Letters to the Editor

Am. J. Hum. Genet. 65:1782, 1999

p53 Codon 72 Polymorphism and Longevity: Additional Data on Centenarians from Continental Italy and Sardinia

To the Editor:

An extensive literature points out that p53 plays a pivotal role in cell cycle, apoptosis, and cell senescence and thus is crucial to a variety of physiological and pathological processes. These data support the view that p53 could be considered a gene that affects human longevity. In a previous letter (Bonafè et al. 1999) we tested the hypothesis that polymorphic variants of p53 have an impact on human longevity, by comparing p53 codon 72 allelic and genotypic frequency distributions between young people and centenarians. A nonsignificant difference emerged between the groups, and several explanations were offered. Following the reply letter of Sun et al. (in this issue), we would like to argue with some of their comments and to provide new data regarding centenarians from continental Italy and Sardinia. We disagree with the conclusion of Sun et al. that a consensus has been reached in the scientific community about the lack of any important role of p53 codon 72 in cancer susceptibility. First, several articles (not quoted by Sun et al.) still claim associations between codon 72 variants and lung cancer (Murata et al. 1998; Wang et al. 1999) and hepatocellular carcinoma (Yu et al. 1999). Second, in some cases, marginal differences regarding allelic and genotypic distributions between cases and controls have been found. In particular, Weston et al. (1997) reported *P* values of .054 and .07 when codon 72 Arg/Pro alleles and genotypes, respectively, were compared between Caucasian controls and patients affected with breast cancer. These authors failed to detect any differences in Mexican Americans or African Americans by use of case-control comparisons. These results cannot be considered conclusive evidence against an association, because the sample sizes analyzed were small (65 patients vs. 117 controls for Caucasians; 18 cases vs. 38 controls for Hispanics; 16 cases vs. 30 controls for African Americans). We doubt that samples of such size can be considered representative of Caucasian, Mexican, or African populations. Third, in several studies, important interactions among the codon 72 polymorphism and age (Murata et al. 1998), sex (Wang et al. 1999), environment, and other gene variants (Yu et al. 1999) have been reported. We tried to model this complex scenario (Bonafè et al. 1999), and methodologically new possible approaches have been proposed (De Benedictis et al. 1998; Yashin et al. 1998, 1999). Using experimental data on ApoB alleles in Italian subjects of different ages, including centenarians, we showed that the genotype relative risk (RR) of death can change with age, thus giving rise to unexpected nonmonotonic age-related trajectories. Comparable approaches to unraveling the possible effect of antagonistic pleiotropy on age-related genotypic changes during aging have been proposed (Toupance et al. 1997), with data on ACE genotypes in French centenarians. On the whole, unexpected results can be obtained by studying the genetics of centenarians. An understanding of the biological meaning of allele and genotype frequency distributions of centenarians requires an integrated approach that combines genetic and demographic data (Yashin et al. 1998, 1999). Calculations such as those proposed by Sun et al. may be inadequate, given that changes, with age, of RR for cancer (and death) have been reported for p53 variants (Weston et al. 1997). We disagree with the assumption that an RR of dying of 1.5–2 can be attributed to people carrying particular p53 codon 72 alleles. Sun et al. first strongly argue in favor of the hypothesis that codon 72 alleles are neutral, but then they assume this specific RR for Pro-allele carriers ("even though we do not agree there is a good evidence for an association between Codon 72 and cancer susceptibility, let us assume"). Because the power analysis performed by Sun et al. is based on this arbitrary assumption, their conclusions do not invalidate our interpretation of the data that we obtained in our study on the frequency of p53 variants in centenarians. We disagree with the conclusion of Sun et al. that the Pro/Arg sequence difference is functionally neutral. Recently, it has been demonstrated that codon 72 alleles differ biochemically and biologically in their ability to bind components of the transcriptional machinery, to activate transcription, to induce apoptosis, and to repress the transformation of primary cells (Thomas et al. 1999). We agree that haplotypes of codon 72 (*Bst*UI, exon 4), *Msp*I site (intron 6), and 16-bp insertion/deletion (intron 3) can help to

