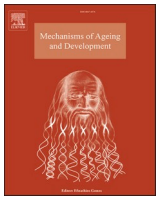
Mechanisms of Ageing and Development 206 (2022) 111691

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev



Blood circulating miR-28-5p and let-7d-5p associate with premature ageing in Down syndrome

Cristina Morsiani [a,*], Maria Giulia Bacalini [b], Salvatore Collura [a], María Moreno-Villanueva [c], Nicolle Breusing [d], Alexander Bürkle [c], Tilman Grune [e,f], Claudio Franceschi [g], Magda De Eguileor [h], Miriam Capri [a,i]

[a] DIMES-Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy
[b] IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
[c] Molecular Toxicology Group, Department of Biology, University of Konstanz, Konstanz, Germany
[d] Department of Applied Nutritional Science/Dietetics, Institute of Nutritional Medicine, University of Hohenheim, Germany
[e] Department of Molecular Toxicology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, Germany
[f] German Centre for Cardiovascular Research (DZHK), Partner Site Berlin, Germany
[g] Laboratory of Systems Medicine of Healthy Aging and Department of Applied Mathematics, Lobachevsky University, Nizhny Novgorod, Russia
[h] Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
[i] Interdepartmental Center "Alma Mater Research Institute on Global Challenges and Climate Change (Alma Climate)", University of Bologna, Italy

ARTICLE INFO

Keywords:
MicroRNAs
Amyloid beta
Down syndrome
Premature ageing

ABSTRACT

Persons with Down syndrome (DS) undergo a premature ageing with early onset of age-related diseases. The main endpoint of this study was the identification of blood circulating microRNAs (c-miRs) signatures characterizing DS ageing process. A discovery phase based on array was performed in plasma samples obtained from 3 young (31 ± 2 years-old) and 3 elderly DS persons (66 ± 2 years-old). Then, a validation phase was carried out for relevant miRs by RT-qPCR in an enlarged cohort of 43 DS individuals (from 19 up to 68 years-old). A group of 30 non-trisomic subjects, as representative of physiological ageing, was compared. In particular miR-628–5p, miR-152–3p, miR-28–5p, and let-7d-5p showed a lower level in younger DS persons (age ≤ 50 years) respect to the age-matched controls. Among those, miR-28–5p and let-7d-5p were found significantly decreased in physiological ageing (oldest group), thus they emerged as possible biomarkers of premature ageing in DS. Moreover, measuring blood levels of beta amyloid peptides, Aβ-42 was assessed at the lowest levels in physiological ageing and correlated with miR-28–5p and let-7d-5p in DS, while Aβ-40 correlated with miR-628–5p in the same cohort. New perspectives in terms of biomarkers are discussed.

1. Introduction

Down syndrome (DS) is considered a segmental progeroid syndrome since persons affected by DS appear to age earlier than non-trisomic people, in particular the immune system and the central nervous systems are those affected early (Gensous et al., 2019). For this reason, DS can represent a model for the study of accelerate ageing, aiming at the identification of molecular markers and potential therapeutic targets.

There are at least three different layers of knowledge supporting the view of DS as a disorder of accelerated ageing, i.e. clinicopathological features, data showing the acceleration of the molecular mechanisms of ageing and various age-related biomarkers at high levels (Franceschi et al. (2019); Jenkins et al., 2017; Borelli et al., 2015; Horvath et al., 2015; Cole and Franke, 2017).

Individuals with DS experience age-related cognitive decline and subsequent dementia more frequently than the general population and the symptoms appear at an earlier age, following a course that is similar to the one occurring in Alzheimer's disease (AD). Dementia is usually preceded by changes in language skills, in executive functions and a loss of adaptive skills (Ghezzo et al., 2014).

In this respect, the gene of amyloid precursor protein (APP) is located in chromosome 21, thus it results triplicated in DS subjects. DS dementia

* Correspondence to: DIMES-Department of Experimental, Diagnostic and Specialty Medicine, ALMA MATER STUDIORUM - University of Bologna, Via S. Giacomo 12, 40126 Bologna Italy.
E-mail address: cristina.morsiani2@unibo.it (C. Morsiani).

https://doi.org/10.1016/j.mad.2022.111691
Received 3 March 2022; Received in revised form 1 June 2022; Accepted 22 June 2022
Available online 30 June 2022

is believed to be related to the overproduction of APP and consequent deposition of beta-amyloid (Aβ) peptides (Lott and Head, 2019). Aβ40 and Aβ42 are considered the most pathogenic amyloid peptides and their accumulation start to develop in the childhood of DS subjects, decades earlier compared to aged control brains (Head et al., 2016).

Moreover, several studies suggest that DS persons display remarkable alterations for the main molecular mechanisms involved in the ageing process, also referred as "the seven pillars of ageing" (Franceschi et al., 2019; Kennedy et al., 2014), i.e. metabolism, stem cells and regeneration, macromolecular damage, inflammation, adaptation to stress, proteostasis, and epigenetics.

Among epigenetic mechanisms non-coding RNAs (ncRNAs) represent both crucial gene regulators and potential biomarkers (Tan et al., 2013). Specifically, microRNAs (miRs) have been described as key regulators of DS transcriptome and DS phenotypes (Brás et al., 2018). Various miRs are encoded on HSA21 and therefore likely over-expressed in DS, and some of them have been implicated in the development of some DS-related pathologies (Gensous et al., 2019).

In this study, blood circulating miRs (c-miRs) and beta-amyloid peptides (Aβ-40 and Aβ-42) were investigated as potential modulators and biomarkers of the ageing process in DS. DS persons were enrolled by the unit of Bologna (Italy) and compared with an age-sex matched non-trisomic control group in the framework of a large-scale European study, the MARK-AGE project, pointing to identify new biomarkers of ageing (Bürkle et al., 2015; Capri et al., 2015).

2. Materials and methods

2.1. Study participants and experimental design

All samples were collected within the FP7 European MARK-AGE project (2008–2013) with the approval of the local ethical committee S. Orsola-Malpighi Hospital (Bologna, Italy). A total of 74 participants enrolled in Bologna were included in the present study as follows: i. 43 individuals affected by DS (24 males and 19 females) aged between 19 and 68 years-old (mean age = 39.8 ± 12.7); ii. 30 control subjects selected randomly after sex partitioning (15 males and 15 females, as Recruited Age-Stratified Individuals from the General population or RASIG) and aged between 35 and 74 years-old (mean age = 56.0 ± 14.8).

Participation in the study was on a totally voluntary basis and written informed consent to participate was obtained from adult DS persons and from parents or authorized tutors. Exclusion criteria were current acute illnesses, hepatic, renal or cardiac insufficiency, consumption of antioxidant or nutraceutical substances (vitamins, lipoic acid, acetylcysteine, omega 3 and 6 fatty acids, probiotics) within the last two months. A diagnosis of DS based on karyotype was obtained in all cases. DS persons underwent neuropsychological function evaluation by means of tests previously described as significantly age-related in DS (Ghezzo et al., 2014), i.e. Tower of London, Token test, Frontal Assessment Battery (FAB), the Visual Object and Space Perception Battery (VOSP), Phonemic fluency (Moreno-Villanueva et al., 2015).

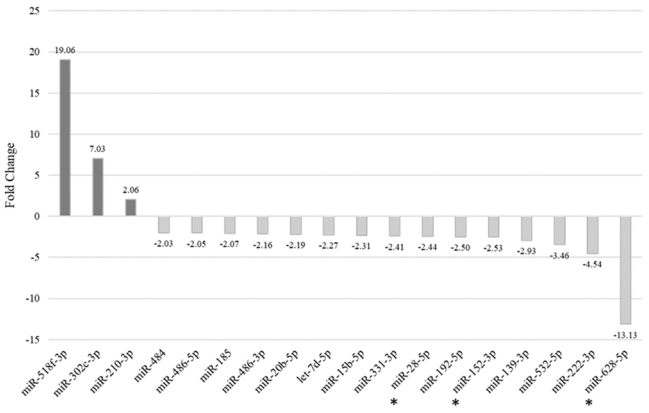


Fig. 1. MiR profiling from the discovery phase. A total of 18 apparently deregulated miRs (fold change ≥ 2 and ≤ −2) was identified in plasma samples of 3 elderly DS females compared with 3 young DS females. Up-regulated c-miRs are displayed in dark grey, while down-regulated c-miRs are displayed in light grey. Fold change values are reported for each miR and significant changes ($p \leq 0.05$, by Student's t-test) are highlighted with an asterisk.

