Functional transcriptomic analysis of centenarians' offspring reveals a specific genetic footprint that may explain that they are less frail than age-matched non-centenarians' offspring.

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ABSTRACT

Centenarians exhibit extreme longevity and compression of morbidity and display a unique genetic signature. Centenarians' offspring seem to inherit centenarians' compression of morbidity, as measured by lower rates of age-related pathologies. We aimed to ascertain whether centenarians' offspring are less frail and whether they are endowed with a "centenarian genetic footprint" in a case-control study, matched 1:1 for gender, age ±5 years, and place of birth and residence. Cases must have a living parent aged 97 years or older, aged 65-80 years, community-dwelling, not suffering from a terminal illness, or less than 6 months of life expectancy. Controls had to meet the same criteria as cases except for the age of death of their parents (not older than 89 years). Centenarians were individuals 97 years or older. Frailty phenotype was determined by Fried's Criteria. We collected plasma and peripheral blood mononuclear cells from 63 centenarians, 88 centenarians' offspring, and 88 non-centenarians' offspring. miRNA expression and mRNA profiles were performed by the GeneChip miRNA 4.0 Array (Thermo Fisher Scientific) and GeneChip Clariom S Human Array (Thermo Fisher Scientific), respectively. We found a lower incidence of frailty among centenarians' offspring when compared to their contemporaries' non- centenarians' offspring (p <0.01). Both miRNA and mRNA expression patterns in centenarians' offspring were more like those of centenarians than those of non-centenarians' offspring (p <0.01). in conclusion, centenarians' offspring are less frail than age-matched non-centenarians' offspring, and this may be explained by their unique genetic endowment.

Keywords: exceptional longevity, genetics, RNA, frailty.

INTRODUCTION

The proportion of people aged over 60 years is growing faster than any other age group, as a result of both longer life expectancy and declining fertility rates (1). Much of the research in this area has shifted to increasing the number of years spent free of disabilities (healthspan), which is often referred to as 'successful ageing'. Centenarians are regarded as model cases for such 'successful ageing' (2), since they seem to avoid or largely delay the onset of age-related diseases or geriatric syndromes (3), thus showing a decelerated trajectory of ageing (4).

Previous work from our group showed that centenarians display a unique miRNA profile and maintained a high rate of miRNA biogenesis pathway when compared to septuagenarians (5, 6). We also demonstrated that the apoptosis-related factors *BCL-xL, FAS, and FAS-L* are overexpressed in centenarians when compared to septuagenarians, with *BCL-xL* playing a critical role in their mRNA profile (7). Moreover, centenarians maintain their stemness better than septuagenarians (8). Results from genomic, epigenomic (9), transcriptomic, metabolomic (10) and lipidomic analysis (11, 12), glycomics (13), and metagenomics (14) indicate that centenarians are endowed with a unique resilience through life that leads to a deceleration of the aging rate (15).

Despite being such an extraordinary model to study longevity, given the lack of an appropriate control group and the impossibility of studying centenarians for long periods for obvious reasons, it becomes necessary to find a similar model to clarify the molecular and genetic basis of healthy aging. One potential model for such a purpose may be centenarians' offspring (CO). Indeed, previous epidemiological studies have reported a strong genetic and familiar component of longevity, thus suggesting that CO displays a significant survival advantage with a higher probability of becoming long-lived and a lower risk for major age-related pathologies, such as cancer, cardiovascular diseases (CVD) and diabetes (16, 17).

Furthermore, two previous studies showed that CO was less frail than the offspring of non-centenarians (NCO) (18, 19), but the authors of these studies did not provide any potential biological reason for this fact.

Thus, we hypothesized that compression of disability, perhaps more than morbidity, is an important marker of exceptional longevity, which may be determined by a particular genetic profile. Based on our previous work on centenarians, we aimed to ascertain whether CO is less frail and is endowed which a particular "genetic footprint" when compared to their contemporaries NCO.