detect associations in case-control studies regarding p53 polymorphisms and cancer susceptibility, but we disagree that the data available in the literature are conclusive. Indeed, in the three molecular epidemiology studies (Sjalander et al. 1996; Wang-Gohrke et al. 1998; Weston 1998 et al.) quoted by Sun et al. as proof of the involvement of a peculiar p53 haplotype in human cancer (breast), some inconsistencies should suggest a more cautious approach in the interpretation of the results. In particular, in the first study (Sjalander et al. 1996), the control sample was composed of a heterogeneous group of placental samples and blood samples from sex- and age-matched women. Moreover, a deficiency of heterozygotes and a slight deviation from Hardy-Weinberg equilibrium were found in the control group. These facts affect the reliability of p53 haplotype estimations. As stated by Sjalander et al., these data on genotypic combinations could "provide some idea" but did not offer definitive results concerning the role of a particular three-locus p53 haplotype in cancer susceptibility. In the second study (Wang-Gohrke et al. 1997), the results appear to be quite blunt (the *Bst*UI–16-bp–*Msp*I haplotype 1-2-1 increases in cases from .108 to .137, the 2-2-1 from 0 to .012) and apparently contradictory. Indeed, a significant difference between cases and controls, with regard to the 16-bp–*Msp*I 2-1 haplotype, appears to be present when a three-locus analysis is performed, but the same difference is not confirmed when a two-locus analysis is used. Thus, the biological role of p53 haplotypes in cancer susceptibility deserves further investigation. In analyses performed by Weston et al. (1997) the OR (odds ratio) estimates on "true" extended haplotypes are based on comparisons of 18 cases versus 38 controls and of 16 cases versus 30 controls. We think that ORs resulting from such small studies cannot provide reliable estimates of the RR of death for people carrying particular genotypes or haplotypes in Italian or other populations. Moreover, these three studies refer only to the risk for breast cancer. In our letter (Bonafè et al. 1999) the difference between the allele/genotype distributions of 176 centenarians and 204 controls was not statistically significant. However, Sun et al. made a number of speculations on the small differences between allele and genotype frequencies of young people versus centenarians. They claimed that these figures were consistent with their a priori hypothesis of an RR of 1.5–2 for death, for Pro/Pro (8.8% in controls vs. 6.8% in centenarians), Pro carriers (53.9% in controls vs. 50.6% in centenarians), and Pro alleles (31.4% in controls vs. 28.7% in centenarians). In association studies, it is not unreasonable to predict that such small, nonsignificant differences can vanish or reverse as the sample size increases or when replications are performed. Thus, we thought that the best approach was to provide experimental data by studying more subjects, either centenarians or controls, and to replicate the study in another population. Unfortunately, we were unable to study the 4,091 centenarians suggested by Sun et al. The reason was quite simple. According to the data of several laboratories, including our own, the prevalence of centenarians is ~80/1 million inhabitants in developed countries—such as Italy, Denmark, Finland, and Sweden, among others—where reliable age validation is possible. The actual number of centenarians living in Italy (which has ~57 million inhabitants) is thought to be ~4,500–5,000 (Capurso et al. 1997), considering the rapid increase of the oldest old in the population. We are not aware of anybody in the world capable of performing studies on 4,000 centenarians from an ethnically and/or geographically homogeneous population. This difficulty would be even higher if only a small minority of centenarians were relatively healthy. In table 1, new data are shown on allele and genotype frequency of p53 codon 72 polymorphisms in a total of 1,005 people. An additional 218 centenarians and 407 controls have been included with respect to the data reported elsewhere (Bonafè et al. 1999). The data reported here confirm, in a larger sample, that no significant difference is detectable between centenarians and young controls.

With the various possible biases inherent in association studies, replications of studies in other ethnically and/or geographically different populations can help us to better understand the obtained results. Thus, we thought it worthwhile to study the allelic and genotypic distributions of p53 codon 72 in Sardinian centenarians and appropriate younger controls (Deiana et al., in press). The data regarding Sardinian centenarians are reported in table 2. In Sardinian controls, the frequencies of p53 Pro and Arg alleles are 20.1% and 79.8%, respectively. This frequency is significantly different from

Table 1

Allelic and Genotypic Frequencies of the p53 Codon 72 Polymorphism in Continental Italy

	No. (%) of	
	Controls (n = 611)	Centenarians (n = 394)
p53 Allele:[a]		
Pro	332 (27.8)	231 (28.3)
Arg	890 (72.2)	557 (71.7)
p53 Genotype:[b]		
Pro/Pro	49 (8.0)	29 (7.4)
Pro/Arg	234 (38.3)	173 (44.0)
Arg/Arg	328 (53.7)	192 (48.6)

NOTE.—Hardy-Weinberg equilibrium (HWE) of p53 genotypes was assessed by exact tests. Both groups were in HWE: younger controls, P = .53; and centenarians, P = .23. χ^2 Tests for comparison of allelic and genotypic distributions were performed by use of Monte Carlo algorithms implemented by means of the Statistical Product and Service Solutions (SPSS) package.