2.2. RNA extraction for miRs evaluation

Total RNA was isolated from 100 μl of serum using the Total RNA purification kit (Norgen Biotek Corporation, Thorold, ON, Canada). The protocol was modified adding 20 fmol of cel-miR-39 (Qiagen, Hilden, Germany) at the lysis step as spike-in control to monitor RNA isolation.

2.3. MiR profiling

MiR microfluidic card, TaqMan Array Human MicroRNA A Card (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA, USA), for the measurements of 377 human miRs, was applied to identify miR-profiles in discovery phase testing 6 plasma samples from 3 young DS females (age: 30, 30 and 34 years old) and 3 elderly DS females (age: 63, 66 and 68 years old).

RNA was converted to cDNA by priming with a mixture of looped primers and then pre-amplified using the MegaPlex primer pools (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. The profiling was run on the Applied Biosystems 7900 HT real-time PCR instrument.

MiRs profiling was normalized with the median of the overall miR expression on each array (ΔCt). Only miRs expressed in at least five of the six analysed samples were selected and Ct values < 32 were established as cut-off. Fold-change ($2^{-\Delta\Delta CT}$) was calculated based on the estimated mean difference and fold changes ≥ 2 and ≤ −2 were selected.

2.4. MiRs validation by RT-qPCR

Real-time quantitative PCR (RT-qPCR) was achieved using TaqMan technologies (Thermo fisher scientific, Waltham, MA, US) following the manufacturer's protocol. RNA was transcribed to cDNA with the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, by Thermo Fisher Scientific, Waltham, MA, USA) and the RT-qPCR was performed with TaqMan MicroRNA Assays. Data were normalized to cel-miR-39 evaluated in each sample and relative expression was calculated with the delta Ct method.

2.5. Amyloid beta peptides analysis

Plasma level of amyloid beta peptides Aβ-40 and Aβ-42 were measured by immunoassay ELISA (DAB140B and DAB142 kit, R&D systems, MN, USA). The quantitative determination of human Aβ-40 and Aβ-42 concentration in plasma samples was obtained following the manufacturer's instruction and all samples were analysed in duplicate.

2.6. Statistical analysis

Statistical analyses were carried out by means of IBM SPSS software, version 26 (SPSS Inc., USA) and p value ≤ 0.05 was considered statistically significant.

Student's t-test was performed to compare relative expression of miRs between the two cohorts included in the discovery phase, i.e. young DS and elderly DS.

Mann Whitney Nonparametric test was applied to evaluated changes in terms of miRs' expression obtained by RT-qPCR in the validation phase and amyloid beta levels.

In addition, correlations between validated miRs expression,

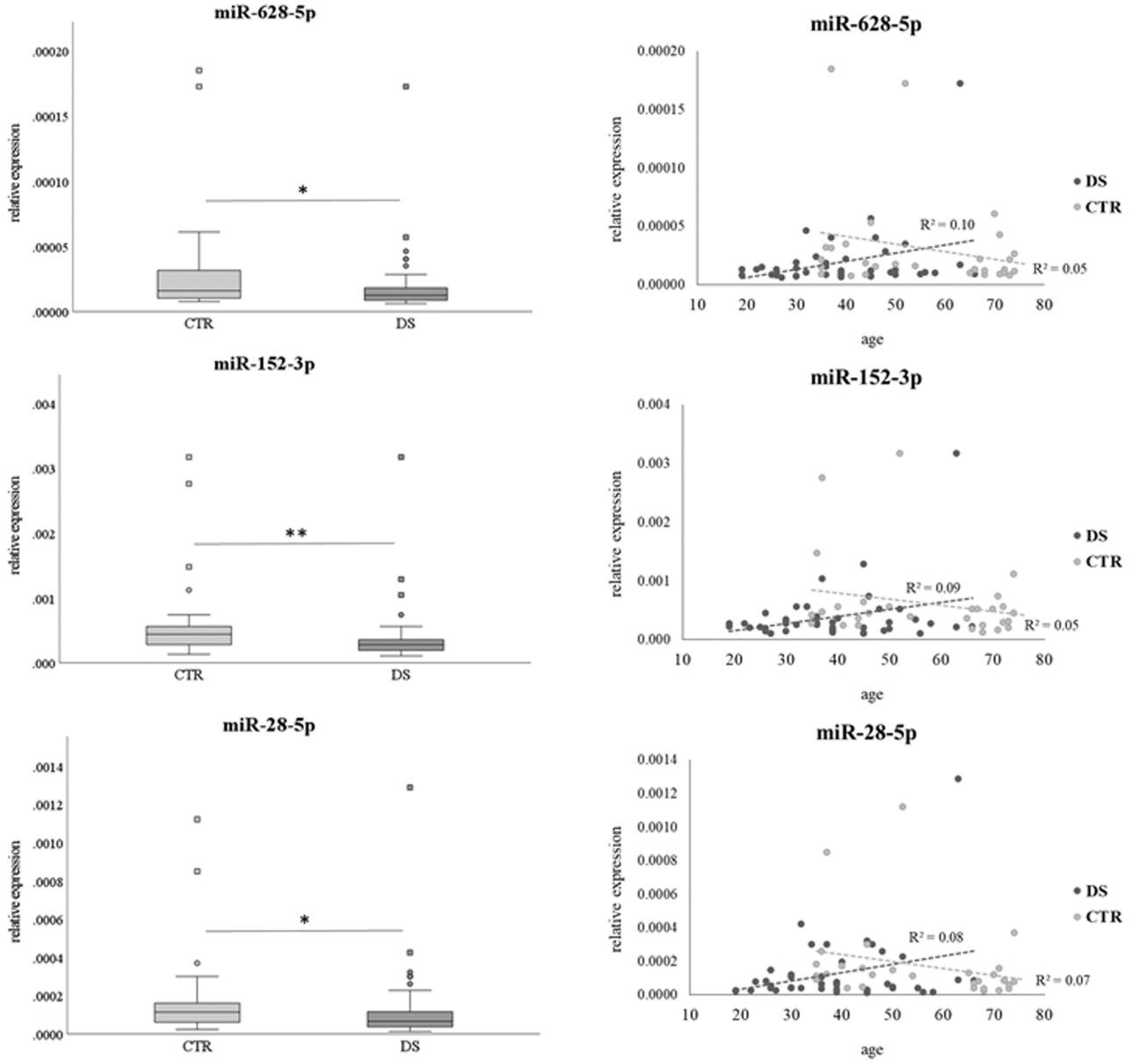


Fig. 2. MiR expression levels comparing DS with controls. C-miRs from DS subjects (N = 43) compared with controls (CTR, N = 30). Three miRs resulted significantly different i.e., miR-628–5p, miR-152–3p, miR-28–5p. Data are reported as box plot of data distribution (median value and quartile ranges). Statistical analysis was performed by Mann-Whitney Nonparametric U test. * = $p \leq 0.05$. Regression analyses alongside the box plots show miR relative expression level with age for both the two cohorts. Coefficients R^2 are indicated.

amyloid beta peptides, i.e. Aβ-40 and Aβ-42, and neuropsychological function tests were investigated using Spearman's Rank-Order Correlation tests.

2.7. Bioinformatic miR targets and pathway analysis

Bioinformatics analysis using the web-server DIANA-Tarbase v8.0 (Karagkouni et al., 2018) was employed to identify common validated targets of the identified miRs. Subsequently, pathway analysis was carried out using the KEGG PATHWAY database.

3. Results

3.1. Discovery phase and miR profiling

MiRs profiling was performed through cards array analysis, aiming at the identification of miRs changes in plasma samples obtained from young and elderly DS. The discovery phase was carried out analysing plasma samples obtained by 3 young and 3 elderly DS females. Differentially expressed miRs were identified according to fold changes, i.e. ≥ 2 and ≤ -2. A total of 18 miRs satisfied such prerequisite, as shown in Fig. 1.

Relative expression of miR profiling was calculated and Student's t-test was performed to verify the statistical significance of miRs variation in elderly DS compared with young DS. Among the 18 identified miRs, 3 miRs were significantly different, i.e. miR-28–5p, miR-152–3p and miR-628–5p, as highlithted with an asterisk in Fig. 1.

