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The Phenotypic Characterization of the Cammalleri Sisters, an Example of Exceptional Longevity

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Abstract

This article shows demographic, clinical, anamnestic, cognitive, and functional data as well as biochemical, genetic, and epigenetic parameters of two exceptional siblings: Diega (supercentenarian) and Filippa (semisupercentenarian) Cammalleri. The purpose of this study is to provide new insights into the extreme phenotypes represented by semisupercentenarians and supercentenarians. Different studies have been published on supercentenarians, but to the best of our knowledge, this is the only concerning two sisters and the most detailed from a phenotypic point of view. Our findings agree with the suggestion that supercentenarians have an increasing relative resistance to age-related diseases, approximating the limits of the functional human reserve to address successfully the acute causes of death. More interestingly, our data agree with, and extend, the suggestion that inflammation and oxidative stress predict centenarian mortality.

Keywords: inflammation, longevity, oxidative stress, semisupercentenarian, supercentenarian

Introduction

Population aging is a global issue that is becoming severe and is posing new challenges for public health.[1] In this context, it is essential to study models of healthy aging and extreme longevity. Among long living individuals (LLIs, ≥90 years), centenarians (≥100 years), semisupercentenarians (≥105 years), and supercentenarians (≥110 years) are object of intense investigation.

Centenarians are considered the most successful aged individuals. They were able to escape diseases or survive to age-related diseases such as cancer, diabetes, cardiovascular diseases, and stroke.[2] Approximately, 1 in 1000 centenarians achieves the age of 110.[3] These individuals can provide useful data to better understand phenotypic aspects of longevity and to identify factors associated with long-term good health. It is a crucial information in an aging society, in which the improvement of the quality of life of the oldest people is becoming a priority because of the continuous increase in number of this population at risk of frailty.[1] Moreover, exceptional longevity is a rare event that may involve mechanisms other than those implicated in common human aging. It is typically characterized by strong genetic familiarity associated with lifestyle factors.[4,5]

This article shows demographic, clinical, anamnestic, cognitive, and functional data as well as biochemical, genetic, and epigenetic parameters of two exceptional siblings: Diega (supercentenarian) and Filippa (semisupercentenarian) Cammalleri. The purpose of this study is to provide new insights into the extreme phenotype represented by semisupercentenarians and supercentenarians.

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Participants and Methods

Participants

The recruitment of the two sisters was conducted within the project ''Discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and comorbidities (DESIGN),'' funded by the Italian Ministry of Education, University and Research. The Ethic Committee of Palermo University Hospital (Sicily, Italy) approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki and its amendments.

On July 27, 2017, well-trained nutritionists and physicians from University of Palermo administered to the sisters a detailed questionnaire to collect demographic, clinical, and anamnestic data of interest as well as cognitive and functional tests. The questionnaire includes nine sections: main pathologies, drugs, smoking, cognitive status (mini-mental state examination [MMSE]), geriatric depression scale (GDS), activities of daily living (ADL), instrumental activity of daily living (IADL), sleep, and eating habits. We also investigate the family history. Before the enrolment, their nephew, and legal guardian, signed the consent form to release the photos and the sensitive family data. The sisters underwent venipuncture, after a fasting period of 12 hours, in the morning (10 AM). The blood was collected in specific tubes containing ethylenediaminetetraacetic acid (EDTA) or without additives.

Anthropometric measurements

To obtain informative and reproducible body composition analysis, weight and height were measured, and bioelectrical impedance analysis (BIA) was performed in the supine position, with light clothes and barefoot. For the tetrapolar measurement, four skin electrodes were applied, one pair on the back of the hand and the other pair on the back of the ipsilateral foot. On the hand, they were placed one on the metacarpophalangeal joint of the third finger (injector electrode) and the other on the radioulnar joint (sensor electrode). On the foot, they were placed one on the metatarsophalangeal joint of the third toe (injector electrode) and the other on the tibiotarsal joint (sensor electrode).

BIA device used was a portable Akern BIA 101, more suitable for at home visit, applying an alternating electrical current of 50 kHz (800 μA), not dangerous for tissues. The resistance and the reactance (in Ohm) and the phase angle were reported, and the body compartments measurements (in percentage by weight and in kg/m) were estimated, using regression equations by BodygramPlus 1.1.4.4 software.