[a] χ^2 = 1.09, df 1, P = .29.

[b] χ^2 = 3.13, df 2, P = .20.

Table 2

Allelic and Genotypic Frequencies of the p53 Codon 72 Polymorphism in Sardinia

	No. (%) of	
	Controls (n = 92)	Centenarians (n = 110)
p53 Allele:[a]		
Pro	37 (20.1)	51 (23.2)
Arg	147 (79.9)	169 (76.8)
p53 Genotype:[b]		
Pro/Pro	4 (4.4)	4 (3.6)
Pro/Arg	29 (31.5)	43 (39.1)
Arg/Arg	59 (64.1)	63 (57.3)

NOTE.—Hardy-Weinberg equilibrium (HWE) of p53 genotypes was assessed by exact tests. Both groups were in HWE: younger controls, P = .75; and centenarians, P = .43. χ^2 tests for comparison of allelic and genotypic distributions were performed by use of Monte Carlo algorithms implemented by means of the Statistical Product and Service Solutions (SPSS) package.

[a] χ^2 = 0.55, df 1, P = .45.

[b] χ^2 = 1.25, df 2, P = .53.

that found in controls from continental Italy (χ^2 = 4.11, df 1, P = .042). Despite this ethnic difference, again, no statistically significant difference was found between Sardinian centenarians and Sardinian controls. The difference between the two groups, with regard to the frequency of the Pro 72 allele, was ~3% in favor of centenarians. Moreover, the Pro 72 allele carriers were slightly increased in Sardinian centenarians (42.7% in centenarians vs. 35.9% in controls), and this trend is similar to that found in centenarians from continental Italy (table 1). We consider these data on Sardinian centenarians to be additional evidence that both p53 codon 72 alleles are equally compatible with extreme longevity.

In conclusion, we consider the study of the genetics of extreme longevity to be worthwhile, even if some important effects can be missed by the limited size of the samples that can reasonably be recruited. These limitations are frequently encountered even in other types of association studies, including cancer studies (Weston et al. 1997). In any case, the study of p53 haplotypes related to cancer susceptibility can be of considerable help in disentangling this difficult topic. We are following this approach, and we hope that new data will emerge soon.

Acknowledgments

This study has been supported by grants from AIRC (Italian Association for Cancer Research); the European GENAGE Project; Ministry of Health "Ricerca Finalizzata to Istituto di Ricovero e Cura a Carattere Sardegna"; MURST (Ministry for University and Scientific and Technological Research) Cofinanziamento 1998; Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno, and Ancona (to C.F.); and Assessorato Regionale alla Sanita (Regiona Autonoma della Sardegna); and by POP (Regional Operative Project) 94/99 from Regione Calabria (to G.D.B.).

MASSIMILIANO BONAFÈ,[1] FABIOLA OLIVIERI,[3] DANIELA MARI,[4] GIOVANNELLA BAGGIO,[4] ROSARIO MATTACE,[7] MAURIZIO BERARDELLI,[7] PAOLO SANSONI,[8] GIOVANNA DE BENEDICTIS,[9] MARIA DE LUCA,[3,9] FRANCESCA MARCHEGIANI,[3] LUCA CAVALLONE,[3] MAURIZIO CARDELLI,[3] SIMONA GIOVAGNETTI,[3] LUIGI FERRUCCI,[3] LOREDANA AMADIO,[3] ROSAMARIA LISA,[3] MARIA GIOVANNA TUCCI,[3] LEONARDA TROIANO,[10] GABRIELLA PINI,[10] PAOLA GUERESI,[2] MARINA MORELLINI,[11] SANDRO SORBI,[12] GIOVANNI PASSERI,[8] CRISTIANA BARBI,[1] SILVANA VALENSIN,[1] DANIELA MONTI,[13] LUCA DEIANA,[6] GIOVANNI MARIO PES,[6] CIRIACO CARRU,[6] AND CLAUDIO FRANCESCHI[1, 3]