METHODS

Participants

The sample consisted of 63 centenarians, 88 centenarian offspring (CO), and 88 non-centenarian offspring (NCO) living within an area near Valencia called La Ribera (11th Health Department of the Valencian Community, Spain). A subsample of 9 centenarians, 8 CO, and 10 NCO with no familiar linkage participated in the transcriptomic analysis. All individuals or their relatives were informed about the aims and scope of the study, including all potential risks. Written informed consent was obtained before participation. The inclusion criteria were: i) centenarians: being aged 97 or more at the beginning of the study (20); ii) CO: community-dwelling individuals aged 65-80 years old having a mother or father who had lived to or beyond the age of 97 years; iii) control NCO, being: age (± 5 years), sex, place of birth and residence, matched controls for CO (1:1) with non-long lived parents (i.e., deceased before 90 years). Furthermore, all individuals had to be habitual residents (more than 6 months a year) in the health department of La Ribera (Valencian Community, Spain) and be included in the population database. Having a terminal illness or a life expectancy of fewer than 6 months by any cause was considered an exclusion criterion for all groups. All

experimental procedures were performed following the principles of the Declaration of Helsinki of the World Medical Association and according to the national and international guidelines. The study protocol was approved by the Committee for Ethics in Clinical Research of the Hospital de la Ribera, Alzira, Spain.

Frailty status

Frailty status was determined according to the Fried criteria (21). Briefly, a subject was considered frail in the presence of 3 or more of the following 5 criteria: weight loss, self-reported exhaustion, weak grip strength, slow walking speed, and low physical activity. Subjects presenting 1 or 2 of these criteria were considered prefrail and those meeting none of them, were non-frail.

Clinical variables

Independence in activities of daily living was determined using the Barthel Index (22), and independence in instrumental activities of daily living using the Lawton Index (23). Cognitive status was assessed using the *Mini-Examen Cognoscitivo* (MEC) (24) and the comorbidity incidence using the Charlson Index (25).

Sampling

Blood samples were drawn from the antecubital vein in VACUTAINER® CPT™ tubes (BD, Franklin Lakes, NJ) containing sodium heparin as an anticoagulant, to obtain peripheral blood mononuclear cells (PBMCs) and with standard tubes (with and without anticoagulant) to perform routine clinical chemistry assays. Within 0.5 hours of collection, blood was processed at the collection site according to the manufacturer's instructions by centrifugation at 3000 × g at room temperature for 15 minutes. After centrifugation, the CPTs were gently

inverted several times to separate plasma, PBMCs, and erythrocytes. We collected the white ring containing PBMCs. PBMCs were then washed twice in PBS and frozen at –80ºC for subsequent RNA isolation. Laboratory personnel was all blinded to the sample´s identity.

Laboratory biochemical assays

Serum IL-6, C-Reactive Protein (CRP), leucocytes, lymphocytes, glucose, total, HDL cholesterol, and triglycerides were determined by standard biochemical assays. The concentration of serum LDL was calculated by using the Friedewald equation: LDL = total cholesterol – HDL – (triglycerides/5).

RNA extraction

Total RNA containing miRNA was isolated by the mirVana miRNA Isolation Kit (Ambion, Austin, TX) according to the manufacturer's directions. RNA concentration and purity were assessed by the 260/280 ratio using a Genequant Pro Classic spectrophotometer (GE Healthcare). RNA integrity was determined by capillary electrophoresis using the RNA 6000 Nano Lab-on-a-Chip kit and the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA).

Expression profiling of miRNAs.

Small non-coding miRNA expression profiling was performed by using the GeneChip miRNA 4.0 Array (Thermo Fisher Scientific, Spain). The array comprised 30,424 mature microRNA sequences from the miRBASE (v20) encoded miRNA coverage of all organisms, 1,996 encompassed snoRNAs and scaRNAs, and 3,770 probe sets unique to human, mouse, and rat pre-miRNA hairpin sequences. Microarray experiments were conducted according to the manufacturer’s instructions. Briefly, 150 ng total RNA was labeled with FlashTag Biotin

HSR RNA Labeling Kit (Thermo Fisher Scientific). The labeling reaction was hybridized on the miRNA Array in Affymetrix Hybridization Oven 645 at 48°C for 17 h. The arrays were stained with Fluidics Station 450 using fluidics script FS450_0003 (Affymetrix) and then scanned on GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA).