3.2. Validation phase of selected miRs by RT-qPCR

The 3 identified miRs from the discovery phase were selected for the following validation phase, together with let-7d-5p, which is thought to

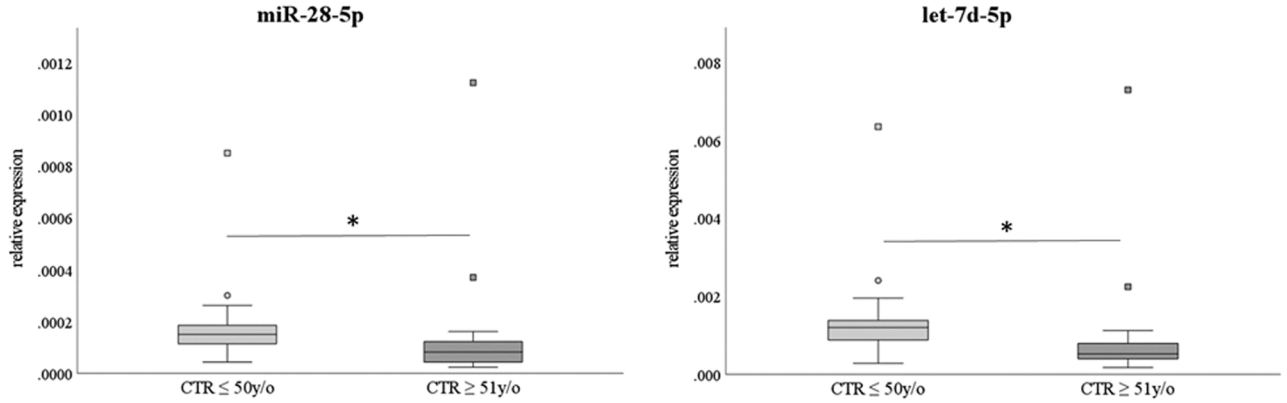


Fig. 3. MiRs and age (≤ 50 and ≥ 51 years) in physiological ageing. The control cohort (CTR) of physiological ageing was divided into two age groups (≤ 50 years, N = 13; ≥ 51 years, N = 17). MiR-28–5p and let-7d-5p significantly decrease in the oldest group. Data are reported as box plot of data distribution (median value and quartile ranges). Statistical analysis was performed by Mann-Whitney Nonparametric U test. * = p ≤ 0.05.

be associated with cognitive decline (Chen et al., 2018; Kumar et al., 2013; Zhao et al., 2013). Furthermore, miR-155–5p and miR-21–5p were added to the validation analysis, given their established role in inflammation (inflamma-miRs) and in the ageing process (Olivieri et al., 2013). Overall, a total of 6 miRs were considered for the subsequent validation phase carried out by RT-qPCR in the enlarged cohort of 43 DS plasma samples. MiRs expression was also evaluated in 30 plasma samples obtained from the control group of age and sex matched subjects.

3.3. MiRs and age analysis

Comparing the two cohorts of DS and control group, three miRs showed significant lower plasma levels in DS, i.e. miR-628–5p, miR-152–3p and miR-28–5p, as reported in Fig. 2. MiRs were also analysed according to age, the regression plots of the above mentioned three miRs are illustrated in Fig. 2 while Fig. 1S shows the regression plots of let-7d-5p, miR-155–5p and miR-21–5p. No significant correlations were found between miR and age in DS, while in the control group only miR-28–5p negatively correlates with age ($p = 0.029$) as well as let-7d, but marginally ($p = 0.054$).

Sex was also considered in both cohorts, 19 females and 24 males in DS group, 15 females and 15 males in control group. No significant differences emerged according to sex (data not shown).

Then, a threshold of 50 years old was set considering the age mean value of the entire population (that corresponds to 46.4 years), according with the previous literature suggesting the premature ageing of DS persons (Ghezzo et al., 2014; Ravaioli et al., 2022). MiRs changes were analysed in these two age groups (≤ 50, ≥ 51). A significant decrease of miR-28–5p and let-7d-5p emerged in non-trisomic elderly subjects (age ≥ 51 years old), as reported in Fig. 3. As far as DS persons are concerned, no significant differences of selected c-miRs were reported comparing the two age groups (data not shown).

Lastly, DS and controls of the same age group (≤ 50 years old and ≥ 51 years old) were compared, as illustrated in Fig. 4. The levels of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p were lower in DS than controls under 50 years old, whereas miR-155–5p and miR-21–5p were not different comparing the two groups. No significant miR variations emerged between the two cohorts aged ≥ 51 years (data not shown).

3.4. Beta amyloid peptides analysis and correlations

Aβ-40 and Aβ-42 peptides were measured in all samples, both DS and control group. The two peptides resulted significantly lower in controls than DS, as shown in Fig. 5 also including the regression plots that confirmed the differences between the two cohorts.

Analysis was also performed considering sex, 19 females and 24 males in DS group, 15 females and 15 males in control group. No significant differences were found (data not shown). The ratio Aβ-42/Aβ-40 was also considered, but no significant difference was found (data not shown).

Following the same method applied for miRs evaluation, amyloid peptides were further analysed considering the threshold of 50 years old. Aβ-42 was significantly lower in physiological ageing (age ≥ 51years old) (Fig. 6 panel A), while Aβ-40 was significantly higher in young DS respect to young controls (≤ 50 years old) (Fig. 6 panel B). Correlation analysis among miRs and beta amyloid peptides in DS cohort are listed in Table 1, showing a positive correlation of Aβ-42 with miR-28–5p and let-7d-5p, while Aβ-40 positively correlates with miR-628–5p. Neuropsychological function tests were also correlated with miRs and beta amyloid peptides, but no significant values were observed (data not shown).

3.5. Bioinformatic analysis considering validated targets and possible pathways involved

Validated and common targets of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p, which showed significant changes in DS persons under 50 years old compared to control group, were considered. The analysis was conducted using DIANA-TarBase v8.0 to identify common validated targets for the 4 selected miRs then, KEGG pathway database was used to investigate the pathways which the identified target genes are involved in.

Interestingly, Insulin-like Growth Factor 1 Receptor (IGFR1) mRNA was found to be a common target of all 4 miRs. According to KEGG analysis, IGFR1 is involved in the Longevity regulating pathway reported in Fig. 7 panel A, having an important role in the ageing process.

Sigma Non-Opioid Intracellular Receptor 1 (SIGMAR1) mRNA was also reported to be a common target of the investigated 4 miRs, and this receptor was found to be involved in multiple pathways of neurodegeneration, as shown in Fig. 7 panel B.

4. Discussion

The premature ageing occurring in DS has been investigated in two cohorts enrolled in the above-mentioned MARK-AGE project i.e., DS individuals and non-trisomic controls aged between 19 and 74 years-old.

Since miRs play an essential role in the epigenetic regulation of gene expression, they have rapidly become a research target to investigate many biological fields, including DS-phenotype and healthy ageing. The aim of this study was to identify a c-miR signature of ageing in plasma