Hematochemical, biochemical, and oxidative stress tests

Hematochemical tests were performed at the department of laboratory medicine, Palermo University Hospital ''P. Giaccone,'' according to standard procedures. The evaluation of inflammatory and oxidative parameters was conducted at the department of biomedical sciences, University of Sassari, Italy, as previously described.[6–9]

Molecular tests

Genomic DNA was extracted from leukocytes through a commercial kit. The single nucleotide polymorphism (SNP) rs2802292 G-allele (G>T) of Forkhead box O3A (FOXO3A) gene was genotyped using an amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR), adopting registered and validated primers (certified at World International Property Organization, on 18/02/2010, n.WO 2010/019519 A2). Three genotypes were analyzed: GG, GT, and TT. The size separation was conducted using agarose gel electrophoresis (2%).

The EzWayTM Direct ApoE Genotyping Kit (Komabiotech Inc) was used to analyze Apolipoprotein (Apo)E polymorphisms through a standard PCR. The genotype was defined by the combination of three alleles $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. The primer mixture of ApoE genes was enabled to perform one-step multiplex PCR. Six genotypes were analyzed: $\varepsilon 2/\varepsilon 2$, $\varepsilon 2/\varepsilon 3$, $\varepsilon 3/\varepsilon 3$, $\varepsilon 3/\varepsilon 4$, $\varepsilon 4/\varepsilon 4$, and $\varepsilon 2/\varepsilon 4$. The size separation was conducted using agarose gel electrophoresis (2.5%).

Relative telomere length (RTL) was determined in the Dana Farber/Harvard Cancer Center Genotyping & Genetics for Population Sciences Facility (Boston, MA), using a modified high-throughput version of the real-time quantitative (Rtq) PCR-based telomere assay that was run on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA).[10] Fifteen nanograms of genomic DNA were required for the protocol. The average RTL was determined as the copy-number ratio between telomere repeats and a single-copy (36B4) reference gene (T/S ratio, $-\Delta$Ct). Leukocyte RTL is reported as the exponentiated T/S ratio corrected for a reference sample. Although this assay measures RTL, the T/S ratio highly correlates with absolute TL provided by Southern Blot ($r = 0.68$–0.85; $p < 0.001$).[11]

The analysis of three circulating microRNAs (mir-146a-5p, mir-126-3p, and mir-21-5p) of the two sisters and 30 controls was conducted in the laboratories of National Research Council of Palermo (Sicily, Italy). The 30 control subjects, subdivided into young (24–39 years, $n = 10$), adult (50–64 years, $n = 10$), and old (66–84 years, $n = 10$), were randomly selected from young, adult, and old people recruited within the DESIGN project. They were checked and judged to be in good health, based on their clinical history and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C-reactive protein [CRP], liver function test, iron, total proteins, cholesterol, and triglycerides). Circulating microRNAs were isolated using the miRNeasy Serum/Plasma kit (Qiagen) according to the manufacturer's protocol. For each sample, the spike-in control, cel-miR-39, was used for normalization and extraction efficiency control.

The analysis was conducted using a TaqMan RT-qPCR microRNA assay. In brief, the isolated microRNAs were retrotranscribed using the miScript Single Cell qPCR kit (Qiagen) according to the manufacturer's protocol. The expression levels of microRNAs were evaluated with a SYBR green-based RT-qPCR using the Step one plus (Applied Biosystem). For the amplification, the miScript SYBR green PCR kit (Qiagen) was used according to the manufacturer's protocol.[12]

Results

Anamnestic and clinical data

Diega and Filippa Cammalleri (Fig. 1) were born and died in Canicattì (Agrigento, Sicily, Italy). Diega was born on

FIG. 1. The picture depicts the two sisters with Prof. Calogero Caruso, Filippa on the left and Diega on the right. Photo used with permission.

October 23, 1905, and died on June 15, 2019. For the validation of her age, see Young and Kroczek.[13] Filippa was born on December 12, 1911, and died on July 6, 2018 (Supplementary Figs. S1 and S2 depict her Italian identity card and death certificate). Their parents, Calogero Cammalleri and Maria Di Pasquale, died, respectively, at the age of 97 years (unknown cause) and at the age of 73 years (colon cancer). Their parents had three other sons, two twins, both died of colon cancer, and a third son died for a traumatic accident (Fig. 2 depicts the Cammalleri's family tree with birth and death dates). Diega earned a high school diploma as teacher for primary school, whereas Filippa received only the primary school diploma (5 years). Diega was a schoolteacher and Filippa was a housewife. Both were unmarried, and they lived in a flat with a live-in caretaker. The sisters had a quite high income.