Gene expression profiling.

mRNA profiling was performed using Clariom D-Human Array (Thermo Fisher Scientific, Spain). This array comprises > 542,000 transcripts constituting over 134,000 gene-level probe sets. Microarray experiments were conducted according to the manufacturer's instructions (Thermo Fisher Scientific, Spain). Briefly, 150 ng total RNA was labeled using GeneChip WT Plus Reagent Kit. The labeling reaction was hybridized on the array using Hybridization Oven 645 at 45°C for 16 hours. The arrays were stained with Fluidics Station 450 using fluidics script FS450_0001, then scanned on GeneChip Scanner 3000 7G. GeneChip® Command Console® software supplied by Thermo Fisher Scientific was used to perform gene expression analysis.

Data analysis of microarrays.

Data (.CEL files) were analyzed and filtered using the Partek Genomic Suite 6.6 software (Partek Inc., St. Louis, MO). Input files were normalized with the RMA algorithm for gene array on core meta probesets or miRNAs. A one-way ANOVA was performed with the Partek Genomics Suite across all samples. Statistically significant small non-coding RNAs and mRNAs between the different groups studied were identified by using a model analysis of variance of p-value < 0.05. The imported data were analyzed by Principal Components Analysis (PCA) to determine the significant sources of variability in the data. Differentially

expressed genes were imported into Pathway Studio v12 (Elsevier) to link the mRNAs with selected cellular processes.

Protein Carbonylation Measured Using Western Blotting.

Oxidative modification of total proteins was assessed by immunoblot detection of plasma protein carbonyl groups using the OxyBlot™ Protein Oxidation Detection Kit (Millipore, Billerica, MA), following the manufacturer's instructions.

Statistical Analysis.

Standard statistical methods were used to obtain the mean and standard deviation of the mean (SD) for quantitative variables, median and inter quarterly range (IQ) when appropriate, and percentages (%) for categorical variables. Normality of variable distribution was checked by the Kolmogorov-Smirnov/ Shapiro-Wilk tests, when appropriate. Comparison between groups was performed with a one-way analysis of variance (ANOVA), two-tailed t-test or Wilcoxon test for quantitative variables, and MCNemar test for categorical variables. A sensitivity analysis was performed for IL-6, CRP, leukocytes, and lymphocytes. To check if the subsamples for the transcriptomic analysis were representative of their respective samples of centenarians, CO, and NCO, comparisons for age, gender, BMI, underweight, obesity, frailty, IL-6, and Charlson index punctuation, were performed. Chi-square test, Yate´s correction, Fisher´s exact test, and Mann-Whitney test, when appropriate, were used. The type I error was established as < 5% ($p < 0.05$). All statistical analyses were performed with SPSS v.21 for Mac (IBM SPSS, Inc., Chicago, IL, USA).

RESULTS

Sample characteristics:

Descriptive statistics, as well as the comparison between groups (i.e., centenarians, CO, and NCO) of the main anthropometric, sociodemographic, and clinical characteristics, are shown in **Table 1**. There were no statistically significant differences in age and height between CO and NCO ($p \geq 0.05$), but CO showed lower weight and BMI than NCO ($p < 0.05$). Both CO and NCO were younger than centenarians and presented a higher weight and height than centenarians ($p < 0.05$).

Regarding the frailty status, only 9.1% of the CO were frail, whereas this percentage reached 21.6% in NCO. Centenarians were more frail by only 31%, a low percentage considering their age. Thus, CO was significantly less frail than NCO ($p < 0.05$), being both groups less frail than centenarians ($p < 0.01$). When frailty criteria were analyzed separately, there was a significantly lower percentage of CO that met the criteria "self-reported exhaustion" ($p < 0.05$), "weakness" ($p < 0.05$), and "slow gait speed" ($p < 0.01$) than NCO, with no differences regarding the other two criteria (although a tendency was found for "low physical activity" in favor of CO).