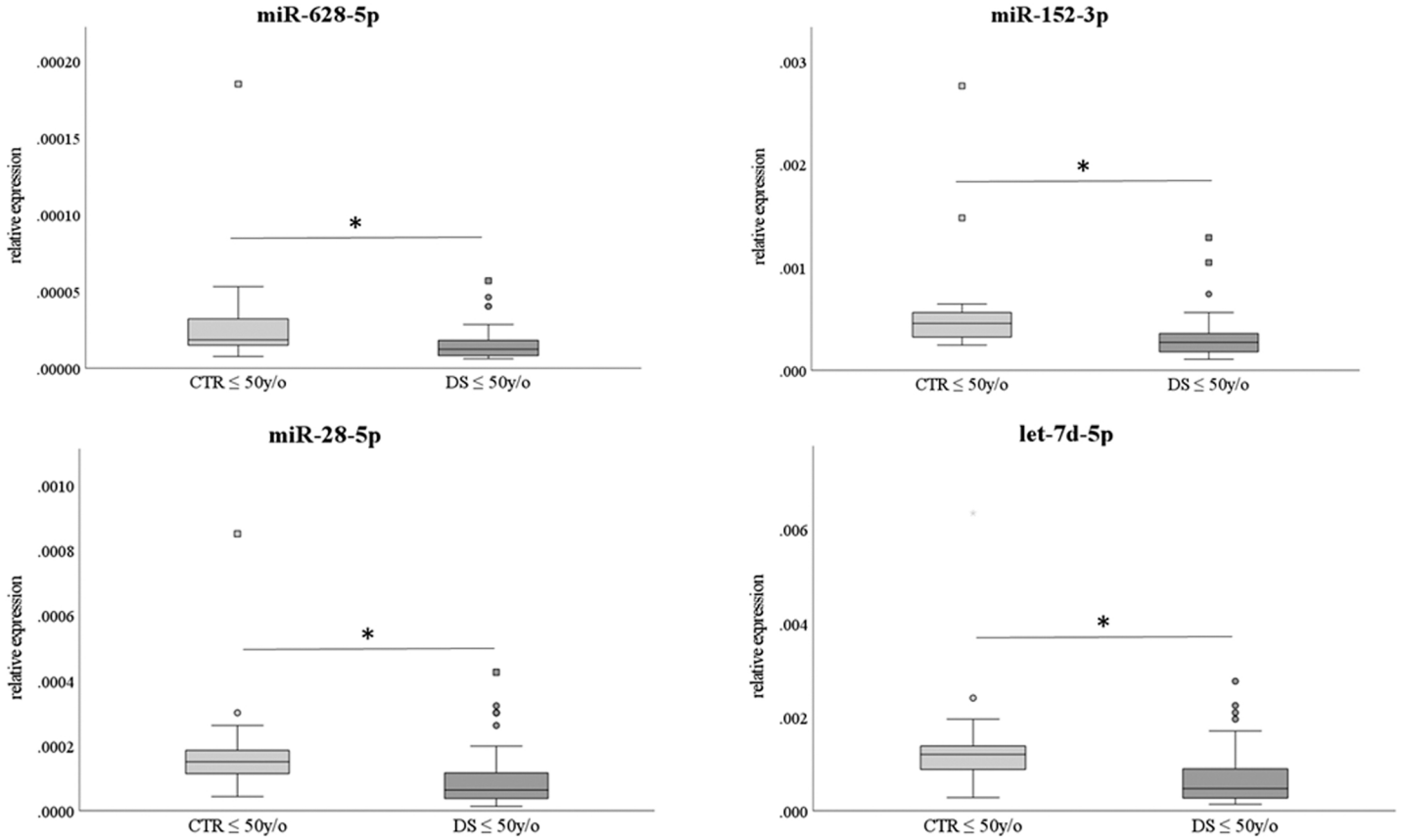


Fig. 4. MiRs significantly differ comparing younger DS with age-matched controls. MiR-628–5p, miR-152–3p, miR-28–5p, and let-7d-5p significantly decrease in DS (N = 36) in comparison with controls (N = 13, CTR) in the younger age group ≤ 50 years. The decreased levels of miR-28–5p, and let-7d-5p occurs early in DS (≤ 50 years) compared to non-trisomic physiological ageing (Fig. 2). Data are reported as box plot of data distribution (median value and quartile ranges). Statistical analysis was performed by Mann-Whitney Nonparametric U test. * = p ≤ 0.05.

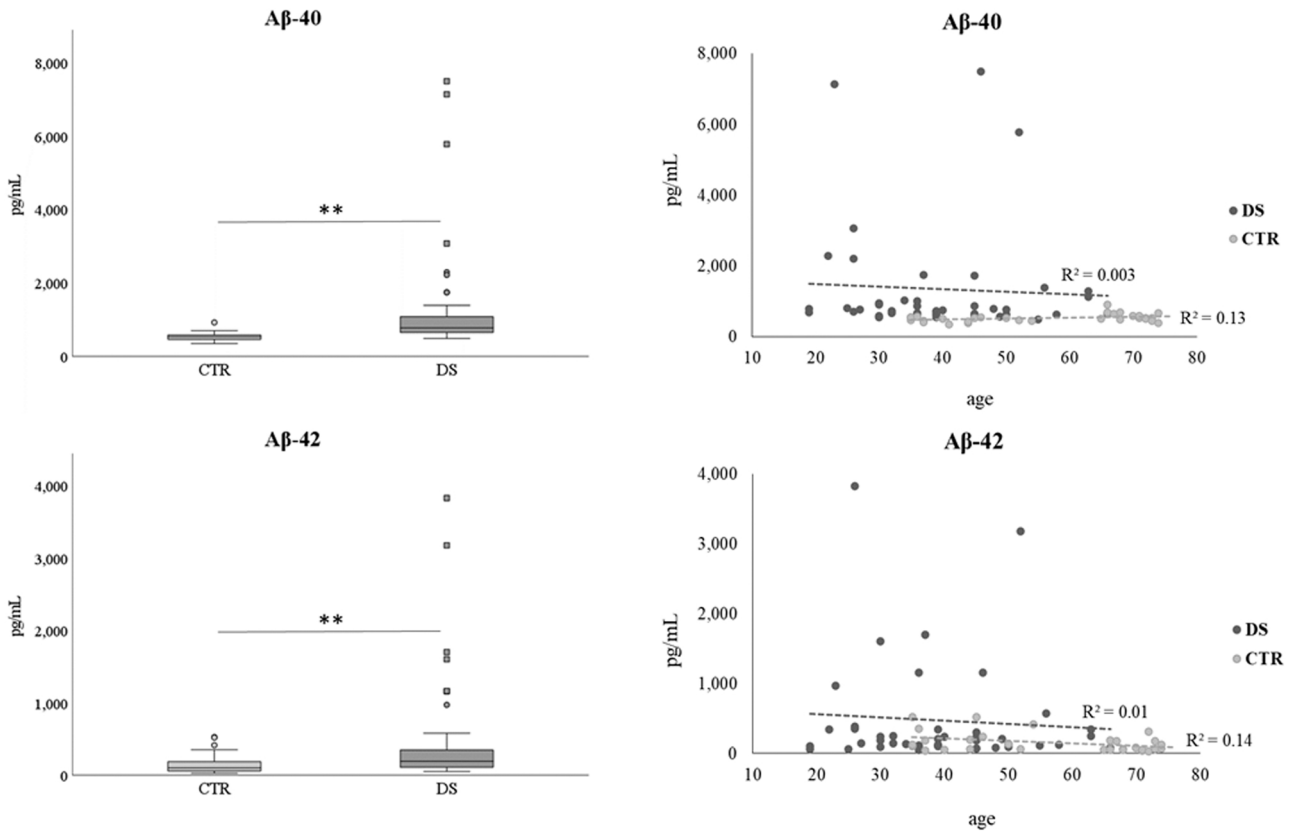


Fig. 5. Beta amyloid peptides concentration in DS and controls. Amyloid peptides concentration in plasma samples from DS subjects (N = 43) and compared to control group (N = 30, CTR). Data are reported as box plot of data distribution (median value and quartile ranges). Statistical analysis was performed by Mann-Whitney Nonparametric U test. * = $p \leq 0.05$. Regression analyses alongside the box plots show amyloid peptides concentration with age for both the two cohorts. Coefficients R^2 are indicated.

samples from DS persons. Initially, miR expression profiling was performed on 3 elderly DS compared to 3 young DS, using arrays. Four miRs were selected for a subsequent RT-qPCR validation analysis, i.e. miR-628–5p, miR-152–3p and miR-28–5p. Additionally, let-7d-5p, miR-155–5p and miR-21–5p were included in the validation phase due to their well-established role in inflammation, ageing (inflamma-miRs) (Olivieri et al., 2013) and cognitive decline (Chen et al., 2018; Kumar et al., 2013; Zhao et al., 2013). The validation phase was conducted in an enlarged cohort of 43 DS together with 30 plasma samples obtained from a control group of age-sex matched subjects. This group was adopted as reference for physiological ageing in terms of blood miRs levels. Comparing the two cohorts of DS and controls, three miRs resulted lower in DS than control group, i.e. miR-628–5p, miR-152–3p and miR-28–5p. Surprisingly, these miRs did not correlate with age in DS, even if a trend of increase was observed. Similarly, stratifying data by the sex variable, no significant differences emerged.

Afterward, a threshold of 50 years old was set considering the age mean value of the entire population, as the literature indicate a premature ageing of DS persons (Ghezzo et al., 2014; Ravaioli et al., 2022). No significant variations resulted comparing DS before and after 50 years old. In this respect, the discrepancy between discovery and validation phases could partially be explained by the different normalization methodology of raw data, being the median of the overall miR expression adopted for arrays, while cel-miR-39 CT values are adopted for the RT-qPCR data normalization (standard procedure in plasma samples). Indeed, the lack of confirmation of profiling data with the validation phase has already been reported for plasma samples (Kangas et al., 2017).

A significant decrease of the expression level of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p, was identified in younger DS ($\leq$ 50 years), when compared with age-matched control, while no significant miR changes were observed between the two different cohorts at older ages ($\geq$51 years). Interestingly, miR-28–5p and let-7d-5p decreased in the oldest control group, while the same miRs were found at lowest levels in younger DS ($\leq$ 50 years), thus they may be proposed as biomarkers of premature ageing in DS. In this respect, miR-628–5p and miR-152–3p seem to be mostly associated with DS phenotype, being not found associated with physiological ageing. These results suggest that the age-acceleration/premature phenomenon characterizing DS could be predominant in the first 50 years of life, when compared with physiological ageing.