Departments of [1]Experimental Pathology and [2]Statistical Science, University of Bologna, Bologna; [3]Italian National Research Centers on Aging, Ancona and Florence; [4]Institute of Internal Medicine, I.R.C.C.S. Maggiore Hospital, University of Milan, Milan; [5]Chair of Geriatrics and Gerontology and [6]Institute of Clinical Biochemistry, University of Sassari, Sassari, Italy; [7]Chair of Geriatrics, University of Reggio Calabria, Catanzaro, Italy; [8]Institute of Internal Medicine and Medical Therapy, University of Parma, Parma, Italy; [9]Department of Cell Biology, University of Calabria, Rende, Italy; [10]Department of Biomedical Sciences, University of Modena, Modena, Italy; [11]Department of Experimental Medicine, Section of Genetics, University "La Sapienza," Rome; and [12]Department of Neurological and Psychiatric Sciences and [13]Institute of General Pathology, University of Florence, Florence

References

Bonafè M, Olivieri F, Mari D, Baggio G, Mattace R, Sansoni P, De Benedictis G, et al (1999) p53 variants predisposing to cancer are present in healthy centenarians. Am J Hum Genet 64:292–295

Capurso A, D'Amelio A, Resta F, Gaddi A, D'Addato S, Galletti C, Trabucchi M, et al (1997) Epidemiological and socioeconomic aspects of Italian centenarians. Arch Gerontol Geriatr 25:149–157

De Benedictis G, Carotenuto L, Carrieri G, De Luca M, Falcone E, Rose G, Yashin AI, et al (1998) Age-related changes of the 3′ APOB VNTR genotype pool in ageing cohorts. Ann Hum Genet 62:115–122

Deiana L, Ferrucci L, Pes GM, Carru C, Delitala G, Ganau A, Mariotti S, et al (1999) AKEntAnnos: the Sardinia study of extreme longevity. Aging (Milano) 11:142–149

Murata M, Tagawa M, Kimura H, Kakisawa K, Shirasawa H, Fujisawa T (1998) Correlation of the mutation of p53 gene and the polymorphism at codon 72 in smoking-related non-small cell lung cancer patients. Int J Oncol 12:577–581

Sjalander A, Birgander R, Hallmans G, Cajander S, Lenner P, Athlin L, Beckman G (1996) p53 polymorphisms and haplotypes in breast cancer. Carcinogenesis 17:1313–1316

Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G (1999) Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 19: 1092–1100

Toupance B, Godelle B, Gouyon PH, Schachter F (1998) A model for antagonistic pleiotropic gene action for mortality and advanced age. Am J Hum Genet 62:1525–1534

Wang YC, Chen CY, Chen SK, Chang YY, Lin P (1999) p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. Clin Cancer Res 5:129–134

Wang-Gohrke S, Rebbeck TR, Besenfelder W, Kreienberg R, Runnebaum IB (1998) p53 germline polymorphisms are associated with an increased risk for breast cancer in German women. Anticancer Res 18:2095–2099

Weston A, Pan CF, Ksieski HB, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, et al (1997) p53 haplotype determination in breast cancer. Cancer Epidemiol Biomarkers Prev 6:105–112

Weston A, Wolff MS, Morabia A (1998) True extended haplotypes of p53: indicators of breast cancer risk. Cancer Genet Cytogenet 102:153–154

Yashin A, Vaupel JW, Andreev KF, Tan Q, Iachine IA, Carotenuto L, De Benedictis G, et al (1998) Combining genetic and demographic information in population studies of aging and longevity. J Epidemiol Biostat 3:289–294

Yashin AI, De Benedictis G, Vaupel JW, Tan Q, Andreev KF, Iachine IA, Carotenuto L, et al (1999) Genes, demography and life span: the contribution of demographic data in genetic studies of aging and longevity. Am J Hum Genet 65: 1178–1193

Yu MW, Yang SY, Chiu YH, Chiang YC, Liaw YF, Chen CJ (1999) A p53 genetic polymorphism as a modulator of hepatocellular carcinoma risk in relation to chronic liver disease, familial tendency, and cigarette smoking in hepatitis B carriers. Hepatology 29:697–702

Address for correspondence and reprints: Dr. Claudio Franceschi, Professor of Immunology, Department of Experimental Pathology, Via S. Giacomo n. 12, University of Bologna, 40126 Bologna, Italy. E-mail: clafra@alma.unibo.it

0002-9297/1999/6506-0037$02.00