Mismatch Repair System and Aging: Microsatellite Instability in Peripheral Blood Cells From Differently Aged Participants

Simona Neri,1 Alessandro Gardini,1 Andrea Facchini,1,2 Fabiola Olivieri,3 Claudio Franceschi,3,4 Giovanni Ravaglia,5 and Erminia Mariani1,2

1Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti, I.O.R., Bologna, Italy.
2Dipartimento di Medicina Interna e Gastroenterologia, Università di Bologna, Italy.
3Istituto Nazionale di Riposo e Cura per Anziani di Ancona, Italy.
4Dipartimento di Patologia Sperimentale e centro Interdipartimentale "L. Galvani," Università di Bologna, Italy.
5Dipartimento di Medicina Interna Cardioangiologia ed Epatologia, Università di Bologna, Italy.

Age-related alterations of DNA repair could be involved in the accumulation of genetic damage with age. Few data suggest a possible alteration with age of the mismatch repair system, evidenced by the acquisition of microsatellite instability. We aimed to point out a possible implication of this repair system in the accumulation of genetic damage with age. Peripheral blood cell DNA from 226 participants, 110 young (25–35 years), 58 old (85–97 years), and 58 centenarian was analyzed at five polymorphic microsatellite loci (CD4, p53, VWA31, TPOX, and FES/FPS) to point out age-related instabilities or modifications in allele frequencies. FES/FPS microsatellite was the most instable, showing both the appearance of trizygosis in DNA from old participants and differences in allele patterns among age groups, thus indicating an association between increased microsatellite instability and aging, one of the possible causes of which being an impairment of mismatch repair system capacity with age.

The somatic theory (1) explains aging in terms of an accumulation of mutations in the genome of somatic cells leading to cell senescence, cell death or transformation, as well as cell loss of function. Age-dependent accumulation of DNA damage (2–6) that seems to affect various tissues at different rates as observed by Ono and colleagues (7) in transgenic mice harboring the LacZ gene and that could contribute to the impaired cellular functions and the increased incidence of diseases, such as cancer, has been widely described to occur with age. The positive correlation observed between life span and DNA repair capacity (8) suggests that age-related alterations in the cellular mechanisms of DNA repair could contribute to the accumulation of genetic damage. The impact of a malfunctioning DNA repair system on genomic integrity is evidenced by progeroid syndromes in which mutations in DNA repair genes induce a phenotype characterized by precocious signs of premature aging such as immunodeficiency and cancer susceptibility (9). It is therefore reasonable that inactivation of one or more DNA repair pathways accelerates in parallel mutation accumulation, age-related deterioration, and death.

Most of the studies on DNA repair alterations during aging focused on the nucleotide excision repair mechanism and its role in repairing ultraviolet light–induced damage. In human peripheral blood lymphocytes, a progressively decreased DNA repair capacity in elderly participants (2) and reduced levels of p53 and other proteins involved in the nucleotide excision repair mechanism have been observed (10). Also the nonhomologous end-joining pathway (the main repair system of double-strand breaks) and the base excision repair pathway appeared to be altered with age (6,11–12).

