The Role of Matrix Metalloproteinases (MMP-2 and MMP-9) in Ageing and Longevity: Focus on Sicilian Long-Living Individuals (LLIs)

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Received 16 December 2019; Revised 29 March 2020; Accepted 13 April 2020; Published 5 May 2020

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Extracellular matrix metalloproteinases (MMPs) are a group of proteins that activate substrates by enzymatic cleavage and, on the basis of their activities, have been demonstrated to play a role in ageing. Thus, in order to gain insight into the pathophysiology of ageing and to identify new markers of longevity, we analysed the activity levels of MMP-2 and MMP-9 in association with some relevant haematological parameters in a Sicilian population, including long-living individuals (LLIs, ≥95 years old). A cohort of 154 healthy subjects (72 men and 82 women) of different ages (age range 20-112) was recruited. The cohort was divided into five subgroups: the first group with subjects less than 40 years old, the second group ranging from 40 to 64 years old, the third group ranging from 65 to 89 years old, the fourth group ranging from 90 to 94 years old, and the fifth group with subjects more than 95 years old. A relationship was observed between LLIs and MMP-2, but not between LLIs and MMP-9. However, in the LLI group, MMP-2 and MMP-9 values were significantly correlated. Furthermore, in LLIs, we found a positive correlation of MMP-2 with the antioxidant catabolite uric acid and a negative correlation with the inflammatory marker C-reactive protein. Finally, in LLIs MMP-9 values correlated directly both with cholesterol and with low-density lipoproteins. On the whole, our data suggest that the observed increase of MMP-2 in LLIs might play a positive role in the attainment of longevity. This is the first study that shows that serum activity of MMP-2 is increased in LLIs as compared to younger subjects. As far as we are concerned, it is difficult to make wide-ranging conclusions/assumptions based on these observations in view of the relatively small sample size of LLIs. However, this is an important starting point. Larger-scale future studies will be required to clarify these findings including the link with other systemic inflammatory and antioxidant markers.
1. Introduction

Ageing is a time-dependent functional decline, which involves a progressive deterioration of organism physiological functions heading to increased susceptibility to disease and death. This process is unavoidable and extremely complex. However, in the last 40 years, a lot of efforts have been made to characterize ageing. As described in literature, there are two ways to age. The first is free of age-related diseases and without disability (successful ageing), while the latter is characterized by a progressive tendency toward inflammaging, disability, and age-related diseases (unsuccessful ageing) [1]. Major age-related diseases include atherosclerosis, Alzheimer’s disease, and diabetes, where the inflammatory components that were prolonged and persisted become damaging [1, 2]. On the other hand, long-living individuals (LLIs) are considered the best example of successful ageing [3].

Extracellular proteinases are a group of proteins that activate substrates by enzymatic cleavage and, on the basis of working mechanisms, are classified into aspartic, metallo-, cysteine, serine, and threonine proteinases [4]. Among different immune cells, macrophage and neutrophils are the main responsible for matrix metalloproteinase (MMP) production. This group of proteins controls a large variety of key physiological and pathological processes, including tissue remodelling, DNA replication, cell-cycle progression, neurodegeneration, and cancer [5].

Moreover, MMPs are responsible for remodelling of extracellular matrix (ECM), which represents a three-dimensional network of extracellular macromolecules such as collagen, enzymes, and glycoproteins, that provides structural and biochemical support of surrounding cells [6], particularly of stem cell niche [7, 8]. The perturbation of ECM remodelling has been associated with ageing and age-related disorders, for example, progeria (an extremely rare, autosomal, dominant genetic disorder in which symptoms, resembling aspects of ageing, are manifested at a very early age) [9], arterial ageing, hypertension-associated vascular changes [10], cancer metastasis, heart failure, and cerebral ischemia and neurological disorders including Parkinson’s and Alzheimer’s diseases [11, 12]. Therefore, the analysis of MMPs can add important information to ageing process and to longevity. As an example, ECM proteins, like elastins, can be a good candidate as a biomarker of ageing due to low turnover and to capacity to accumulate damage during ageing process [13], as in a vascular one. Also, collagen, another ECM protein, is deeply involved in ageing since its accumulation drives both vascular and lung ageing [14]. In addition, in Caenorhabditis elegans, collagen, among other ECM proteins, is influenced by the insulin/IGF-1-like signalling, which in turn extend life span in a worm model [15].