Immunoassay of beta amyloid peptides Aβ-40 and Aβ-42 was performed in all plasma samples. The two peptides resulted significantly different comparing the two entire cohorts of DS and controls regardless of age. Using the same threshold of 50 years old, Aβ-42 significantly decreased in physiological ageing ($\geq$ 51 years group), while Aβ-40 increased in young DS respect to age-matched controls ($\leq$ 50 years). It was already reported that amyloid peptides are higher in DS respect to controls (Fortea et al., 2020) and they doesn't change with age (Startin et al., 2019).

Aβ-42 was found negatively correlated in physiological ageing (Lue et al., 2019), even if discordant data are reported with fluctuations of the protein plasma levels, likely influenced by age (Zecca et al., 2021). Aβ-42 positively correlated with miR-28–5p and let-7d-5p, while Aβ-40 positively correlated with miR-628–5p. These data suggest a different role of circulating amyloid peptides in the two cohorts, accordingly with the finding that in plasma of DS without dementia, only Aβ-40 is increased respect to controls (Fang et al., 2020). Moreover, it is necessary to highlight that the current data obtained in plasma samples are not necessarily the mirror of what it might be found in cerebrospinal

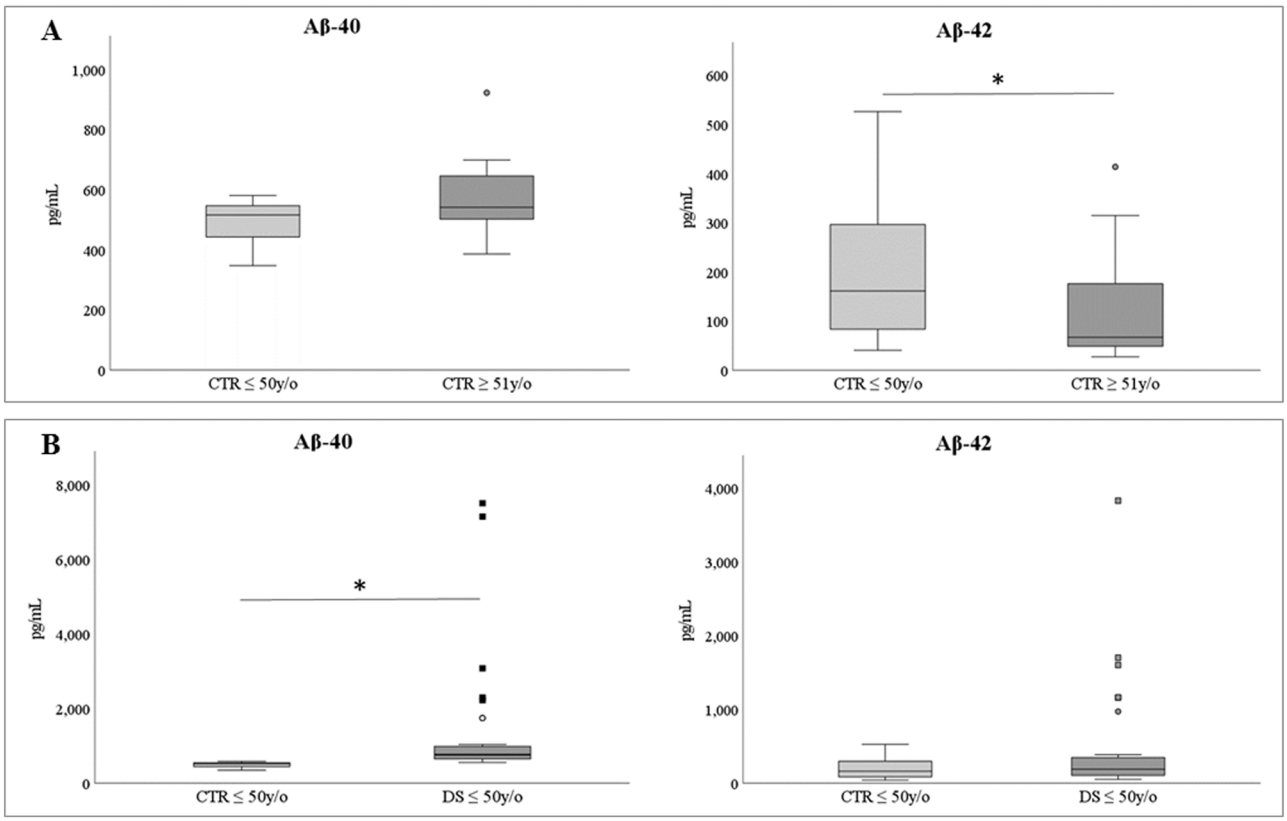


Fig. 6. Amyloid peptides both in physiological ageing and in younger DS vs age-matched controls. Panel A. Aβ-40 and Aβ-42 plasma concentrations in physiological ageing were reported. Panel B. Aβ-40 and Aβ-42 plasma concentrations in younger DS subjects were compared with age-matched controls (CTR) (≤ 50 years). Data are reported as box plot of data distribution (median value and quartile ranges). Statistical analysis was performed with Mann-Whitney Nonparametric U test. * = p ≤ 0.05.

Table 1
Crrelations of Aβ-40 and Aβ-42 and selected miRs in DS cohort.

Sperman's rho		Aβ-40	Aβ-42
miR-628–5p	Correlation coefficient	**0.316**	0.266
	Sig. (2-tailed)	**0.04**	0.09
	N	43	43
miR-152–3p	Correlation coefficient	0.087	0.149
	Sig. (2-tailed)	0.58	0.34
	N	43	43
miR-28–5p	Correlation coefficient	0.255	**0.304**
	Sig. (2-tailed)	0.15	**0.05**
	N	43	43
let-7d-5p	Correlation coefficient	0.254	**0.301**
	Sig. (2-tailed)	0.10	**0.05**
	N	43	43

fluid or in brain (Zlokovic, 2011). In fact, no significant correlations were found among neuropsychological function tests and beta amyloid peptides expression, as already described (Yun et al., 2021). Various evidences have shown that the ratio between the two peptides, Aβ-42/40, is not only an important marker for AD both in CSF (Lewczuk et al., 2020) and in plasma (Pérez-Grijalba et al., 2019), but also in cognitive impairment for the prediction of dementia progression. Nevertheless, this ratio was not found to be significantly modified in the current work, likely suggesting a non-specific role in DS.

Literature indicates that the most relevant differences according to premature ageing phenotype can be found in early life of DS. In a study by Ghezzo et al., older DS persons show lower neuropsychological functions (short memory skills, frontal lobe functions, visuo-spatial abilities, adaptive skills) compared to younger ones and the decline of these skills starts very early, preceding by about 20–30 years the onset of dementia in DS persons, while it occurs mostly after the age of 60 years in physiological ageing. Therefore, the study suggests that anti-dementia treatment may not be effective in older DS persons, as their neuropsychological functions and adaptive skills have already declined (Ghezzo et al., 2014). Similarly, a recent study indicates that DS persons younger than 35 years tend to show DNA methylation levels of ribosomal DNA similar to that of older euploid subjects (Ravaioli et al., 2022).

As far as the identified miRs are concerned, miR-628–5p expression levels was found decreased with ageing in skeletal muscle tissue. A possible role of miR-628–5p in the STARS (Striated Muscle Activator of Rho) signalling pathway was predicted, and it was described critical for skeletal muscle growth, repair, and function over the lifespan (Russell et al., 2017). Moreover, a recent study by Liu et al. (2021) reported that miR-628–5p overexpression may inhibit progressive neurodegeneration in AD by targeting TYROBP. TYROBP is a type I transmembrane protein necessary for the activation signal transduction and typically expressed on microglial cells in the brain (Paloneva et al., 2000). TYROBP-mediated signalling participates in regulating the expression levels of multiple genes in the brain, including TREM1 and TREM2 (Satoh et al., 2012). It was proposed that activated microglia acts as contributor to the progression of chronic neurodegeneration in AD, rather than simply being a consequence of the pathology. In AD progression, chronic microglia activation leads to chronic neuroinflammation, which likely contributes to neuronal dysfunction, injury, and loss (Streit et al., 2004). Therefore, the significant lower expression level of miR-628–5p found in DS subjects may lead to an increased expression of TYROBP, potentially contributing to the early onset of neurodegeneration (or AD). Overall, these findings suggest that miR-628–5p might have an important role in fundamental age-related processes, making it a potential biomarker of biological age.