DNA replication is an important source of somatic mutations, and the mismatch repair (MMR) system is the main postreplicative correction pathway: it contributes to genomic stability by repairing replicative and recombinational errors (13) leading to mispaired bases (mismatches). The system scans and repairs newly replicated DNA by excising the mutated strand in either direction to the mismatch (13). Until now, six human genes involved in DNA MMR have been cloned and studied: hMSH2, hMSH3, hMSH6 (all homologues of the Escherichia coli mutS gene), hMLH1, and hPMS1 and hPMS2 (human postmeiotic segregation 1 and 2) (all homologues of the E. coli mutL gene) (14,15). These six genes confer to the genome a 100- to 1000-fold protection against replication-induced mutations (16). Mismatch recognition is performed in human cells by two complexes, hMUTSx (a heterodimer of hMSH2 and hMSH6) and hMUTSβ (formed by hMSH2 and hMSH3). Defects in this pathway are associated with a substantial destabilization of microsatellites, highly polymorphic, tandemly repeated sequences (from 1 to 6 bp) interspersed in the genome and particularly prone to slippage during replication. Slippages determine changes of allele length either for insertion or deletion of repeating units, and lead either to the appearance of additional bands of different length or to modification of the expected bands. These genetic alterations are experimentally evidenced as microsatellite instability (MSI). Mutations are observed in
repeated sequences, but can also occur randomly in all the genome, therefore MSI indicates a higher susceptibility to mutations. Because of inactivation of the MMR system, mutator phenotypes have been demonstrated in a number of cancers and cancer cell lines. MSI, caused by mutations in several members of MMR genes (17–20), was initially described in hereditary nonpolyposis colorectal cancer (21,22). Subsequently, it was also described in sporadic tumors of the colon, endometrium, and stomach (15,23–25), where inactivation of the MMR system is frequently caused by MLH1 promoter methylation (24,26,27).

The activity of this repair system during aging has not been sufficiently investigated. Dysfunctions of the MMR system can lead to increased mutation rates and the accumulation of mutations that play a role in carcinogenesis. MSH2-deficient mice succumb within one year to cancer with lymphomas, which is a common cause of death in normally aged mice (28). An increase in the methylation status of several gene promoters was observed with age in normal colon mucosa. Among the promoters, the MLH1 promoter was found hypermethylated with a related inhibition of its expression (29). In patients with gastric lymphomas, the frequency of MSI in the pathologic tissue showed a tendency to increase with age, as did microsatellite variability (30). In a preliminary study by Yehuda and colleagues (31), MSI was evidenced by comparing, at a 10-year time interval, the DNA obtained from blood cells of aged participants; no instability was observed in DNA from younger donors. CD8 lymphocytes from aged individuals develop MSI during repeated population doublings in culture, and CD4 clones develop significant MSI during in vitro aging (32,33).

Because of the importance of the MMR system in the conservation of genomic stability, and its emerging relevance to malignancy, this study aims to evidence a putative role of this system in the accumulation of genetic damage occurring with advancing age. In fact, most malignancies occur in old people, and decreased DNA repair capacity with age may contribute to this high incidence or affect efficiency of cellular functions. For this reason, DNA from total peripheral blood of 110 young, 58 old, and 58 centenarian participants was analyzed for MSI or modifications in allele frequencies. Five different polymorphic tetra- and pentanucleotide microsatellite loci (CD4, p53, VWA31, TPOX, and FES/FPS) were investigated.

**Methods**

**Participants**

One hundred ten young (25–35 years old), 58 old (85–97 years old), and 58 centenarian (≥100 years old) healthy participants from Northern-Central Italy were enrolled in this study. The sample of centenarians was composed of 49 women and 9 men, whereas, in young and old groups, males and females were equally represented. Donors gave informed consent for enrollment in the study.

**Analysis of MSI**

Total DNA from whole peripheral blood (about 1.5 ml) collected in heparin and stored at −20°C was extracted using the QIAamp DNA Blood Midi Kit (Qiagen GmbH, Hilden, Germany), following manufacturer's instructions, and was quantified by spectrophotometric determination at 260 nm.

For MSI analysis, five loci containing tetra- and pentanucleotide polymorphic tandem repeat sequences were used: CD4 (12p13), a TTTTC repeat located in the 5′ nontranscribed region of the T-cell surface antigen gene; p53 (17p13), an AAAT repeat in the first intron of the TP53 gene; VWA31 (12p12-pter), an AGAT repeat in intron 40 of the von Willebrand factor gene; TPOX (2p23-pter), an AATG repeat in intron 10 of the thyroid peroxidase gene; and FES/FPS (15q25-pter), an ATTT repeat in intron 5 of the c-fes proto-oncogene (33). These markers are widely used in population studies (34–38) and also in instability studies in sporadic cancers (39). They were selected to cover different chromosomal regions, some of them associated with human cancers.