MMP-2 (a type of gelatinase A, 72 kDa) and MMP-9 (a type of gelatinase B, 92 kDa) are composed of 3 domains, distinguished by the presence of type II additional fibronectin domain inserted into the catalytic domain. They are able to degrade collagen, elastin, fibronectin, gelatin, and laminin and have both proinflammatory and anti-inflammatory impacts on numerous tissues [16–18]. In particular, MMP-2 is constitutively expressed in several tissues and is regulated by tumor necrosis factor-α under the influence of NF-κB transcription factor [19], while redox-regulated p38 phosphorylation and subsequent AP-1 activation appear to be critical for lipopolysaccharide-induced MMP-9 expression, at least in murine macrophages [20]. MMP-2 is tightly associated with inflammatory states such as osteoarthritis [21, 22]; besides, MMP-2 protects from hypertensive heart disease by suppressing the transcription and activity of 3-hydroxy-3-methylglutaryl-CoA reductase in the early stages of the hypertensive response [23]. Interestingly, it was demonstrated that in atherosclerotic plaque, MMP-2 levels decreased, compared to nonatherosclerotic human tissues [24], whereas MMP-9 levels increased, showing that MMP-9 activity contributes to endothelial dysfunction [25].

MMP-9 is also implicated in lipid metabolism [26], and in a mouse model, it plays an important role for atheroma formation [27, 28]. Moreover, the protein levels are detected in the acute phase after stroke, whereas MMP-2 protein levels were increased several days after when barrier leakage is presumably restored.

Thus, in our study, in order to gain insight into ageing, age-related disease, and longevity, the activity levels of MMP-2 and MMP-9 were analysed in association with some relevant haematochemical parameters in a Sicilian population.

2. Materials and Methods

2.1. Subject Recruitment and Study Design. A cohort of 154 healthy subjects (72 men and 82 women) of different ages (age range 20-112) was recruited. Donors were all Sicilians, living in Western Sicily. A group of well-trained nutritionists and physicians administered a questionnaire to collect demographic and anamnestic data of interest. Participants were selected on the basis of their health status since none of them had neoplastic, infective, or autoimmune diseases and none was prescribed drugs known to interfere with immune-inflammatory responses. Participants (or their relatives for some LLIs) signed an informed consent before the enrolment. To respect the privacy, everyone was identified with an alphanumeric code. A database was created to handle the collected information. The study protocol, conducted in accordance with the Declaration of Helsinki and its amendments, was approved by the Ethics Committee of Palermo University Hospital (Nutrition and Longevity, No. 032017).

The suitability of the sample size was checked using free software (http://ps-powerand-sample-sizecalculation.software.informer.com) on the basis of the results of our previous studies. The analysed cohort was divided into five subgroups: the first group included people less than 40 years old (19%, group 1—young people; N = 29), the second group ranging from 40 to 64 years old (25%, group 2—adult people; N = 39), the third group ranging from 65 to 89 years old (32%, group 3—old people; N = 50), the fourth group ranging from 90 to 94 years old (8%, group 4—oldest old people; N = 12), and the fifth group characterized by subjects 95 or more years old (16%, group 5—formed by LLIs; N = 24). The recruitment was performed in accordance with the relevant guidelines and regulations.
The recruited participants underwent vein puncture after a fasting period of 10–12 hours. The fasting blood samples were obtained in the morning (between 8:30 and 10 a.m.) and were collected in serum tubes with no additives.

The following haematochemical parameters were performed for all participants: albumin, alanine and aspartate transaminases, alkaline phosphatase, bilirubin, calcium, creatinine, C-reactive protein (CRP), ferritin, glycaemia, high-density lipoproteins, iron, low-density lipoproteins (LDL), magnesium, potassium, total cholesterol (CHO), total proteins, transferrin, triglycerides, urea, and uric acid (UA) as well as complete blood count tests. The tests were carried out at the Department of Laboratory Medicine, "P. Giaccone" University Hospital, Palermo, according to standard procedures. In particular, CRP measurement was performed by immunoturbidimetry methods, UA by colorimetric test, and lipid parameters by enzymatic colorimetric test through Roche/Hitachi Cobas system.

Table 1 shows the baseline characteristics of the studied cohort with haematochemical parameters shown to be associated with MMPs.