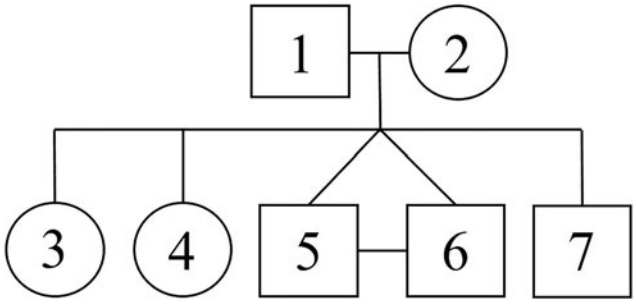

FIG. 2. The figure depicts the Cammalleri's family tree. The birth and death dates are, respectively, (1) June 19, 1875 to March 18, 1972 (Calogero Cammalleri, father); (2) June 24, 1886 to March 1, 1960 (Maria Di Pasquale, mother); (3) October 23, 1905 to June 15, 2019 (Diega Cammalleri); (4) December 12, 1911 to July 6, 2018 (Filippa Cammalleri); (5) May 5, 1918 to January 31, 2000 (Giuseppe Cammalleri, one of the twin brothers); (6) May 5, 1918 to May 4, 2002 (Antonio Cammalleri, the other twin brother); (7) May 15, 1914 to February 2, 2001 (Luigi Cammalleri, the third brother).

They never smoked, slept 5–6 hours per night, and were treated with antihypertensive drugs, diuretics, and antiplatelet agents. Diega was affected by macular degeneration and Filippa by cataract. Both suffered from arthrosis and osteoporosis. Over the last years, Filippa was admitted twice to the hospital. The first time, at the age of 100 years, for femur fracture, and then, at the age of 105 years, for fecal impaction. It was not possible to administer the GDS to both sisters, whereas the MMSE was administered to Filippa, receiving a score of 23 out of 30 (mild dementia). The assessment of ADL (*e.g.*, personal hygiene, dressing, toileting/continence, ambulating, and eating) showed that the sisters needed help for <10 years in terms of basic physical needs. The IADL (*e.g.*, food preparation, financial administration, housekeeping, use of telephone, and responsibility for own medication) showed that the sisters were not able to perform this complex behavior of domestic functioning in the last 10 years.

About their eating habits, Cammalleri sisters did not show close adherence to the Mediterranean diet except for the assumption of cereals, such as pasta, extra virgin olive oil, milk, and fruits that they consumed twice a day, and legumes that they consumed twice a week. On the contrary, it was detected low consumption of vegetables (two/three times a week), high assumption of sweets (such as biscuits, small pastries, and sugar) twice a day, and eggs and potatoes once a day. About meat consumption, it was noticed very low intake of red and cured meat (one or two times in a month),

frequent assumption of white meat (such as chicken) once a day, and moderate consumption of bluefish, once a week.

Anthropometric measurements

Table 1 shows anthropometric and bioimpedance values. For Diega, body mass index (BMI), fat mass (FM), both in percentage on weight and in effective measure, and free fat mass (FFM) in percentage and total body water, both in percentage on weight and in effective measure, were inside the range. For Filippa, BMI, FM in effective measure, and total body water in effective measure were inside the range. The rest of the values, including PhA, were out of range.

Hematochemical and biochemical tests

Table 2 shows the hematochemical values. It is possible to observe a slight anemia in Filippa, whose total and low-density lipoprotein (LDL) cholesterol levels were higher than the reference values. Both sisters showed values of thyroid-stimulating hormone (TSH) and osteocalcin higher than the reference values, whereas albumin and protein as well as high-density lipoprotein (HDL) cholesterol levels were lower than the reference values.

Table 3 depicts the oxidative and inflammatory parameters. The obtained results pointed out in both sisters lower values than the reference ones (obtained from subjects between 50 and 65 years old) for paraoxonase (PON), arginine, taurine (TAU), tryptophan (TRP), and blood reduced glutathione (GSH), and higher than reference value for malondialdehyde (MDA), asymmetric dimethylarginine (ADMA), kynurenine (KYN), KYN/TRP ratio, homocysteine (HCY), and cysteine (CYS).