Polymerase chain reaction (PCR) amplifications were carried out using the following specific primers: CD4-pF: 5′-TGGAGTGCGAAGCTGAATGACA-3′; CD4-pR: 5′-GGCTAGTGACAGTAGGAAACC-3′ (34); p53-pF: 5′-ACTTCAAAGCTGGGAAATAAGCT-3′; p53-pR: 5′-ACAAACATCCTCCACACAGC-3′ (40); VWA31-pF: 5′-CCCTAGTGATGAATAAATCATGATG-3′; VWA31-pR: 5′-GGACAGATGTAATAATCATAAGGTAGTGG-3′ (36); TPOX-pF: 5′-ACTGCAACAGACAGCACTTAGG-3′; TPOX-pR: 5′-GGAGAAGTGGGAAACACCAGAGT-3′ (37); and FES/FPS-pF: 5′-GGGATTCCTGATGGATGAGACT-3′; FES/FPS-pR: 5′-GGGAAAGAATGAGACT-3′ (38).

Template DNA (50 ng), 1 μM each primer, 200 μM each deoxynucleotide triphosphate, 1.5 mM (for CD4, p53, TPOX, and FES) or 2.5 mM (for VWA31) MgCl2, 1X PCR buffer II (50 mM KCl, 10 mM Tris-HCl, pH 8.3) and 1.25 U AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA) were combined in 25-μl reactions. Amplification profiles were, respectively: 10 cycles (1 minute at 94°C, 45 seconds at 62°C) followed by 20 cycles (1 minute at 90°C, 45 seconds at 62°C) for CD4; 30 cycles (1 minute at 94°C, 45 seconds at 65°C, 45 seconds at 72°C) for p53; 30 cycles (1 minute at 94°C, 1 minute at 57°C, 1 minute at 72°C) for VWA31; 30 cycles (1 minute at 94°C, 1 minute at 64°C, 1 minute at 72°C) for TPOX; 30 cycles (1 minute at 94°C, 1 minute at 54°C, 1 minute at 72°C) for FES/FPS. A sample without template, as negative control, was included in all reactions. To verify amplification, 8 μl of each PCR product were analyzed on a 2% agarose gel, then adjusted amounts of PCR product underwent electrophoresis (100–150 V for 16–18 hours) on 10% nondenaturing polyacrylamide vertical gels (0.75 mm thick, 20 cm long) containing 5% glycerol in Tris-Borate-EDTA buffer, together with the DNA Molecular Weight Marker VIII (Roche Molecular Systems, Branchburg, NJ) and allelic ladders prepared with known mixed alleles. Gel staining was performed at room temperature with continuous shaking as described by Budowle and colleagues (41), with partial modification. Briefly, the gels were fixed in 10% EtOH for 10 minutes, oxidized in 1% nitric acid for 2 minutes, stained in 0.02% AgNO3 for 20 minutes, rinsed in distilled water, and finally reduced in developing solution (3% sodium carbonate and 0.1% formaldehyde).
Table 1. CD4 Alleles and Genotypes in Young, Old, and Centenarian Participants

<table>
<thead>
<tr>
<th>Genetic Distribution</th>
<th>Young (N = 110)</th>
<th>Old (N = 58)</th>
<th>Centenarian (N = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
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<tr>
<td>4 (88 bp)</td>
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<td>31</td>
<td>37</td>
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<tr>
<td>5 (93 bp)</td>
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<td>9 (113 bp)</td>
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<td>28</td>
</tr>
<tr>
<td>10 (118 bp)</td>
<td>11</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>11 (123 bp)</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
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<td>116</td>
<td>106</td>
</tr>
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</tr>
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<tr>
<td>Total</td>
<td>109</td>
<td>58</td>
<td>53</td>
</tr>
</tbody>
</table>