### Table 1: Baseline characteristics of the studied cohort with haematochemical parameters.

<table>
<thead>
<tr>
<th>Age</th>
<th>n (%)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td></td>
<td>&lt;40</td>
<td>40-64</td>
<td>65-89</td>
<td>90-94</td>
<td>≥95</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>72 (47%)</td>
<td>13 (45%)</td>
<td>19 (49%)</td>
<td>28 (56%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>82 (53%)</td>
<td>16 (55%)</td>
<td>20 (51%)</td>
<td>22 (44%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>CRP &lt;5 g/dL</td>
<td></td>
<td>127 (82%)</td>
<td>26 (90%)</td>
<td>34 (87%)</td>
<td>43 (86%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>CRP ≥5 g/dL</td>
<td></td>
<td>27 (18%)</td>
<td>3 (10%)</td>
<td>5 (13%)</td>
<td>7 (14%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>UA &lt;2.4 g/dL</td>
<td></td>
<td>15 (10%)</td>
<td>4 (14%)</td>
<td>4 (10%)</td>
<td>1 (2%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>UA ≥2.4 g/dL</td>
<td></td>
<td>114 (74%)</td>
<td>23 (79%)</td>
<td>33 (85%)</td>
<td>37 (74%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>CHO &lt;200 g/dL</td>
<td></td>
<td>113 (73%)</td>
<td>27 (93%)</td>
<td>22 (56%)</td>
<td>35 (70%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>CHO ≥200 g/dL</td>
<td></td>
<td>41 (27%)</td>
<td>2 (7%)</td>
<td>17 (44%)</td>
<td>15 (30%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>LDL &lt;70 g/dL</td>
<td></td>
<td>15 (10%)</td>
<td>5 (17%)</td>
<td>0 (0%)</td>
<td>4 (8%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>LDL ≥70 g/dL</td>
<td></td>
<td>106 (69%)</td>
<td>23 (79%)</td>
<td>23 (59%)</td>
<td>34 (68%)</td>
<td>8 (66%)</td>
</tr>
</tbody>
</table>

3.1. Activity Levels of MMP-2 and MMP-9 in Serum Samples and Their Correlation with Age. Sera from all subjects enrolled in this study were subjected to gelatin zymography to determine the relative levels of activity attributable to MMP-2 and MMP-9. Figure 1 shows a panel of 36 zymograms randomly selected among the collection of our samples. Parallel SDS polyacrylamide gels were run in order to ascertain the correct protein loading. The two prominent...
gelatinolytic bands represent the proenzymatic forms of MMP-2 (Pro-MMP-2) (72 kDa) and of MMP-9 (Pro-MMP-9) (92 kDa). Two additional lytic bands of 200 and 116 kDa, identified as MMP-9 dimers (220 kDa) and as MMP-9/TIMP1 complex (116 kDa), are also evident [40, 41]. In all serum samples, no activity levels were evident for the active forms of the MMPs.

The activity levels of Pro-MMP-9 in all samples appear more intense than Pro-MMP-2. In order to assess the relative variations of the two Pro-MMP levels in all subjects (Figure 2), gels, containing samples, run in triplicate were subjected to densitometric analysis by using the ImageJ software, as described in Materials and Methods.

The subjects are allocated in the gels as follows: group 1—young people, \( N = 29 \): lanes 12, 44, 45, 46, 75, 77, 79, 80, 81, 82, 85, 89, 90, 92, 98, 99, 100, 106, 109, 110, 115, 118, 122, 125, 128, 130, 136, 142, and 145; group 2—adult people, \( N = 39 \): lanes 11, 14, 15, 16, 25, 27, 33, 34, 39, 50, 52, 54, 60, 64, 66, 69, 72, 74, 78, 88, 91, 93, 95, 104, 105, 108, 116, 117, 121, 123, 124, 127, 131, 134, 135, 138, 139, 144, and 152; group 3—old people, \( N = 50 \): lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 17, 19, 20, 22, 23, 28, 29, 38, 42, 43, 47, 51, 55, 57, 63, 68, 76, 83, 84, 86, 87, 94, 97, 101, 102, 103, 113, 114, 119, 120, 126, 132, 137, 140, 141, 146, 149, 151, and 154; group 4—oldest old people, \( N = 12 \): lanes 31, 35, 53, 56, 58, 61, 62, 70, 71, 73, 129, 133; and group 5—LLIs, \( N = 24 \): lanes 18, 19, 20, ...
In the present study, the possible role played by MMPs in ageing and longevity has been focused. MMPs are a family of gelatinases and collagenases that play a crucial role in tissue remodelling and in the pathophysiology of inflammatory processes [45]. They are involved in various pathological conditions, such as atherosclerosis, cancer, and inflammation [46]. MMPs are produced by various cell types, including endothelial cells, smooth muscle cells, and immune cells [47].