Table 1. Anthropometric and Body Composition Values

Parameters	*D.C.*	*F.C.*	*Reference range values*
Age (years.days)	111.277	105.227	—
Height (cm)	155	153	—
Weight (kg)	53	50	—
BMI (kg/m^2)	22.1	21.4	18–24.9
FM (%)	36.9	***30.4***	31.2–42.5
FM (kg/m)	12.6	9.9	7–14
FFM (%)	63.1	***69.6***	57.5–68.8
FFM (kg/m)	***21.6***	***22.8***	23–28
TBW (%)	54.2	***59.6***	46–57
TBW (L/m)	18.5	19.5	15–22
ECW (%)	***72.5***	***69.3***	42–53
BCM (%)	***25.1***	***28.6***	38–70
BCM (kg/m)	***5.4***	***6.5***	10–17
BMR (Kcal)	993.4	1039.1	—
Rz (Ω)	620	551	—
Xc (Ω)	25	25	—
PhA (°)	***2.3***	***2.6***	3.4–5.8

Values out of range are in bold, italic and underlined.

BCM, body cell mass; BMI, body mass index; BMR, basal metabolic rate; D.C., Diega Cammalleri; ECW, extracellular water; F.C., Filippa Cammalleri; FFM, free fat mass; FM, fat mass; PhA, phase angle; Rz, resistance; TBW, total body water; Xc, reactance.

Table 2. Hematochemical Values

Variable (U)	*D.C.*	*F.C.*	*Reference range value*
Endocrine system			
TSH (μIU/mL)	***5.01***	***5.13***	0.27–4.20
FT3 (pg/mL)	2.56	2.23	2.00–4.40
FT4 (ng/dL)	1.00	1.12	0.93–1.70
Insulin (μU/mL)	5.29	4.39	2.60–24.90
Osteocalcin (ng/mL)	***82.3***	***58.3***	14.00–46.00
Electrolytes			
Potassium (mmol/L)	4.51	5.05	3.50–5.10
Calcium (mg/dL)	8.58	8.15	8.40–10.20
Magnesium (mg/dL)	2.09	2.40	1.60–2.60
Liver markers			
ALT (U/L)	7	8	<41
AST (U/L)	12	17	<40
ALP (U/L)	81	71	40–129
GGT (U/L)	8	8	8–61
Bilirubin (mg/dL)	0.35	0.52	<1.20
Conjugated bilirubin (mg/dL)	0.16	0.16	<0.30
Albumin (g/L)	***34.7***	***34.1***	38–48
Proteins (g/L)	***49.7***	***73.4***	66–87
Iron markers			
Iron (μg/dL)	42	63	37–145
Ferritin (ng/mL)	28	31	15–400
Transferrin (mg/dL)	213	250	200–360
Lipid values			
Cholesterol (mg/dL)	171	***248***	<200
LDL (mg/dL)	115	***178***	70–129
HDL (mg/dL)	***38***	***50***	>50
Triglycerides (mg/dL)	92	102	<200
Other values			
Creatinine (mg/dL)	0.7	1.2	0.5–1.2
Urea (mg/dL)	***49.7***	***73.4***	16.8–48.5
Glycemia (mg/dL)	89	74	70–100
Uric acid (mg/dL)	4.1	6.9	2.4–7.0
CRP (mg/dL)	1.14	1.59	<5
Hematological values			
Red blood cells (10^{6}μL)	5.09	4.09	3.80–5.00
Hemoglobin (g/dL)	14.30	***11.90***	12–16
Platelets (10^{3}μL)	250	215	150–450
Leukocytes (10^{3}μL)	6.14	5.95	4.00–11.00
Neutrophils (10^3 μL)	4.33	3.22	2.00–8.00
Lymphocytes (10^3 μL)	1.13	1.80	1.00–5.00
Monocytes (10^3 μL)	0.52	0.68	0.16–1.00
Eosinophils (10^3 μL)	0.12	0.22	0.00–8.00
Basophils (10^3 μL)	0.04	0.03	0.00–1.50

Values out of range are in bold, italic, and underlined.