Notes: Allele nomenclature refers to the number of repeats and length of polymerase chain reaction products is indicated in parentheses. No difference in the overall allele frequency distribution was found by comparing either young and pooled old and centenarian participants (Pearson’s χ² = 4.398, p = 0.502, 99% CI = 0.489–0.514) or young, old, and centenarians separately (Pearson’s χ² = 8.620, p = 0.580, 99% CI = 0.567–0.592). No difference in the overall genotype frequency distribution was found by comparing either young and pooled old and centenarian participants (Pearson’s χ² = 3.546, p = 0.951, 99% CI = 0.945–0.956) or young, old, and centenarians separately (Pearson’s χ² = 11.626, p = 0.886, 99% CI = 0.877–0.894).

Ra = rare alleles (n.6, 7, corresponding to amplification products of 98 and 103 bp, respectively); Rg = rare genotypes (4-6, 4-11, 5-6, 5-7, 5-10, 9-11, 10-10, 10-11, 11-11); AR = adjusted residuals; CI = confidence interval.

Development lasted until an optimal band staining was obtained, and was stopped by adding 5% acetic acid for 3 minutes; then, the gels were placed in distilled water. Images of the gels were acquired using the Kodak Electrophoresis Documentation and Analysis System (EDAS) 120. Genotyping was made by side-by-side comparison with allele ladders. To control results, a second PCR analysis was done for samples that were trizygotic.

Statistical Analysis

Allele frequencies were calculated for every microsatellite by counting genes from the observed genotypes within each age group. Pearson’s chi-square test calculated by the Monte Carlo algorithm was used to compare allele and genotype frequency distributions among groups of different ages and to evaluate the appearance of trizygotic samples. Spearman’s correlation calculated by Monte Carlo algorithm was also used. Rare alleles and genotypes (with a frequency lower than 2%) were pooled (42) and identified as Ra and Rg, respectively. Allele nomenclature referred to the number of repeat units for each microsatellite. Adjusted residuals (AR), which represent the difference between expected and observed frequencies under the null hypothesis of independence, were used for qualitative evaluations of the allele and genotype frequency distributions. Statistical analysis was performed using the SPSS package for Windows (SPSS, Inc., Chicago, IL).

RESULTS

The distribution of CD4, p53, VWA31, TPOX, and FES/FPS allele frequencies was, in general, in accordance with previously published data obtained by population screenings (not shown). No sex difference was found when allelic frequencies and genotype distributions of men and women were compared.

The allele and genotype distribution of CD4 microsatellite in young, old, and centenarian participants is reported in Table 1. A total of seven different CD4 microsatellite alleles, ranging from 4 to 11 repeat units (88–123 bp as PCR product length) were observed in the three age groups. Two rare alleles (n.6 and 7, corresponding to amplification products of 98 and 103 bp, respectively) and nine rare genotypes (4-6, 4-11, 5-6, 5-7, 5-10, 9-11, 10-10, 10-11, 11-11) were pooled. No difference in allele and genotype (Table 1) frequency distributions was ob-
Seven different VWA31 microsatellite alleles, ranging from 14 to 20 repeat units (139–163 bp as PCR product length), were observed. Only an allele with 20 repeats was rare. Overall allele frequency distribution (Table 3) showed some differences between groups of different age, as evidenced by the observation of AR. In particular, a trend for the overall allele frequency distribution was found by comparing either young and pooled old and centenarian participants (Pearson’s $\chi^2 = 3.979$, $p = .565$, 99% CI = 0.553–0.578) or young, old, and centenarians separately (Pearson’s $\chi^2 = 13.354$, $p = .187$, 99% CI = 0.177–0.197). No difference in the overall genotype frequency distribution was found by comparing either young and pooled old and centenarian participants (Pearson’s $\chi^2 = 13.417$, $p = .137$, 99% CI = 0.128–0.146) or young, old, and centenarians separately (Pearson’s $\chi^2 = 22.676$, $p = .192$, 99% CI = 0.182–0.202).