When compared to centenarians, there were significantly more CO and NCO people meeting "low physical activity" ($p < 0.01$), whereas more centenarians showed "weakness" and "slow walking speed" criteria ($p < 0.01$).

When analyzing clinical variables, both CO and NCO obtained higher scores for the functional ability and cognitive scales (i.e., Barthel Index, Lawton Index, and *Mini-Examen Cognoscitivo* (MEC) than centenarians ($p < 0.01$) but no differences in any of these variables were found between CO and NCO ($p \geq 0.05$). The incidence of comorbidities was similar between NCO and CO ($p \geq 0.05$).

Concerning biochemical variables, both NCO and CO showed lower levels of the pro-inflammatory markers IL-6 and CRP than centenarians ($p < 0.01$), with CO displaying lower levels of the IL-6 than NCO ($p < 0.05$). Fasting glucose was higher in the younger groups (i.e., CO and NCO) than in centenarians ($p < 0.01$), with no differences between them, whereas CO showed higher levels of total and LDL cholesterol ($p < 0.05$), but similar levels of HDL cholesterol ($p \geq 0.05$) when compared to NCO controls, thus showing an impaired lipid profile.

No differences were found for leucocytes, lymphocytes, and triglycerides between groups ($p \geq 0.05$).

Regarding oxidative damage markers, NCO presented a protein damaged profile, as indicated by higher levels of protein carbonylation than CO and centenarians ($p < 0.01$). Finally, subsamples for transcriptomic analysis did not display statistically significant differences in age, gender, BMI, underweight, obesity, frailty, IL-6, and Charlson index punctuation, with their respective samples.

miRNA expression profile

The principal component analysis (PCA) of the non-coding RNAs showed that the miRNA expression profile in CO was very close to that of centenarians but very different from that of NCO (**Figure 1A**). Indeed, NCO directionality largely differed from that of CO and centenarians. When we performed the 1-way ANOVA analysis to check how many miRNAs significantly changed ($p < 0.01$) between contrasts (i.e., centenarians vs CO, centenarians vs NCO, and CO vs NCO), we observed that the greatest difference was when comparing centenarians and NCO (i.e., 237). Interestingly, we observed less miRNA that changed between CO and centenarians (i.e., 136) than between CO and NCO (i.e., 197) (**Figure 1B**).

When we performed the unsupervised hierarchical clustering analysis on the miRNA expression profiles of centenarians, CO, and NCO, we observed that dendrograms of centenarians overlapped with those of CO, whereas NCO resulted to be the most different cluster (**Figure 1C**).

mRNA expression profile

The results derived from our functional transcriptomic analysis array revealed that the mRNome of CO was different from that of NCO, as indicated by a distinct directionality in the principal component analysis (PCA) (see **Figure 2A**). This finding was further confirmed by a higher number of mRNAs that were differentially expressed ($p < 0.01$) when comparing CO vs NCO (i.e., 2939) than when comparing the rest of the groups: centenarians versus CO (1360), centenarians versus NCO (1711) (**Figure 2B)**. This suggests that CO is closer to centenarians than to NCO in terms of mRNA profile.

The unsupervised hierarchical clustering analysis shows a clear separation between C, CO, and NCO. Interestingly, CO was between C and NCO (**Figure 2C)**.

Supervised interactions analysis of genes associated with frailty

We performed a supervised analysis to find direct interactions between the list of genes that we found to be differentially expressed between CO and NCO and selected cell processes associated with frailty (**Figure 3**). Those selected cell processes were bone growth, myogenesis, skeletal development, and cell differentiation, all of them related to frailty. We found twenty-six genes that were related to some of those biological processes. Almost all those genes were overexpressed in CO vs NCO except one, STC1 (*Stanniocalcin 1*), which was under-expressed in CO vs NCO. Furthermore, as shown in Figure 3, most of the genes overexpressed in CO activate bone growth, myogenesis, skeletal development, and cell differentiation pathways.