MiR-152–3p expression level was found significantly decreased in

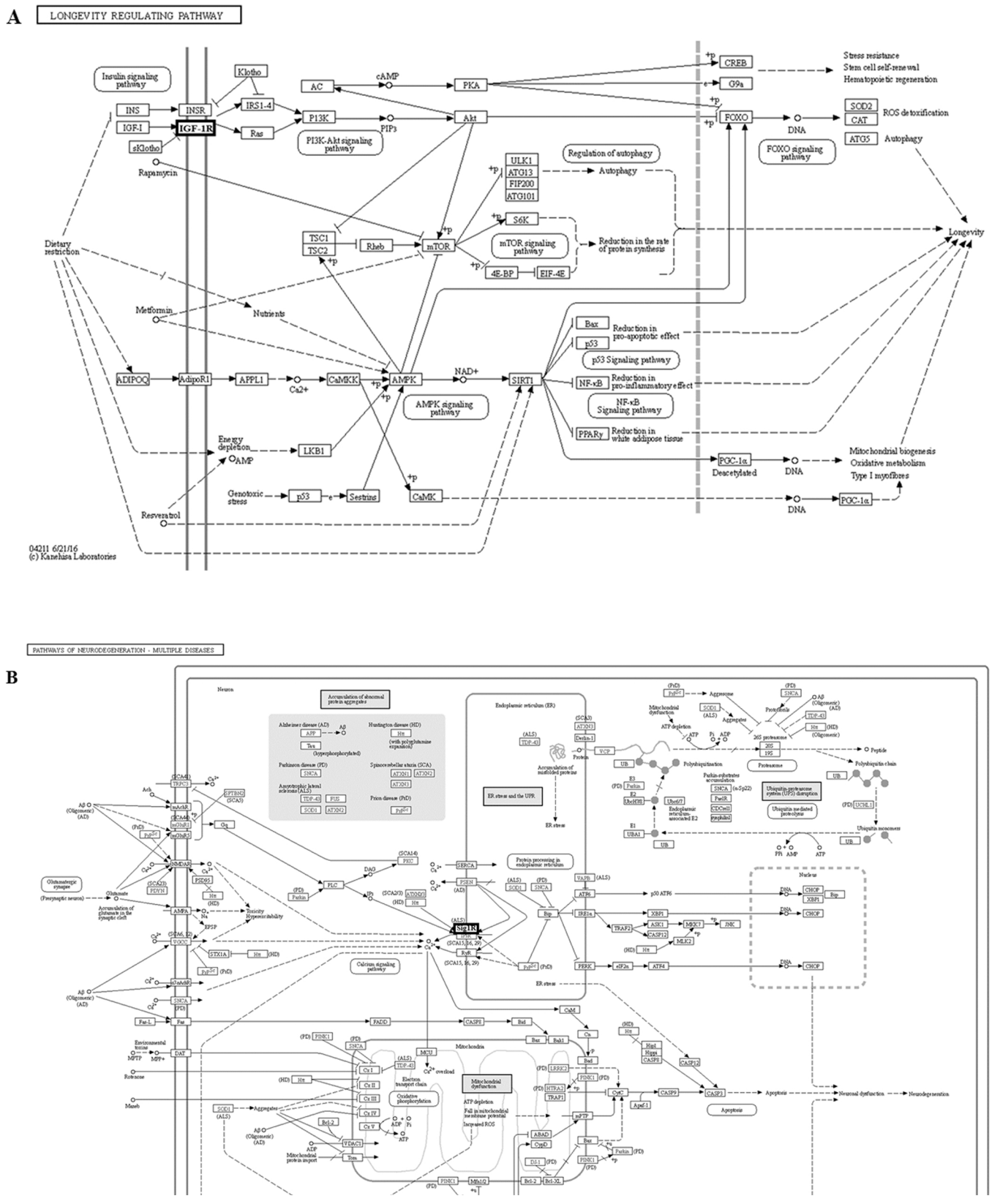


Fig. 7. MiR targets and pathway analysis. Panel A. A schematic representation of longevity regulating pathway (KEGG). The figure displays the genes involved in the regulation of longevity, and IGF1R (highlighted gene) is a validated target of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p. Panel B. A schematic representation of neurodegenerative pathways (KEGG). The figure displays some of the genes involved in the neurodegeneration process, and SIGMAR1 (highlighted gene) is a validated target of miR-628–5p, miR-152–3p, miR-28–5p, and let-7d-5p.

NK cells of patients affected by Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) in comparison with non-fatigued controls (Brenu et al., 2012). Clinically, CFS/ME is characterized by chronic, multi-system symptoms including post-exertional malaise (PEM), unrefreshing sleep, significant fatigue, pain, muscle weakness, and cognitive impairment (Jason et al., 2015). Proposed risk factors include altered immunity, infection, environmental exposures, allergies, genetics, and stress acting through alterations in immune and inflammatory responses. The symptoms and risk factors for CFS/ME have features in common with those for accelerated ageing/premature immunosenescence (Rajeevan et al., 2018). Therefore, the significantly decreased expression level of miR-152–3p in DS subjects may be associated with a similar age-acceleration phenomenon and may serve as an effective biomarker of ageing in DS.

MiR-28–5p has been reported to be involved in numerous kinds of cancer, but its role in DS and ageing has to be investigated yet. Thus, the results obtained in the current work may reveal a possible involvement of miR-28–5p in the ageing process and its potential function as a biomarker of premature ageing in DS.

Numerous studies investigated the role of let-7d-5p in the pathogenesis of AD. For instance, a systematic bioinformatics analysis found a significant down-regulation of let-7d-5p in patients affected by AD compared to healthy controls (Chen et al., 2018). Similar results were achieved revealing significantly decreased expression levels of let-7d-5p in plasma samples from AD patients compared to healthy control donors (Kumar et al., 2013). It has been demonstrated that let-7d-5p can regulate multiple aspects of neurogenesis, including neural stem cell proliferation, differentiation, and neuronal migration in the mammalian brain (Zhao et al., 2013). Moreover, a study by Luo et al. (2015) demonstrated that amyloid precursor protein (APP) mRNA is a direct target of let-7d-5p and reported a significant down-regulation of this miR in an animal model, where expression levels of APP and Aβ were higher in the learning and memory impaired rats. Overall, these findings suggest that low expression level of let-7d-5p may participate in the development of age-related neurodegeneration and likely may have also a role in premature ageing of DS persons.

To further investigate the role of the identified miR signature in ageing and DS, a bioinformatic analysis was performed, with the aim of finding common validated targets. Interestingly, Insulin-like Growth Factor 1 Receptor (IGF1R) mRNA was found to be a validated target of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p, significantly modified in DS $\leq$ 50 years. The crucial role of IGF1R as regulator of longevity is already well described in literature. It has been reported that centenarians, the best example of successful ageing, have lower plasma IGF-I levels (Paolisso et al., 1997) and genetic variants of IGF1R are significantly associated with longevity (Franceschi et al., 2020). According to these findings, the significant down-regulation of miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p reported in DS subjects compared to controls under 50 years old may contribute to an up-regulation of IGF1R, but further experiments are needed to verify this issue, which could be associated to life span average of DS persons and its possible therapeutic approach.

The bioinformatic analysis identified another interesting common target of the 4 miRs i.e., Sigma-1 Receptor (SIGMAR1). This receptor has a neuroprotective activity demonstrated in various models of neurodegenerative disorders and some of the neuroprotective effects mediated by SIGMAR1 have been attributed to anti-inflammatory actions of its ligands (Ruscher and Wieloch, 2015). These findings seem to be in contrast with the results obtained, but current data report that SIGMAR1 is regulated by at least 62 miRs (Tarbase v8.0) and, interestingly, it is targeted by let-7c-5p, miR-125b-5p and miR-155–5p, which are located on human chromosome 21. Therefore, it is possible that reduced expression of miR-628–5p, miR-152–3p, miR-28–5p, and let-7d-5p does not directly reflect an overexpression of SIGMAR1 in the brain of DS individuals, considering that the analysis was performed on c-miRs and the results obtained do not necessarily mirror what happens in different districts of body.

Data emerged from this study suggest that miR-628–5p, miR-152–3p, miR-28–5p and let-7d-5p may represent a blood miRs signature for the characterization of the segmental ageing in DS persons. Particularly, miR-28–5p and let-7d-5p, could be potential biomarkers of the premature ageing in DS before the age of 50, since they decrease later in non-trisomic physiological ageing.