The correlation between the serum activity levels of Pro-MMP-2 and CRP and UA was also analysed. In fact, MMP-2 activity plays a relevant role in tissue remodelling and in the pathophysiology of inflammation, CRP is an inflammatory marker [45], and serum UA is considered to have an antioxidant effect [46]. Interestingly, a significant correlation was found only in LLIs. In particular, we found an inverse correlation between CRP levels and Pro-MMP-2 activity in LLIs \( (r = -0.39, p < 0.05; \text{Figure 5(a)}) \). On the contrary, we found a positive correlation between UA levels and Pro-MMP-2 activity in LLIs \( (r = 0.41, p < 0.05; \text{Figure 5(b)}) \).

The data in Figure 6 are depicted also according to the gender where it is clear that in the female LLI population, Pro-MMP-2 activity inversely correlates with CRP \( (r = -0.5680, p = 0.0139) \) and positively correlates with UA \( (p = 0.512, p = 0.0305) \).

Since MMP-9 modulates cholesterol metabolism [26], we analysed the correlation between Pro-MMP-9 activity and CHO levels. Interestingly, we found a positive correlation between cholesterol levels and Pro-MMP-9 activity in LLIs \( (r = 0.52, p < 0.001) \).

We also found a positive correlation between LDL levels and Pro-MMP-9 activity in LLIs \( (r = 0.43, p < 0.05; \text{Figures 7(a) and 7(b)}) \).

The data in Figure 8 show the same results according to gender. In the male LLI population, Pro-MMP-9 activity correlates positively with CHO \( (r = 0.81, p = 0.0479) \) and with LDL \( (p = 0.77, p = 0.049) \).

4. Discussion

In the present study, the possible role played by MMPs in ageing and longevity has been focused. MMPs are a family of gelatinases and collagenases that play a crucial role in tissue remodelling and in the pathophysiology of inflammatory processes [45]. They are involved in various pathological conditions, such as atherosclerosis, cancer, and inflammation [46]. MMPs are produced by various cell types, including endothelial cells, smooth muscle cells, and immune cells [47].

The correlation between the serum activity levels of Pro-MMP-2 and CRP and UA was also analysed. In fact, MMP-2 activity plays a relevant role in tissue remodelling and in the pathophysiology of inflammation, CRP is an inflammatory marker [45], and serum UA is considered to have an antioxidant effect [46]. Interestingly, a significant correlation was found only in LLIs. In particular, we found an inverse correlation between CRP levels and Pro-MMP-2 activity in LLIs \( (r = -0.39, p < 0.05; \text{Figure 5(a)}) \). On the contrary, we found a positive correlation between UA levels and Pro-MMP-2 activity in LLIs \( (r = 0.41, p < 0.05; \text{Figure 5(b)}) \).
of structurally and functionally related zinc-dependent proteases with a wide range of substrates, including ECM components, cytokines, receptors, and cell motility factors. It is widely recognized that MMPs play a role in the pathophysiology of various tissues during growth, development, and ageing. Traditionally, the gelatinase members of the MMP family, MMP-2 and MMP-9, have been the easiest to detect using gelatin zymography; therefore, there are much more available data on them [47–49].

Previous studies demonstrated that ageing is associated with increased activities of MMP-2 [50] or of MMP-2 and MMP-9 [51] and concentration of active MMP-9 decreases with age [48].

Many studies on ageing showed that LLIs are the best models of ageing with success, so this old population is the best population to be studied. On the average of onset of age-associated diseases, centenarians have been divided into three profiles, survivors, delayers, and escapers. Survivors had a diagnosis of an age-associated disease prior to the age of 80. Delayers were affected by an age-associated disease after the age of 80. Escapers attained 100th year of life without the diagnosis of age-associated diseases [52]. Therefore, the extreme longevity is often characterized by a not unique and unequivocal phenotype, because there may be multiple routes to achieve exceptional longevity. However, each LLI can represent a model of “positive biology” by which it is possible to explain the biological mechanisms of health and well-being [44].