DISCUSSION

The results of the current study show that CO displays a lower prevalence of frailty when compared to their matched controls. In parallel, we report that both miRNA and mRNA expression patterns in CO are like those of centenarians and different from those of NCO and found a differential expression of genes involved in cellular processes related to frailty. To the best of our knowledge, this is the first study to compare the functional (i.e., frailty status) and genetic (i.e., miRNA and mRNA expression patterns) profiles from CO and NCO. Our results reinforce the idea that CO is functionally and genetically distinct from their contemporaries and resembles centenarians' unique genetic characteristics.

CO is less frail than age-matched NCO

Regarding functional status, we found that CO is significantly healthier (i.e., lower prevalence of frailty, lower levels of the pro-inflammatory cytokine IL-6, lower protein carbonylation levels) than NCO matched controls. This is in agreement with previous studies reporting that CO is significantly healthier than offspring of short-lived parents (16, 26, 27), likewise, one of them has further tried to explain the bad lipid profile in terms of lower pharmacological therapy requirements among these patients (28). Our results showing lower oxidative stress and inflammatory biomarkers are of importance, since they may indicate that centenarians' offspring (i.e. lower protein carbonylation levels) may show a decelerated ageing

Nevertheless, only two previous studies have evaluated the frailty status in the same three experimental groups as the current one (i.e. CO, age-matched controls, and centenarians) using the Frailty Index (18, 19), with similar results to the present study, this is, centenarians' offspring are less frail than their contemporaries and centenarians. In particular, we found that 'slow gait speed' and 'exhaustion' in the CO group exhibited a lower % compared to the

NCO group. In this regard, it is well-established that slowness can be considered a major biomarker of healthspan, hence it can predict multiple adverse health outcomes, such as physical function and cognitive decline, disability, and even death in older adults (29-31). Furthermore, it has been reported, both in humans and mice (32, 33), that gait speed decline appears to be fully explained by age and frailty, particularly after mid-age when the energetic cost of walking increases exponentially. These changes have been related to extensive and differential metabolic remodeling of several organs in response to specific functional demands (33). Our results thus suggest that CO might display a better pattern of metabolic remodeling than NCO, thus coping with the increased energetic cost to maintain health, energy, and redox balance. Further studies are needed to confirm this hypothesis. **miRNA and mRNA expression in CO are closer to centenarians than to NCO.**

miRNA are important regulators of the aging process and modulators of longevity (34). In this regard, we previously reported that centenarians show a characteristic miRNA profile that differs from that of normal ageing (i.e. septuagenarians) (5), which may be due to centenarians' preserved miRNA biogenic pathway (6). In the current study, we show that the miRNA expression profile in CO is very close to that of centenarians (with no familiar linkage) but very different from that of NCO. These results highlight the importance to consider miRNA as powerful epigenetic biomarkers to trace successful ageing, as previously suggested (35).

We also studied mRNA expression. Aging has been classically associated with alterations in mRNA levels, which may reflect changes in gene expression, mRNA stability, or both (36). However, we previously demonstrated that centenarians show an mRNA expression profile closer to young people than to septuagenarians (7), which suggests that they somehow avoid the detrimental effects of normal ageing on mRNA levels. When analyzing here the mRNA expression profile of CO, similar results to those of the miRNA profile were depicted: CO is

closer to centenarians than to NCO in terms of mRNA profile. Continuing with the argument described in the previous subsection, dysregulated metabolism often leads to premature aging and certain diseases in humans (37). In contrast, CO may inherit somehow "healthy" metabolic profiles displayed by centenarians. This hypothesis is supported by a recent study based on genome- wide precision metabolic modeling showing that the most significant metabolic feature observed in centenarians (i.e., elevated fatty acid oxidation) seem to be inherited by their offspring (37).

Taken together, our results show that CO displays different miRNA and mRNA transcriptomic profiling than NCO, which may reinforce the idea that they may inherit centenarians' unique genetic signature.