Future researches should be focused on therapeutic approaches to counteract or slow-down the ageing process in DS persons. Until now, no approved treatment exists, even if metformin was proposed as promising candidate to counteract the cognitive decline in DS, due to its anti-ageing effects (Franceschi et al., 2019). Here, new identified miRs are proposed for a further enlarged analysis aiming at the identification of gene/epigenetic changes that contribute to abnormalities in cognitive function and behaviour and likely to the identification of successful treatments applicable across the life span for people with DS (Bartesaghi et al., 2022).

Funding sources

This work was supported by the European Union's Seventh Framework Programme [grant HEALTH-F4–2008-200880 MARK-AGE] to CF and to the coordinator AB; by CARIPLO (Fondazione Cassa di Risparmio delle Province Lombarde) [grant number 2016-0835] Ricerca malattie invecchiamento 2016 to MdE, and by the Italian Ministry of Health Ricerca Finalizzata Young Researchers (under 40)-Giovani Ricercatori [grant number 2019-12369983] to MGB.

Conflicts of interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2022.111691.

References

Bartesaghi, R., Vicari, S., Mobley, W.C., 2022. Prenatal and postnatal pharmacotherapy in down syndrome: the search to prevent or ameliorate neurodevelopmental and neurodegenerative disorders. Annu. Rev. Pharm. Toxicol. 62, 211–233. https://doi.org/10.1146/annurev-pharmtox-041521-103641.

Borelli, V., Vanhooren, V., Lonardi, E., Reiding, K.R., Capri, M., Libert, C., Garagnani, P., Salvioli, S., Franceschi, C., Wuhrer, M., 2015. Plasma N-Glycome Signature of Down Syndrome. J. Proteome Res. 14, 4232–4245. https://doi.org/10.1021/acs.jproteome.5b00356.

Brás, A., Rodrigues, A.S., Gomes, B., Rueff, J., 2018. Down syndrome and microRNAs. Biomed. Rep. 8, 11–16. https://doi.org/10.3892/br.2017.1019.

Brenu, E.W., Ashton, K.J., van Driel, M., Staines, D.R., Peterson, D., Atkinson, G.M., Marshall-Gradisnik, S.M., 2012. Cytotoxic lymphocyte microRNAs as prospective biomarkers for chronic fatigue syndrome/myalgic encephalomyelitis. J. Affect. Disord. 141, 261–269. https://doi.org/10.1016/j.jad.2012.03.037.

Bürkle, A., Moreno-Villanueva, M., Bernhard, J., Blasco, M., Zondag, G., Hoeijmakers, J. H.J., Toussaint, O., Grubeck-Loebenstein, B., Mocchegiani, E., Collino, S., Gonos, E. S., Sikora, E., Gradinaru, D., Dollé, M., Salmon, M., Kristensen, P., Griffiths, H.R., Libert, C., Grune, T., Breusing, N., Simm, A., Franceschi, C., Capri, M., Talbot, D., Caiafa, P., Friguet, B., Slagboom, P.E., Hervonen, A., Hurme, M., Aspinall, R., 2015. MARK-AGE biomarkers of ageing. Mech. Ageing Dev. 151, 2–12. https://doi.org/10.1016/j.mad.2015.03.006.

Capri, M., Moreno-Villanueva, M., Cevenini, E., Pini, E., Scurti, M., Borelli, V., Palmas, M.G., Zoli, M., Schön, C., Siepelmeyer, A., Bernhardt, J., Fiegl, S., Zondag, G., de Craen, A.J.M., Hervonen, A., Hurme, M., Sikora, E., Gonos, E.S., Voutetakis, K., Toussaint, O., Debacq-Chainiaux, F., Grubeck-Loebenstein, B., Bürkle, A., Franceschi, C., 2015. MARK-AGE population: from the human model to new insights. Mech. Ageing Dev. 151, 13–17. https://doi.org/10.1016/j.mad.2015.03.010.

Chen, J., Qi, Y., Liu, C.-F., Lu, J.-M., Shi, J., Shi, Y., 2018. MicroRNA expression data analysis to identify key miRNAs associated with Alzheimer's disease. J. Gene Med. 20, e3014 https://doi.org/10.1002/jgm.3014.

Cole, J.H., Franke, K., 2017. Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. Trends in Neurosciences 40, 681–690. https://doi.org/10.1016/j.tins.2017.10.001.

Fang, W.-Q., Hwu, W.-L., Chien, Y.-H., Yang, S.-Y., Chieh, J.-J., Chang, L.-M., Huang, A.-C., Lee, N.-C., Chiu, M.-J., 2020. Composite scores of plasma tau and β-amyloids correlate with dementia in down syndrome. ACS Chem. Neurosci. 11, 191–196. https://doi.org/10.1021/acschemneuro.9b00585.

Fortea, J., Vilaplana, E., Carmona-Iragui, M., Benejam, B., Videla, L., Barroeta, I., Fernández, S., Altuna, M., Pegueroles, J., Montal, V., Valldeneu, S., Giménez, S., González-Ortiz, S., Muñoz, L., Estellés, T., Illán-Gala, I., Belbin, O., Camacho, V., Wilson, L.R., Annus, T., Osorio, R.S., Videla, S., Lehmann, S., Holland, A.J., Alcolea, D., Clarimón, J., Zaman, S.H., Blesa, R., Lleó, A., 2020. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. Lancet 395, 1988–1997. https://doi.org/10.1016/S0140-6736(20)30689-9.

Franceschi, C., Garagnani, P., Gensous, N., Bacalini, M.G., Conte, M., Salvioli, S., 2019. Accelerated bio-cognitive aging in Down syndrome: State of the art and possible deceleration strategies. Aging Cell 18, e12903. https://doi.org/10.1111/acel.12903.

Franceschi, C., Garagnani, P., Olivieri, F., Salvioli, S., Giuliani, C., 2020. The contextualized genetics of human longevity: JACC focus seminar. J. Am. Coll. Cardiol. 75, 968–979. https://doi.org/10.1016/j.jacc.2019.12.032.

Gensous, N., Franceschi, C., Salvioli, S., Garagnani, P., Bacalini, M.G., 2019. Down syndrome, ageing and epigenetics. In: Harris, J.R., Korolchuk, V.I. (Eds.), Biochemistry and Cell Biology of Ageing: Part II Clinical Science, Subcellular Biochemistry. Springer, Singapore, pp. 161–193. https://doi.org/10.1007/978-981-13-3681-2_7.

Ghezzo, A., Salvioli, S., Solimando, M.C., Palmieri, A., Chiostergi, C., Scurti, M., Lomartire, L., Bedetti, F., Cocchi, G., Follo, D., Pipitone, E., Rovatti, P., Zamberletti, J., Gomiero, T., Castellani, G., Franceschi, C., 2014. Age-related changes of adaptive and neuropsychological features in persons with down syndrome. PLoS One 9, e113111. https://doi.org/10.1371/journal.pone.0113111.

Head, E., Lott, I.T., Wilcock, D.M., Lemere, C.A., 2016. Aging in down syndrome and the development of Alzheimer's disease neuropathology. Curr. Alzheimer Res. 13, 18–29.

Horvath, S., Garagnani, P., Bacalini, M.G., Pirazzini, C., Salvioli, S., Gentilini, D., Di Blasio, A.M., Giuliani, C., Tung, S., Vinters, H.V., Franceschi, C., 2015. Accelerated epigenetic aging in Down syndrome. Aging Cell 14, 491–495. https://doi.org/10.1111/acel.12325.

Jason, L.A., Zinn, M.L., Zinn, M.A., 2015. Myalgic encephalomyelitis: symptoms and biomarkers. Curr. Neuropharmacol. 13, 701–734. https://doi.org/10.2174/1570159×13666150928105725.

Jenkins, E.C., Marchi, E.J., Velinov, M.T., Ye, L., Krinsky-McHale, S.J., Zigman, W.B., Schupf, N., Silverman, W.P., 2017. Longitudinal telomere shortening and early Alzheimer's disease progression in adults with down syndrome. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 174, 772–778. https://doi.org/10.1002/ajmg.b.32575.

Kangas, R., Morsiani, C., Pizza, G., Lanzarini, C., Aukee, P., Kaprio, J., Sipilä, S., Franceschi, C., Kovanen, V., Laakkonen, E.K., Capri, M., 2017. Menopause and adipose tissue: miR-19a-3p is sensitive to hormonal replacement. Oncotarget 9, 2279–2294. https://doi.org/10.18632/oncotarget.23406.