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

Molecular findings

Table 4 reports FOXO3A, APOE, and RTL data. Concerning FOXO3A gene, only Filippa carried one copy of the allele associated with longevity (the G SNP).[14] Regarding ApoE, both Diega and Filippa carried ε3/ε3. The RTL of these two sisters fitted in the range of 60–69 Sicilian women (unpublished observations).

Table 3. Oxidative and Inflammatory Tests

Variable (U)	*D.C.*	*F.C.*	*Reference range values*
PON (U/L)	***43***	***52***	147–340
MDA (μmol/L)	***3.19***	***5.78***	2.46–3.23
Arginine (μmol/L)	***23.9***	***55.0***	68.3–90.4
ADMA (μmol/L)	***0.762***	***0.725***	0.454–0.544
ADMA/Arg ratio	***0.0320***	***0.0132***	0.0056–0.0077
KYN (μmol/L)	***1.256***	***2.193***	0.54–0.96
TRP (μmol/L)	***27.9***	***34.4***	56.0–71.2
KYN/TRP ratio	***0.0450***	***0.0638***	0.0080–0.0140
HCY (μmol/L)	***33.9***	***36.5***	3.1–30.3
CYS (μmol/L)	***333***	***533***	170–294
TAU (μmol/L)	***21.6***	***26.7***	49–76.8
GSH (μmol/L)	***544***	767	621–1085

Values out of range are in bold, italic, and underlined.
For explanation see text.
ADMA, asymmetric dimethylarginine; CYS, cysteine; GSH, blood reduced glutathione; HCY, homocysteine; KYN, kynurenine; MDA, malondialdehyde; PON, paraoxonase; TAU, taurine; TRP, tryptophan.

The profile of three circulating microRNAs, mir-146a-5p, mir-126-3p, and mir-21-5p, known to be involved in inflammation, senescence, and carcinogenesis,[15] has been also characterized. The levels of these microRNAs in the sisters were comparable with those measured in young (24–39 years) and middle-aged individuals (50–64 years), rather than those found in old subjects (66–84 years) (Fig. 3a–c). However, a caution note needs to be added for the small number of comparisons.

Discussion

The Cammalleri sisters case is intriguing because three members of this family were long living (the two sisters and the father), whereas other three died of colon cancer (two brothers and the mother). It might be relevant to the suggestion of Sebastiani et al.,[16] based on the whole genomic sequences of two supercentenarians, that several genes may have pleiotropic effects, and the same gene(s) that can increase risk for disease might also increase the ability to live long, depending on the genetic background. Another hypothesis might be that the two sisters have inherited from father alleles that protected them from age-related diseases.[17]

About the diet of the two sisters, it was rich in bioactive Mediterranean foods as fruits and legumes but no close adherent to traditional Mediterranean diet. Since the 1960s, the consumption of meat, fish, fats, and sugars had significantly increased in the Italian Southern regions, whereas the consumption of bread, pasta, cereals, vegetables, and olive oil had decreased. It is likely that during this nutritional transition there was a great change in the diet of the two sisters, as in the rest of the Italians. Accordingly, the preliminary analysis of our survey in Sicilian LLIs confirms their adherence to Mediterranean diet, during young age, for scarcity of food rather than for choice.[18] Necessarily, the nutritional options were strictly seasonal, and the amount of food was enough but never in excess. There were no variety of dishes but rather a monotonous diet, often based on legumes as main choice.[14,19] This dietary habit might influence the ability of an individual to reach extreme longevity by epigenetic modifications.[20]

Table 4. Genetic Tests

Test	*D.C.*	*F.C.*
FOXO3A	T/T	G/T
ApoE	ε3/ε3	ε3/ε3
RTL	0.83	0.79

For explanation see text.
RTL, relative telomere length.

The analysis of body composition demonstrated hyperhydration in extracellular compartment (Diega Cammalleri [DC] + 25%, Filippa Cammalleri [FC] + 21.8%) and lower PhA (PhA mean = 4.6°, DC −2.3°, FC −2°). Using the PhA we should classify the two sisters as cachectic. These data are not surprising because are in line to that we saw in Sicilian LLIs (mean age 101.3 ± 4.9, range 2–4.5°).[14] The higher is the PhA value, the better is the healthy condition. PhA decreases when the body cell mass lowers. Moreover, it depends on the extracellular water with an inverse proportionality. Lower PhA appears to be consistent with either cell death or a breakdown in the selective permeability of the cell membrane, in accordance with hyper extracellular hydration (edema). About FFM, the expectation is a decline of this value with age and increase in body fat, due to disability and overall physical inactivity. In this case, it is possible to observe contrasting results between the two sisters. For Diega it was observed a percentage of FFM on weight perfectly in the range (63.1%) even if no physical activity nor ADL or IADL was performed.