A better understanding of the molecular pathways involved in frailty and its clinical consequences may contribute to improving the possibility of achieving healthy aging. Nevertheless, there is still controversy and no consensus about the exact molecular mechanisms and pathways taking part in this geriatric syndrome due to its multifactorial nature. Inflammation and oxidative stress have been posited as two potential drivers of frailty pathogenesis. As frailty is related to the presence of chronic diseases and functional decline, the increased amount of energy required to cope with them could be the reason why mitochondria produce excessive quantities of free radicals. This increase could also activate the NFkB pathway, and lead to inflammation (38). Accordingly, systemic inflammatory biomarkers, such as C-reactive protein, interleukin-6 (IL-6), and tumor necrosis α have been related to frailty (38). Furthermore, we and others reported that frail people exhibit increased oxidative damage when compared with robust people (39-41). In the same line, a previous study showed that a reduced expression of several genes implicated in the cellular response to oxidative stress or hypoxia was significantly associated with the presence of frailty (42).

Besides the plausible implication of these pathways in the frailty phenotype, impairments in mitochondrial function have been related to the development of sarcopenia, the age-related loss of muscle mass and performance, and all of the processes related to frailty (43, 44). Furthermore, together with sarcopenia, osteoporosis has been proposed as a major contributor to disability and frailty (45). Our supervised analysis of the differentially expressed genes associated with selected cellular processes related to some of those pathways (i.e., bone growth, myogenesis, skeletal development, and cell differentiation) revealed that most of the genes activating bone growth, myogenesis, skeletal development, and cell differentiation pathways were overexpressed in CO when compared to NCO.

Thus, the biologically plausible explanation for the picture depicted in these results is that CO individuals display increased bone growth, myogenesis, skeletal development, and cell differentiation when compared to NCO. Interestingly, all these processes involve adaptive responses that challenge the endogenous cellular defense mechanisms. In this regard, frailty has been associated with lower expression of genes involved in the cellular stress response (46). Cellular stress response, in turn, involves the activation of pro-survival pathways that result in the production of molecules endowed with antioxidant and anti-apoptotic activities (47). It has been suggested that these pathways are controlled by protective genes called vitagenes (48). Given the relationship between redox oxidative status and the vitagene network and its possible biological relevance in survival and cellular protection, our results may indicate that CO may have better endogenous cellular defense mechanisms than their contemporaries.

Although reporting novel findings, the present study has some limitations that should be acknowledged. The main limitation was that the frailty status of centenarians is not fully comparable, since they are 30 years older and therefore obviously frailer than the younger groups. Furthermore, the sample size of the random subsample selected to perform biological

determinations (i.e., transcriptomic analysis) was rather small, yet this subsample did not differ from the original one and thus was considered representative of all groups.

In conclusion, we found that CO is functionally and genetically distinct from their contemporaries and resembles centenarians' unique genetic endowment resulting in less frailty. Consequently, the results reported here may contribute to shed light on key functional and genetic features that may be considered biomarkers of successful ageing. Further studies are needed to confirm our findings in a larger population.

DECLARATIONS

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Conflict of interest

The authors declare that they have no conflict of interest

Availability of data and material

Data analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

MI, AB, and ES performed the experimental work and analysis. FJT directed the clinical experimental work. MI, CB, and JV wrote and reviewed the paper.

Ethics approval

The study was conducted in agreement with legal requirements and international norms (Declaration of Helsinki 1964). The study protocol was approved by the Ethics Committee of the Hospital Universitario de la Ribera de Alzira.

Consent to participate

Before enrolment, all participants provided written informed consent

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TABLE AND FIGURE LEGENDS

Table 1. Baseline comparisons of individuals' demographic, anthropometric and clinical data.