$Ra$ = rare alleles (allele n,6, corresponding to an amplification product of 226 bp); $Rg$ = rare genotypes (9–9, 9–12, 10–10, 10–12, 11–12, 12–12); AR = adjusted residuals; CI = confidence interval.
alleles n. 10, 13, and 16; C3 alleles n. 10, 12, and 15; C4 alleles n. 10, 12, and 15.

Figure 1. Pattern of FES/FPS alleles in 1 old (O) and 4 centenarian (C1–C4) participants that were trizygotic at this locus. Polymerase chain reaction products were subjected to electrophoresis on 10% nondenaturing polyacrylamide gels and were silver stained. Allele length (indicated on both sides of the figure) was determined by comparison with the allelic ladder (L) made up from a mixture of known alleles. Allele nomenclature refers to the number of repeats. The old participant was trizygotic for alleles n. 10, 12, and 13, whereas centenarians presented, respectively, the following alleles: C1 and C2 alleles n. 10, 11, and 12; C2 alleles n. 10, 11, and 13; C3 alleles n. 10, 12, and 15; C4 alleles n. 10, 12, and 15.

of AR (Table 3), overall distribution was homogeneous in the three age groups.

In the studied groups, 6 different TPOX microsatellite alleles (Table 4) were observed, ranging from 6 to 12 repeat units (226–250 bp as PCR product length); only one was rare (allele n.6). The allele and genotype distributions of the TPOX microsatellite (Table 4) showed no differences among young, old, and centenarian participants, either when analyzed separately or when old and centenarian groups were pooled.

The more interesting data were obtained by the analysis of the FES/FPS microsatellite. Only for this microsatellite did we observe five trizygotic participants among the 226 analyzed (Pearson χ² = 8.944, p = .006); this observation was a direct indication of instability. All samples that were trizygotic belong to the old (1 case) and to the centenarian groups (4 cases) (Figure 1), whereas no trizygosis was observed among young participants. In addition, in three of the four cases of trizygosis in centenarians, one allele was rare (allele n.15 in 1 case, and n.16 in 1 case); the presence of this allele was never observed in homo- or heterozygosis. Allele n.15 has been described in African population studies, but it seemed to be absent in Caucasians (43), whereas allele n.16 was not previously described in the literature. We observed 8 FES/FPS alleles, ranging from 8 to 16 repeat units (210–242 bp as PCR product length); 4 of these 8 alleles were rare (alleles n.8, 14, 15, and 16), and were pooled for statistical analysis (Table 5). Significant differences in FES/FPS allele frequency distribution between young participants and pooled old and centenarian participants were also observed (Pearson’s χ² = 10.985, p = .025), whereas comparison of young, old, and centenarian participants separately failed to reach statistical significance (Table 5). Allele n.11 seemed to be typical of the young group; in fact, its frequency dropped from 50.5% in the young group to 41.9% and 34.2% in the old and centenarian groups, respectively, in agreement with the obtained AR that showed a decreased frequency of this allele in old (AR = −0.6) and, particularly, in centenarian participants (AR = −2.5) compared with young participants (AR = +2.6). On the contrary, rare alleles were absent in the young group (AR = −2.3), whereas they were typical of the centenarian group (AR = +2.1). As far as FES/FPS genotypes are concerned (Table 5), significant differences were observed by both comparing young and pooled old and centenarian participants (Pearson’s χ² = 18.707, p = .033) and comparing young and centenarian separately (Pearson’s χ² = 34.993, p = .016). In particular, 12-12 homozygotes were present in old (AR = +1.0) and centenarian (AR = +1.3) participants, and were rare in young participants (AR = −2.0); 11–13 genotypes were typical in the old (AR = +2.8) but not the young (AR = −1.0) or centenarian participants (AR = −1.7); 8–11 genotypes were observed only in centenarian participants (AR = +2.6).