BIA specifically evaluates hydration in any condition (clinical or not) and it is a suitable method for nutritional assessment. However, it presents limitations due to substantial prediction errors associated with the use of calculation for the evaluation of body compartments.[21] Current models have been developed starting from analyses in healthy subjects. Indeed, it is important to highlight that the measurement of body composition with BIA permits to directly measure reactance and resistance only. All the rest of measurements are estimated, rather than directly measured, using formulas that are probably not suitable for centenarian population that likely suffers massive fluids and electrolysis changes.[22]

In relation to BMI, usually centenarians are underweight but, in this case, both have normal weight. Moreover, their estimated FM (kg/m) is within the range. These data are interesting considering that underweight and overweight conditions, as well as reduction or increase of FM (compared with normal range), are unfavorable for longevity. It is likely that, in certain people, such as old individuals, fat accumulation protects from death. This is the so-called obesity paradox that implies an inverse correlation between BMI and mortality, as demonstrated by several studies, although it could be related to a low specificity of BMI for certain people.[23–27]

BIA analysis would be a piece of the puzzle to better detail the two extreme longevous sisters. It is important to note that the use of reference range values could be unsuitable for centenarians. In fact, they usually have a body composition different from young, adult, and old people, due to the unavoidable reduction of muscle mass.

In 2008, a study was published in which, in a sample of 381 old subjects (between 65 and 85 years) and 120 healthy

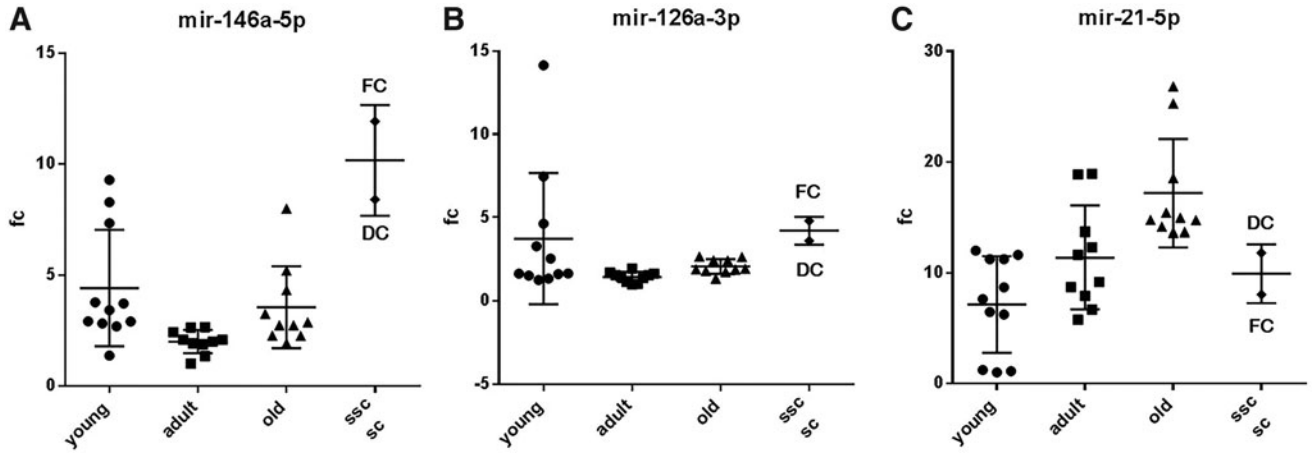

FIG. 3. **(a–c)** Comparison of expression variance of mir146a-5p **(a)**, mir-126a-3p **(b)**, and mir-21-5p **(c)** in plasma samples of the sisters (semisupercentenarian, ssc; supercentenarian, sc), (◆) and 10 young (•), 10 adults (■), 10 old (▲) healthy individuals. All values were normalized with respect to the lowest samples and plotted on the Y-axis as folds change (fc). FC showed higher values than DC in **(a)** and **(b)**, whereas Diega showed a higher value than Filippa in **(c)**. Data from RTq-PCR assays. DC, Diega Cammalleri; FC, Filippa Cammalleri; RTq-PCR, real-time quantitative polymerase chain reaction.