Figure 1. miRNA expression profiles in mononuclear cells from centenarians (n=9), centenarians' offspring (n=8), and offspring of non-centenarians (n=10). A) PCA of the miRNA profiles. The ellipsoids (in red, NCO=offspring of non-centenarians; in green, C=centenarians, and in blue, CO=centenarians' offspring) show a distinct directionality in different groups based on similarities and differences between them. The axes correspond to principal component 1 (PC1, x-axis), PC2 (y-axis), and PC3 (z-axis). B) Representation of 1-way ANOVA analysis between different contrasts: centenarians versus centenarians' offspring (C vs CO), centenarians versus offspring of non-centenarians (C vs NCO), and centenarians' offspring versus offspring of non-centenarians (CO vs NCO). Statistically significant miRNAs were filtered using a p-value < 0.01. C) Heatmap of the unsupervised hierarchical clustering analysis on the miRNA expression profiles of the miRNA array analysis. Individuals' samples (raws) are labeled as centenarians (green), centenarians' offspring (blue), or offspring of non-centenarians at the left of the figure, whereas miRNA differential expression (columns) is represented as indicated by the color bar on the bottom (i.e., blue represents low expression, red high expression, and yellow intermediate expression). The dendrograms on the left show the similarities between the miRNA expression profiles of centenarians, centenarians' offspring, and offspring of non-centenarians.

Figure 2. mRNA expression profiles in mononuclear cells from centenarians (n=9), centenarians' offspring (n=8), and offspring of non-centenarians (n=10). A) PCA of the mRNA profiles. The ellipsoids (NCO in red, C in green, and CO in blue) show a distinct directionality in different groups based on similarities and differences between them. The

axes correspond to principal component 1 (PC1, x-axis), PC2 (y-axis), and PC3 (z-axis). B) Number of mRNAs that are expressed significantly differently ($p < 0.01$) when comparing the different groups: centenarians versus centenarian's offspring (C vs CO), centenarians versus offspring of non-centenarians (C vs NCO), and centenarians' offspring versus offspring of non-centenarians (CO vs NCO). C) Heatmap of the unsupervised hierarchical clustering analysis on the mRNA expression profiles of the mRNA array analysis of centenarians (green), centenarians' offspring (blue,) and control offspring of non-centenarians (red). Samples are identified on the y-axis, whereas mRNA expression is on the x-axis, where blue represents low expression, red high expression, and yellow intermediate expression. The dendrograms on the left show the similarities between the mRNA expression profiles of centenarians, centenarians' offspring, and offspring of non-centenarians.

Figure 3. Cellular processes related to transcriptomic analyses (CO versus NCO). Gray rectangles represent the cellular processes, whereas the figures with different shapes are the genes related to those processes. The red color indicates overexpression and blue under-expression. Green and red arrows that connect the gene to the cellular process indicate whether the gene activates or suppresses that process, respectively.

Table 1.

	C (n=63)	**CO (n=88)**	**NCO (n=88)**	***p (C:CO)***	***p (C: NCO)***	***p (CO: NCO)***
Female, n (%)	49 (77.8)	53 (60.2)	53 (60.2)	0.023*	0.023*	1
Age *(years), median (IQR)*	98 (1.3)	70 (6)	69 (7)	0.000**	0.000**	0.695
Weight *(kg), mean* (SD)	61.7 (15.7)	71.2 (14.6)	76.9 (13.6)	0.000**	0.000**	0.008**
Height *(cm), median (IQR)*	150 (10)	158 (13)	160 (13)	0.000**	0.000**	0.557
BMI *(kg/m^2), mean* (SD)	26.5 (14.5)	27.7 (4.5)	29.7 (4.9)	0.057	0.001**	0.007**
Frailty, n (%)	23 (36.5)	8 (9.1)	19 (21.6)	0.000**	0.039*	0.019*
Weight loss, n (%)	7 (11.1)	6 (6.8)	5 (5.7)	0.354	0.216	1
Exhaustion, n (%)	6 (10)	4 (4.5)	15 (17)	0.317	0.238	0.013*
Low physical activity, n (%)	16 (25.4)	43 (49.4)	54 (61.4)	0.003**	0.000**	0.082
Weakness, n (%)	62 (98.4)	38 (43.7)	50 (56.8)	0.000**	0.000**	0.045*
Slowness, n (%)	62 (98.4)	9 (10.3)	22 (25)	0.000**	0.000**	0.004**
Barthel, *median (IQR)*	45 (75)	100 (0)	100 (0)	0.000**	0.000**	0.305
Lawton, *median (IQR)*	1 (2)	8 (0)	8 (0)	0.000**	0.000**	0.719