**DISCUSSION**

Because of the importance of the MMR system in the conservation of genome stability (as supported by hereditary nonpolyposis colorectal cancer and sporadic cancers caused by mutations in MMR genes) (25), it was interesting to...
evaluate the possible contribution of the MMR system to the age-related accumulation of mutations affecting cellular function or favoring neoplastic transformation in elderly participants. The analysis of MSI, evidenced by the appearance of alleles different from the ones characteristic of the individual (plurizygosity or substitution of the original alleles) caused by expansions or contractions of repeated sequences interspersed in the genome, represents the experimental approach for studying the efficiency of the MMR system. Despite the large amount of data evidencing an accumulation of genetic damage with age and an impairment of DNA repair pathways (2–6), the importance of the MMR system is still underevaluated, although some evidence has suggested a possible role of the MMR system in aging (29,31–33). Our study represents a preliminary approach aiming to ascertain whether aging and longevity are associated with modifications of microsatellite stability or allelic patterns.

The presence of FES/FPS trizygotic allelic patterns in 5 elderly participants (1 old and 4 centenarian) and the lack of plurizygosity in young ones, suggest both an increased instability in very advanced age and an age-dependent genetic damage affecting repeated sequences, the stability of which is guaranteed predominantly by the MMR system. Because MSI is an indirect effect of MMR inefficiency, we cannot exclude that other mechanisms are involved in its generation. In particular, low levels of microsatellite mutations could be due to the involvement of the base excision repair pathway (in MSI, the role of which was postulated in chronic inflammation) (44) or to an altered polymerase activity, the efficiency and fidelity of which seem to be compromised by aging (45). The data regarding the FES/FPS microsatellite are in accordance with those data obtained in lymphocyte clones that, analyzed at different times of in vitro aging, showed instability particularly at this locus and plurizygosity only in this sequence (33). These results therefore suggest either an augmented instability, possibly due to decreased MMR system repair efficiency, or a weaker ability to balance the increased rate of genetic damage, due to advancing age. Cells that underwent several duplications were more exposed to the repair activity of the MMR system and possibly have accumulated greater genetic damage than were virgin cells, because of the inefficiency of the MMR system. It has been demonstrated that a major characteristic of immunosenescence is the decline of virgin T cells as well as the accumulation of clones, mostly of memory and effector T cells, caused by chronic exposure to a variety of antigens (46,47) (mainly Epstein–Barr and cytomegalovirus antigens) (48,49). In addition, MSI has been associated with in vitro replicative senescence of CD8 cells from aged donors (32). It is conceivable that cells of these clones, which have repeatedly replicated in vivo, may have simultaneously accumulated genetic damage not evident in cells from young participants in whom, conversely, naive cells are more represented. The level of expansion of such clones, frequently dramatically high (49), could explain the experimental evidence of additional alleles (trizygosis) in DNA from heterogeneous cell populations, as those obtained by total peripheral blood. In fact, to be detectable, the phenomenon should involve a discrete proportion of cells from which DNA is obtained. The possible appearance of new alleles might be missed in underrepresented cellular subpopulations (below 5%–10%); this under-representation could induce an underestimation of MSI.