centenarians of Sicilian and Italian ancestry, laboratory parameters were evaluated. Compared with old people, significant differences were observed in blood urea nitrogen levels increased in centenarians, whereas blood glucose, cholesterol, and platelet levels were reduced in centenarians.[28] In the ongoing DESIGN research on Sicilian LLIs preliminary results suggest no differences concerning lipid profile, glucose, and insulin levels when compared with young (mean age 30.7 ± 4.8) individuals, whereas creatinine was increased (unpublished observations). However, Filippa displayed quite high levels of cholesterol and LDL. At this regard, it is noteworthy that Filippa, but not Diega, showed very high levels of serum matrix metalloproteinases (MMP)-9 activity (unpublished observations) that correlates with cholesterol and LDL values, since it is known that MMP-9 affects cholesterol metabolism.[29]

Regarding the increase of TSH observed in both sisters, familial longevity maybe associated with increased basal TSH secretion.[30] In contrast, the two sisters should have subclinical hypothyroidism (high TSH, normal thyroid hormones) that could explain dyslipidemia (together with eggs consumed once a day), as well as low albumin levels and overhydration at BIA. In fact, as extensively discussed, clinical or latent hypothyroidism is rising both in general and in old population. Accordingly, it has been suggested that the reduction of thyroid signaling during older age might be beneficial for optimal aging, perhaps by lowering metabolism.[31] Regarding osteocalcin, the increase is not surprising because osteocalcin, an osteoblast-specific secreted protein expressed by mature osteoblasts, is used in clinical practice and in research as a marker of bone turnover.[32] Although the blood urea nitrogen levels were out of range, the creatinine levels were low likely due to their sarcopenia. It is noteworthy that CRP values were in the reference range, although other inflammatory markers were increased. Most of these values fall within the range of the values of the few hematochemical data studied in the only two reports published on hematochemical data of supercentenarians.[33,34]

Aging is associated with a rise in pro-oxidant factors and to a decline in antioxidant mechanisms. Oxidative stress plays an important role in determining and maintaining the low-grade inflammation, which in turn contributes to oxidative stress.[2,35,36] However, in several centenarian groups, from different geographical areas, some indices of oxidative stress were lower than in aged subjects. In contrast, centenarians show an increase in many inflammatory molecules, and it has been suggested that the proinflammatory status should be an adaptive mechanism that occurs throughout life and might coexist with longevity especially if counterbalanced by an anti-inflammatory component.[2,36,37]

The sisters show a suffering status in the oxidative stress profile. They showed low levels of antioxidant PON and GSH (no for Filippa) as well as of sulfur amino acids CYS, HCY, and TAU. On the contrary, they showed high levels of MDA, the main product of the polyunsaturated fatty acids peroxidation, and ADMA.[6–9] Since oxidative stress increases the activity of arginine methylating enzymes involved in ADMA synthesis and reduces ADMA degrading enzymes, increased ADMA concentrations and increased ADMA/arginine ratio are the mirror of increased oxidative stress.[38] They simultaneously showed a worsened inflammatory condition as demonstrated by high levels of KYN, a marker of immune system activation, and KYN/TRP ratio, a sensitive biomarker of systemic inflammation.[6–9] However, both sisters showed levels of mir-21-5p, considered a biomarker of aging inflammatory status, similar to that of young people.[15]

Recent studies have, in fact, revealed that human aging can be characterized by a profile of circulating microRNAs that is predictive of chronological age and that can be used as a biomarker of risk for age-related outcomes. In particular, three microRNAs, mir-146-5p, mir-126-3p, and mir-21-5p, involved in pathways related to inflammation, senescence, and carcinogenesis, seem to be characteristic of the longevity phenotype.[15,39] The three microRNA plasmatic levels observed in sisters suggest that the two subjects were experiencing healthy aging conditions relatively to

age-related diseases. Accordingly, it has been demonstrated that the offspring of semisupercentenarians have a lower epigenetic age than age-matched controls.[40] However, it is intriguing that mir-146-5p and mir-126-3p are involved in endothelial dysfunctions and that supercentenarians have been claimed to markedly delay and even escape clinical expression of vascular disease toward the end of their exceptionally long lives.[15,41]