MEC, *median (IQR)*	17 (19)	32 (5)	33 (3)	0.000**	0.000**	0.592
Charlson Index, *median (IQR)*	1 (1)	0 (1)	0 (1)	0.000**	0.000**	0.145
IL-6 (pg/mL), *median (IQR)*	3.55 (3.49)	1.03 (0.96)	1.45 (1.38)	0.000**	0.000**	0.044*
CRP (mg/L), *median (IQR)*	3.86 (6.84)	1.18 (2.14)	1.92 (2.24)	0.000**	0.000**	0.155
Leucocytes (x10^9/L), *mean* (SD)	6.55 (1.20)	6.60 (1.70)	6.84 (1.89)	0.846	0.352	0.475
Lymphocytes (x10^9/L), *median (IQR)*	1.80 (0.70)	1.80 (0.78)	1.90 (0.80)	0.334	0.124	0.733
Glucose (mg/dL), *median (IQR)*	85 (18)	93 (21)	96 (27)	0.000**	0.000**	0.113
Cholesterol (mg/dL), *mean* (SD)	184.3 (39.4)	206.4 (40.4)	191.7 (37.3)	0.001**	0.242	0.015*
LDL (mg/dL), *mean* (SD)	109.7 (33.8)	121.3 (37.5)	110.1 (32.1)	0.052	0.932	0.043*
HDL (mg/dL), *mean* (SD)	52.4 (13.7)	59.4 (15.7)	57.1 (14.9)	0.005**	0.048*	0.213
Triglycerides (mg/dL), *mean* (SD)	111.2 (53.1)	128.7 (65.5)	122.4 (53.5)	0.083	0.203	0.360
Protein carbonylation (a.u.)	57.4 (21.5)	54.0 (23.4)	64.9 (23,9)	0.431	0.045*	0.013*

*Data are presented as mean ± standard deviation (SD), median and interquartile range (IQR), percentage or n, as indicated. C = Centenarians; CO = Centenarians' Offspring; NCO = Offspring of non-centenarians; BMI = body mass index. Statistical significance is set at ** = p <0.01; * = p <0.05.*

Figure 1

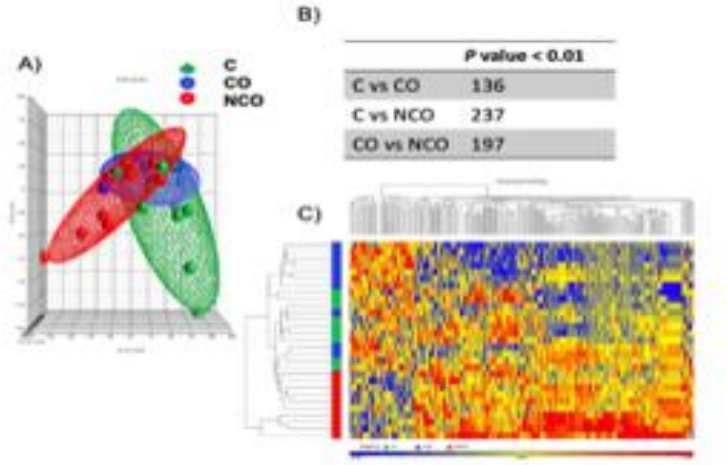

Figure 2

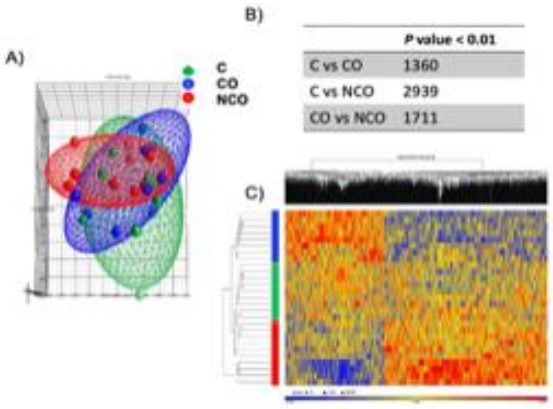

Figure 3