Mutant factor V (Arg506Gln) in healthy centenarians. *Lancet* **347,** 1044.
6. Sacchi, E., Duca, F., Franceschi, C., and Mari, D. (1999) Prothrombin gene mutation (G20210A) in healthy Centenarians. *Thromb. Haemost.* **81,** 990–991.
7. Schachter, F., Faure-Delaneff, L., Guenot, F., Rouger, H., Froguel, P., Lesueur-Ginot, L., and Cohen, D. (1994) Genetic associations with human longevity at the APOE and ACE loci. *Nat. Genet.* **6,** 29–32.
8. De Benedictis, G., Carotenuto, L., Carrieri, G., De Luca, M., Falcone, E., Rose, G., Cavalcanti, S., Corsonello, F., Feraco, E., Baggio, G., Bertolini, S., Mari, D., Mattace, R., Yashin, A. I., Bonafe, M., and Franceschi, C., (1998) Gene/longevity association studies at four autosomal loci (REN, THO, PARP, SOD2). *Eur. J. Hum. Genet.* **6,** 534–541.
9. De Benedictis, G., Rose, G., Carrieri, G., De Luca, M., Falcone, E., Passarino, G., Bonafè, M., Monti, D., Baggio, G., Bertolini, S., Mari, D., Mattace, R., and Franceschi, C. (1999) Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans *FASEB J.* **13,** 1532–1536.
10. d'Errico, A., Taioli, E., Xhen, X., and Vineis, P. (1996) Genetic metabolic polymorphisms and the risk of cancer: A review of the literature. *Biomarkers* **1,** 149–73.
11. Tan, E. K. (2000) Variability and validity of polymorphism association studies in Parkinson's disease. *Neurology.* **55**(4), 533–538.
12. Agundez, J. A. G., Rodriguez, I., Olivera, M., Ladero, J. M., Garcia, M. A., Ribera, J. M., and Benitez, J. (1997) CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians. *Age Ageing* **26,** 147–151.
13. Muiras, M-L., Verasdonck, P., Cottet, F., and Schachter, F. (1998) Lack of association between human longevity and genetic polymorphisms in drug-metabolizing enzymes at the NAT2, GSTM1, and CYP2D6 loci. *Hum. Genet.* **102,** 526–532.
14. Bathum, L., Andersen-Ranberg, K., Boldsen, J., Brosen, K., and Jeune, B. (1998) Genotypes for the cytochrome P450 enzymes CYP2D6 and CYP2C19 in human longevity. *Eur. J. Clin. Pharmacol.* **54,** 427–430.
15. Bonafé, M., Olivieri, F., Mari, D., Baggio, D., Mattace, R., Sansoni, P., De Benedictis, G., De Luca, M., Bertolini, S,, Barbi, C., Monti, D., and Franceschi, C. (1999) P53 variants predisposing to cancer are present in healthy centenarians. *Am. J. Hum. Genet.* **64,** 292–295.
16. Bonafè, M., Olivieri, F., Mari, D., Baggio, G., Mattace. R., Berardelli, M., Sansoni, P., De Benedictis, G., De Luca, M., Marchegiani, F., Cavallone, L., Cardelli, M., Giovagnetti, S., Ferrucci, L., Amadio, L., Lisa, R., Tucci, M. G., Troiano, L., Pini, G., Guresi, P., Morellini, M., Sorbi, S., Passeri, G., Barbi, C., Valensin, S., Monti, D., Deiana, L., Pes, G. M., Carru, C., and Franceschi, C. (1999) p53 Codon 72 polymorphism and longevity: Additional data on centenarians from continental Italy and Sardinia. *Am. J. Hum. Genet.* **65,** 1782–1785.
17. Taioli, E., Ford, J., Trachman, J., Li, Y., Demopoulos, R., and Garte, S. (1998) Lung cancer risk and CYP1A1 genotype in African-Americans. *Carcinogenesis* **19,** 813–817.
18. Kelsey, K. T., Spitz, M. R., Zuo, Z. F., and Wiencke, J. K. (1997) Polymorphisms in the glutathione *S*-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, US). *Cancer Cause Control* **8,** 554–559.
19. Roff, D. A., and Bentzen, P. (1989) The statistical analysis of mitocondrial DNA polymorphisms: Chi square and the problem of small samples. *Mol. Biol. Evol.* **6,** 539–545.
20. Bladbjerg, E. M., Andersen-Ranberg, K., de Maat, M. P., Kristensen, S. R., Jeune, B., Gram, J., and Jespersen, J. (1999) Longevity is independent of common variations in genes associated with cardiovascular risk. *Thromb. Haemost.* **82**(3), 1100–105.
21. Yashin, A. I., De Benedictis, G., Vaupel, J. W., Tan, Q., Andreev, K. F., Iachine, I. A., Bonafe, M., De Luca, M., Valensin, S., Carotenuto, L, and Franceschi, C. (1999) Genes, demography, and life span: The contribution of demographic data ingenetic studies on aging and longevity. *Am. J. Hum. Genet.* **65**(4), 1178–1193.
22. De Benedictis, G., Carotenuto, L, Carrieri, G., De Luca, M., Falcone, E., Rose, G., Yashin, A. I., Bonafe, M., and Franceschi, C. (1998) Age-related changes of the 3′APOB-VNTR genotype pool in ageing cohorts. *Ann. Hum. Genet.* **62,** 115–22.
23. London, S..J., Yuan, J-M., Chung, F-L., Gao, Y-T., Coetzee, G. A., Ross, R. K., and Yu, M. C. (2000) Diet-gene interaction in relation to lung cancer: Prospective study of isothiocyanates and glutathione S-transferase M1 and T1 genetic polymorphisms among middle-aged men in Shanghai, China. *Proc. Am. Assoc. Cancer Res.* **41** (n. 3516), 552.