Oxidative stress and inflammation are also important telomere modulators and these processes accelerate the telomere attrition. Therefore, short telomeres represent a marker of the cumulative load of inflammation and oxidative stress. In fact, a larger study has demonstrated that short telomeres are associated with a higher risk of all-cause mortality. Conversely, individuals who lead a healthy lifestyle have been shown to have longer telomeres than those who do not adhere to this lifestyle.[42,43] The RTL of the sisters fitted in the range of 60–69 Sicilian women RTL. In fact, in our sample of Sicilians, LLI women have RTL not significant different from those observed in women in the 60–69 years old range (unpublished observations).

FOXO3A, a member of the Forkhead family of transcription factors, is an evolutionary conserved transcription factor that plays important regulatory roles in insulin/insulin-like growth factor signaling. The FOXO3A rs2802292 was associated with longevity in different populations, likely due to an increased expression of FOXO3A, involved in homeostatic responses.[44,45] Only one of the two sisters carried the G allele associated with longevity. However, this is not surprising because in our analysis of FOXO3A SNP in Sicilian LLIs, we did not observe an association with longevity.[14]

ApoE has multiple effects on aging and longevity. The $\varepsilon 4$ allele is a risk factor for the onset of Alzheimer's disease and has a deleterious effect on longevity. $\varepsilon 2$ is the allele that promotes longevity and healthy aging, whereas the $\varepsilon 3$ allele is a neutral allele.[14,46] Both sisters were homozygotes for ApoE $\varepsilon 3$; therefore, they carried neither the protective allele $\varepsilon 2$ nor the detrimental $\varepsilon 4$. It is noteworthy that in our small sample of Sicilian LLIs, we did not observe an association of $\varepsilon 2$ allele with longevity, but we did not find $\varepsilon 4$ alleles in our LLI population.[14] These results are consistent with a recent analysis on a large group of 1309 subjects enriched of Southern Italian genetic ancestry with weaker protective effect of $\varepsilon 2$ and no detrimental effect of $\varepsilon 4$. The author hypothesis is that the Mediterranean diet that was followed in Italy by generation under study contributes to the weaker role of $\varepsilon 2$ and $\varepsilon 4$ alleles.[46]

Supercentenarians generally delay or escape age-related diseases and disability well beyond the age of 100 and this exceptional survival has been claimed to be influenced by a genetic predisposition that includes both common and rare genetic variants.[16] However, Gierman et al. have sequenced the genomes of 13 supercentenarian Caucasian women. From this relatively small sample size, they found no significant evidence of enrichment for a single rare protein-altering variant or for a gene, harboring different rare protein altering variants with extreme longevity.[47]

Conclusion

Compared with centenarians, supercentenarians are extremely rare. Unlike so-called blue zones, where centenarians are said to cluster, seemingly there is no geographical shortcut for netting supercentenarians. However, geographic area where supercentenarians are more numerous have been reported (*i.e.*, Japan that is considered the country with the highest number of supercentenarians[48]). In 2020, the number of living validated supercentenarians by the ''Gerontology Research Group'' is 27 worldwide.[13] In Sicily, according to the Italian supercentenarians database, the number of living is estimated to be one (https://www.supercentenariditalia.it/persone-viventi-piu-longeve-in-italia). Nevertheless, it is difficult to estimate the exact number of supercentenarians in the world, because some individuals lack a birth certificate, or it might be difficult to obtain a reliable birth certificate.

The analysis of these individuals who have survived to extremely old ages should provide insight into the processes that contribute to the maintenance of health during aging, which has assumed a central position in terms of refining preventive intervention for health promotion at advanced ages.

Different studies have been published on this topic, but to the best of our knowledge this is the only concerning two sisters and the most detailed from a phenotypic point of view.[3,16,33,34,41,47,49–54]

Our findings agree with the suggestion that supercentenarians and semisupercentenarians have increasing relative resistance to age-related diseases, approximating the limits of the functional human reserve to successfully address the acute causes of death.[49] More interestingly, our data agree with and extend the suggestion that inflammation and oxidative stress predict centenarian mortality.[51]

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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