Changes associated with ageing affect the immune-inflammatory responses as shown by decline in immune
function and increase in the systemic proinflammatory status, i.e., immunosenescence, linked not only to the functional decline associated with the passage of time but also to antigen burden to which an individual has been exposed during lifetime [53]. The long-life chronic antigenic stress contributes to the chronic state of low-grade inflammation, inflammageing, observed in old people. Inflammageing is characterized by an increase in the levels of proinflammatory mediators. That, in turn, represents a negative prognostic factor for all causes of death. Oxidative stress plays an important role in determining and maintaining low-grade inflammation, which contributes to oxidative stress [54]. So, centenarians show an increase in many inflammatory molecules comparing to adults, but this condition is compensated by a concomitant activation of anti-inflammatory responses. This suggests that inflammageing may coexist with longevity.
especially if counterbalanced by an anti-inflammatory com-
ponent. Someone who will (probably) become a centenarian
should be able to keep inflammation down for longer [44].

The knowledge coming from these studies might provide
valuable information to achieve healthy ageing by modulat-
ing the ageing rate and pointing out a sort of longevity signa-
ture. The identi-
fi-
cation of the factors that predispose to a
successful ageing is of enormous interest for translational
medicine [3].

In our paper, the results show that serum activity of
MMP-2 increases in LLIs as compared to younger subjects.
Furthermore, in LLIs, we
fi-
nd a positive correlation of
MMP-2 with UA and an inverse correlation with CRP.

UA is the end product of endogenous and exogenous
purine metabolism, and some epidemiological studies sug-
gest that increased serum levels of UA are a risk factor for
diseases where oxidative stress plays an important role. On
the contrary, other evidence shows that UA might play a role
as an antioxidant [46]. The possible explanation lies in the
fact that UA might behave as oxidant getting older.

CRP, primarily secreted by the liver, is the most impor-
tant biomarker of inflammation, commonly evaluated for
monitoring treatment response and predicting long-term
outcome in inflammatory diseases. The serum levels of CRP
increase in an age-dependent manner and are good predic-
tors of physical and cognitive performance and of the risk
of mortality in both the entire old population and in success-
fully aged individuals [45].

Despite their name, MMP enzymes are not just bulk
degraders of matrix proteins but they also provide a mecha-
nism to add an additional layer of regulation to intercellular
communication, including inflammation. In particular,
MMP-2 regulates the processing of the chemokine
monocyte-chemotactic protein 3 generating a chemokine
receptor antagonist, which, in turn, might provide a regula-
tion of the inflamma-
tory signalling cascades directly exerted
by MMP-2 [55]. More interestingly, MMP-2 activation is
responsible for degrading the alarmin S100A9, thus limiting
inflammation-inducing signals. It is relevant that alarmins
are mediators of sterile inflammation in ageing and age-
related diseases [56].

Therefore, the anti-inflammatory activity of MMP-2 can
explain the negative and positive associations, respectively,
with CRP and UA as well as the increased level in LLIs when
compared to other younger groups. However, a note of cau-
tion in the interpretation of these results should be added
because it has been demonstrated that antihypertensive drugs
may reduce Pro-MMP-2 activity [57] and we do not have this
information for most subjects under study.

MMP9 affects cholesterol metabolism, at least in part,
through a MMP-9 plasma-secreted phospholipase A2 axis
that affects the hepatic transcriptional responses to dietary
cholesterol. Therefore, it has been proposed that the dysreg-
ulation of MMP-9 can contribute to the development of meta-
abolic disorders that could, ultimately, lead to atherosclerosis
and coronary heart disease [26]. The MMP-9 results show

Figure 8: Histograms of Pro-MMP-9 activity levels and hematological values of CHO in the female (a) and male (b) LLI group and of LDL in
the female (c) and male (d) LLI group.
that their levels do not increase in LLIs and that the levels are highly variable in the whole population.

On the whole, the observed increase of MMP-2 in LLIs might suggest a positive role in the attainment of longevity. Interestingly, MMPs have been characterized as bifunctional proteins in Alzheimer’s disease, with some of them, such as MMP-2 and MMP-9, displaying protective roles during disease progression, while others promote disease evolution [7].

However, it is difficult to make wide-ranging conclusions/assumptions based on these observations in view of the relatively small sample size of LLIs. Nonetheless, we believe that this is an important starting point for future larger-scale studies required to warrant these findings including the link with other systemic inflammatory and antioxidant markers.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to privacy reasons but are available in anonymized form from the authors on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Patrizia Cancemi and Anna Aiello contributed equally to this work.

Acknowledgments

This research was funded by PRIN (PRIN-20157ATSLF_009; DESIGN: Discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and comorbidities) to C.C. and G. C. and by Stiftelsen Olle Engkvist Byggmästare to VS.

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