With regard to the analysis of allele frequencies, two of five microsatellites (VWA31 and FES/FPS) showed a different distribution among age groups, further suggesting modifications in microsatellite stability evidenced as shifts in allele frequency patterns from young (representative of a control population) to old populations. On the contrary, CD4 and p53 microsatellite sequences appeared to be more stable (in agreement with previous observations) in cloned lymphocytes (33). Similar results with the p53 microsatellite were obtained in another larger casistic (50): In tests of three different polymorphisms at the p53 locus (in addition to the pentanucleotide microsatellite at intron 1 tested in our study), no differences among young, old, and centenarian participants were observed. These results exclude a significant role of p53 variants in the alteration of gene frequency in old people and centenarians. Due to the absence of an internal control, the analysis of MSI in DNA from normal participants is quite different than that from tumor patients. Excluding the appearance of plurizygosity, which represents a direct indication of instability, but whose frequency is expected to be low in healthy participants without germ-line mutations in MMR genes, it is not possible to compare the allelic pattern with a control (as between normal and tumor DNA from the same patient). For this reason, shifts in allele length were evaluated only in terms of age-related modifications of allele frequencies, as indirect indications of genetic instability, possibly induced by a defect of genomic conservation mechanisms such as the MMR system. This is in accordance with the appearance of new alleles (as 15 and 16 FES/FPS alleles) only in the centenarians. This hypothesis, even if not demonstrated by the comparison with an internal control, is supported by data obtained in lymphocyte clones used in in vitro aging, where in vitro proliferation (mimicking replications accumulated in vivo during the course of life) was able to point out modifications of allelic patterns occurred and not corrected during repeated replications (32,33). An increase in allele variability and an augmented MSI were observed with age in patients with diffuse large B-cell lymphomas (30) and also in microsatellite sequences obtained from total peripheral blood DNA of old participants analyzed at a 10-year time interval (31); these observations further support our hypothesis. Another possibility, in accordance with observed alterations in only two of five analyzed sequences, is that particular alleles are associated with reaching older ages. The particular results obtained at the FES/FPS locus could be, therefore, explained in different ways. One can assume a different sensitivity of this region to the putative inefficiency of the MMR system (in fact, not all sequences are susceptible to instability; different loci may show different rates of MSI) (39). Accordingly, a specific FES/FPS somatic instability was described in sporadic gastric cancer (39) and in lymphocyte clones after in vitro aging (33). Another possibility is that some alleles undergo a selection with age and therefore disappear in the
oldest participants due to a relationship with the FES/FPS microsatellite sequence or other sequences in linkage disequilibrium with it. The contributions of genetic backgrounds to interindividual differences in human longevity have been evidenced in recent years, and associations between longevity and allelic variants of polymorphic markers associated to putative age-related loci have been observed. This is the case of HLA (51), ACE (52), APO B (53), APO C (54), APO E (52–54), TH (55), HRAS1-3 VNTR (42) and 3’ APO B-VNTR (56) loci. The analyzed FES/FPS microsatellite lies at intron 5 of the coding region of the FES/FPS proto-oncogene that encodes for a non-receptor protein–tyrosine–kinase, activation of which can mediate cellular transformation. Several growth factors, cytokines, and immunoglobulins, after engaging their receptors, induce the activation of the cellular FES/FPS that has been shown to play important roles in the regulation of inflammation and immune response (57,58), particularly for survival and terminal differentiation of hematopoietic cells of the myeloid lineage (59). Taking into account the involvement of this factor in the homeostasis of the immune system and modifications of FES/FPS-associated microsatellite allele distributions with age, we cannot exclude a possible relationship among allelic variants and aging. Because FES/FPS microsatellite alleles are not expressed, it is likely that modifications in allele frequencies depend on coding sequences in linkage disequilibrium with the analyzed ones, but we should not discard the possibility of a correlation between the number of repeats and the aging process. This correlation influences, for example, the expression of the gene where the repeated sequence is located, as proposed for VNTR (60). Accordingly, polymorphic alleles of inflammatory cytokines, by determining changes in cytokine production, play an important role in age-related inflammatory diseases, i.e., in unsuccessful aging (61).

**Conclusion**

Our data evidence an association between MSI and aging. One of the possible causes of the observed instability could be the alteration of MMR system efficiency that, with advancing age, might contribute to the accumulation of genetic damages that, in turn, can be involved in the alterations of cellular functions and the increased incidence of neoplastic diseases described in elderly persons.

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A. Gardini is now affiliated with IFOM, Fire Institute of Molecular Oncology, Milan, Italy.

Address correspondence to Erminia Mariani, MD, Istituto di Ricerca Codivilla Putti, IOR Via di Barbiano 1/10, 40136 Bologna, Italy. E-mail: marianiec@alma.unibo.it

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