Karagkouni, D., Paraskevopoulou, M.D., Chatzopoulos, S., Vlachos, I.S., Tastsoglou, S., Kanellos, I., Papadimitriou, D., Kavakiotis, I., Maniou, S., Skoufos, G., Vergoulis, T., Dalamagas, T., Hatzigeorgiou, A.G., 2018. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions. Nucleic Acids Res. 46, D239–D245. https://doi.org/10.1093/nar/gkx1141.

Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt, E.E., Wyss-Coray, T., Sierra, F., 2014. Geroscience: linking aging to chronic disease. Cell 159, 709–713. https://doi.org/10.1016/j.cell.2014.10.039.

Kumar, P., Dezso, Z., MacKenzie, C., Oestreicher, J., Agoulnik, S., Byrne, M., Bernier, F., Yanagimachi, M., Aoshima, K., Oda, Y., 2013. Circulating miRNA biomarkers for Alzheimer's disease. PLoS One 8, e69807. https://doi.org/10.1371/journal.pone.0069807.

Lewczuk, P., Łukaszewicz-Zając, M., Mroczko, P., Kornhuber, J., 2020. Clinical significance of fluid biomarkers in Alzheimer's Disease. Pharmacol. Rep. 72, 528–542. https://doi.org/10.1007/s43440-020-00107-0.

Liu, M., Li, S., Ma, C., Wang, X., Shuang, Y., Lin, H., Guan, Z., Yang, L., 2021. Bioinformatics analysis indicates that microRNA-628–5p overexpression may alleviate Alzheimer's disease by targeting TYROBP. Mol. Med. Rep. 23, 142. https://doi.org/10.3892/mmr.2020.11781.

Lott, I.T., Head, E., 2019. Dementia in down syndrome: unique insights for Alzheimer disease research. Nat. Rev. Neurol. 15, 135–147. https://doi.org/10.1038/s41582-018-0132-6.

Lue, L.-F., Pai, M.-C., Chen, T.-F., Hu, C.-J., Huang, L.-K., Lin, W.-C., Wu, C.-C., Jeng, J.-S., Blennow, K., Sabbagh, M.N., Yan, S.-H., Wang, P.-N., Yang, S.-Y., Hatsuta, H., Morimoto, S., Takeda, A., Itoh, Y., Liu, J., Xie, H., Chiu, M.-J., 2019. Age-dependent relationship between plasma Aβ40 and Aβ42 and total tau levels in cognitively normal subjects. Front. Aging Neurosci. 11, 222. https://doi.org/10.3389/fnagi.2019.00222.

Luo, T., Yin, S., Shi, R., Xu, C., Wang, Y., Cai, J., Yue, Y., Wu, A., 2015. miRNA expression profile and involvement of Let-7d-APP in aged rats with isoflurane-induced learning and memory impairment. PLoS One 10, e0119336. https://doi.org/10.1371/journal.pone.0119336.

Moreno-Villanueva, M., Capri, M., Breusing, N., Siepelmeyer, A., Sevini, F., Ghezzo, A., Craen, A.J.M., de, Hervonen, A., Hurme, M., Schön, C., Grune, T., Franceschi, C., Bürkle, A., 2015. MARK-AGE standard operating procedures (SOPs): a successful effort. Mech. Ageing Dev. Biomark. Hum. Ageing 151, 18–25. https://doi.org/10.1016/j.mad.2015.03.007.

Olivieri, F., Rippo, M.R., Procopio, A.D., Fazioli, F., 2013. Circulating inflamma-miRs in aging and age-related diseases. Front. Genet. 4 https://doi.org/10.3389/fgene.2013.00121.

Paloneva, J., Kestilä, M., Wu, J., Salminen, A., Böhling, T., Ruotsalainen, V., Hakola, P., Bakker, A.B.H., Phillips, J.H., Pekkarinen, P., Lanier, L.L., Timonen, T., Peltonen, L., 2000. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat. Genet. 25, 357–361. https://doi.org/10.1038/77153.

Paolisso, G., Ammendola, S., Del Buono, A., Gambardella, A., Riondino, M., Tagliamonte, M.R., Rizzo, M.R., Carella, C., Varricchio, M., 1997. Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function. J. Clin. Endocrinol. Metab. 82, 2204–2209. https://doi.org/10.1210/jcem.82.7.4087.

Pérez-Grijalba, V., Romero, J., Pesini, P., Sarasa, L., Monleón, I., San-José, I., Arbizu, J., Martínez-Lage, P., Munuera, J., Ruiz, A., Tárraga, L., Boada, M., Sarasa, M., The AB255 Study Group, 2019. Plasma Aβ42/40 ratio detects early stages of Alzheimer's disease and correlates with CSF and neuroimaging biomarkers in the AB255 study. J. Prev. Alzheimers Dis. 6, 34–41. https://doi.org/10.14283/jpad.2018.41.

Rajeevan, M.S., Murray, J., Oakley, L., Lin, J.-M.S., Unger, E.R., 2018. Association of chronic fatigue syndrome with premature telomere attrition. J. Transl. Med. 16, 44. https://doi.org/10.1186/s12967-018-1414-x.

Ravaioli, F., Zampieri, M., Morandi, L., Pirazzini, C., Pellegrini, C., De Fanti, S., Gensous, N., Pirazzoli, G.L., Sambati, L., Ghezzo, A., Ciccarone, F., Reale, A., Monti, D., Salvioli, S., Caiafa, P., Capri, M., Bürkle, A., Moreno-Villanueva, M., Garagnani, P., Franceschi, C., Bacalini, M.G., 2022. DNA methylation analysis of ribosomal DNA in adults with down syndrome. Front. Genet. 13, 792165 https://doi.org/10.3389/fgene.2022.792165.

Ruscher, K., Wieloch, T., 2015. The involvement of the sigma-1 receptor in neurodegeneration and neurorestoration. J. Pharm. Sci. 127, 30–35. https://doi.org/10.1016/j.jphs.2014.11.011.

Russell, A.P., Wallace, M.A., Kalanon, M., Zacharewicz, E., Della Gatta, P.A., Garnham, A., Lamon, S., 2017. Striated muscle activator of Rho signalling (STARS) is reduced in ageing human skeletal muscle and targeted by miR-628-5p. Acta Physiol. 220, 263–274. https://doi.org/10.1111/apha.12819.

Satoh, J., Shimamura, Y., Tabunoki, H., 2012. Gene expression profile of THP-1 monocytes following knockdown of DAP12, a causative gene for Nasu-Hakola disease. Cell Mol. Neurobiol. 32, 337–343. https://doi.org/10.1007/s10571-011-9769-z.

Startin, C.M., Ashton, N.J., Hamburg, S., Hithersay, R., Wiseman, F.K., Mok, K.Y., Hardy, J., Lleó, A., Lovestone, S., Parnetti, L., Zetterberg, H., Hye, A., LonDownS Consortium, Strydom, A., 2019. Plasma biomarkers for amyloid, tau, and cytokines in down syndrome and sporadic Alzheimer's disease. Alzheimers Res. Ther. 11, 26. https://doi.org/10.1186/s13195-019-0477-0.

Streit, W.J., Mrak, R.E., Griffin, W.S.T., 2004. Microglia and neuroinflammation: a pathological perspective. J. Neuroinflamm. 1, 14. https://doi.org/10.1186/1742-2094-1-14.

Tan, Lin, Yu, J.-T., Hu, N., Tan, Lan, 2013. Non-coding RNAs in Alzheimer's disease. Mol. Neurobiol. 47, 382–393. https://doi.org/10.1007/s12035-012-8359-5.

Yun, G., Kim, H.J., Kim, H.-G., Lee, K.M., Hong, I.K., Kim, S.H., Rhee, H.Y., Jahng, G.-H., Yoon, S.S., Park, K.-C., Hwang, K.S., Lee, J.S., 2021. Association between plasma amyloid-β and neuropsychological performance in patients with cognitive decline. Front. Aging Neurosci. 13.

Zecca, C., Pasculli, G., Tortelli, R., Dell'Abate, M.T., Capozzo, R., Barulli, M.R., Barone, R., Accogli, M., Arima, S., Pollice, A., Brescia, V., Logroscino, G., 2021. The role of age on beta-amyloid1–42 plasma levels in healthy subjects. Front. Aging Neurosci. 13.

Zhao, C., Sun, G., Ye, P., Li, S., Shi, Y., 2013. MicroRNA let-7d regulates the TLX/microRNA-9 cascade to control neural cell fate and neurogenesis. Sci. Rep. 3, 1329. https://doi.org/10.1038/srep01329.

Zlokovic, B.V., 2011. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat. Rev. Neurosci. 12, 723–738. https://doi.org/10.1038/nrn3114.