

## A geroscience approach for Parkinson's disease: Conceptual framework and design of PROPAG-AGEING project

Chiara Pirazzini<sup>a</sup>, Tiago Azevedo<sup>b</sup>, Luca Baldelli<sup>c</sup>, Anna Bartoletti-Stella<sup>a</sup>,  
 Giovanna Calandra-Buonaura<sup>a,c</sup>, Alessandra Dal Molin<sup>d</sup>, Giovanna Maria Dimitri<sup>b</sup>,  
 Ivan Doykov<sup>e</sup>, Pilar Gómez-Garre<sup>f,g</sup>, Sara Hägg<sup>h</sup>, Jenny Hällqvist<sup>e</sup>, Claire Halsband<sup>i,j</sup>,  
 Wendy Heywood<sup>e,k</sup>, Silvia Jesús<sup>f,g</sup>, Juulia Jylhävä<sup>h</sup>, Katarzyna Malgorzata Kwiatkowska<sup>l</sup>,  
 Miguel A. Labrador-Espinosa<sup>f,g</sup>, Cristina Licari<sup>m</sup>, Maria Giovanna Maturo<sup>n</sup>,  
 Giacomo Mengozzi<sup>a</sup>, Gaia Meoni<sup>o</sup>, Maddalena Milazzo<sup>l</sup>, Maria Teresa Perinán-Tocino<sup>f,g</sup>,  
 Francesco Ravaioi<sup>l</sup>, Claudia Sala<sup>p</sup>, Luisa Sambati<sup>a,c</sup>, Sebastian Schade<sup>i</sup>,  
 Sebastian Schreglmann<sup>q</sup>, Simeon Spasov<sup>b</sup>, Leonardo Tenori<sup>r</sup>, Dylan Williams<sup>h</sup>,  
 Luciano Xumerle<sup>d</sup>, Elisa Zago<sup>d</sup>, Kailash P. Bhatia<sup>q</sup>, Sabina Capellari<sup>a,c</sup>, Pietro Cortelli<sup>a,c</sup>,  
 Paolo Garagnani<sup>l</sup>, Henry Houlden<sup>s</sup>, Pietro Liò<sup>b</sup>, Claudio Luchinat<sup>m,t</sup>, Massimo Delledonne<sup>u</sup>,  
 Kevin Mills<sup>e,k</sup>, Pablo Mir<sup>f,g</sup>, Brit Mollenhauer<sup>v,w</sup>, Christine Nardini<sup>x</sup>, Nancy L. Pedersen<sup>h</sup>,  
 Federica Provini<sup>a,c</sup>, Stephen Strom<sup>y</sup>, Claudia Trenkwalder<sup>v,z</sup>, Paola Turano<sup>m,t</sup>,  
 Maria Giulia Bacalini<sup>a,\*</sup>, Claudio Franceschi<sup>a,A</sup>, on behalf of PROPAG-AGEING Consortium

<sup>a</sup> IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

<sup>b</sup> Department of Computer Science and Technology, University of Cambridge, Cambridge, United Kingdom

<sup>c</sup> Department of Biomedical and NeuroMotor Sciences (DiBiNeM), University of Bologna, Italy

<sup>d</sup> Personal Genomics s.r.l, Italy

<sup>e</sup> Centre for Inborn Errors of Metabolism, UCL Institute of Child Health, London, United Kingdom

<sup>f</sup> Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Instituto de Biomedicina de Sevilla, Seville, Spain

<sup>g</sup> Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

<sup>h</sup> Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

<sup>i</sup> Department of Clinical Neurophysiology, University Medical Center Göttingen, Göttingen, Germany

<sup>j</sup> Department of Gerontopsychiatry, Rhein-Mosel-Fachklinik, Andernach, Germany

<sup>k</sup> NIHR Great Ormond Street Biomedical Research Centre, Great Ormond Street Hospital and UCL Great Ormond Street Institute of Child Health, London, United Kingdom

<sup>l</sup> Department of Experimental, Diagnostic, and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

<sup>m</sup> CERM, University of Florence, Sesto Fiorentino, Florence, Italy

<sup>n</sup> Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

<sup>o</sup> Giotto Biotech srl, Florence, Italy

<sup>p</sup> Department of Physics and Astronomy, University of Bologna, Bologna, Italy

<sup>q</sup> Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, United Kingdom

<sup>r</sup> Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), Florence, Italy

<sup>s</sup> Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, WC1N 3BG, United Kingdom

<sup>t</sup> Department of Chemistry "Ugo Schiff", University of Florence, Italy

<sup>u</sup> Department of Biotechnology, University of Verona, Italy

<sup>v</sup> Paracelsus-Elena-Klinik, Kassel, Germany

<sup>w</sup> Department of Neurology, University Medical Centre Goettingen, Goettingen, Germany

<sup>x</sup> Istituto per le Applicazioni del Calcolo Mauro Picone, CNR, Roma, Italy

<sup>y</sup> Department of Laboratory Medicine, Karolinska Institute and Karolinska Universitetssjukhuset, 171 76, Stockholm, Sweden

<sup>z</sup> Department of Neurosurgery, University Medical Center Göttingen, Germany

<sup>A</sup> Laboratory of Systems Medicine of Healthy Aging and Department of Applied Mathematics, Lobachevsky University, Nizhny Novgorod, Russia

\* Corresponding author at: IRCCS Istituto delle Scienze Neurologiche di Bologna, Via Altura 3, 40139, Bologna, BO, Italy.

E-mail address: [mariagiulia.bacalini@ausl.bologna.it](mailto:mariagiulia.bacalini@ausl.bologna.it) (M.G. Bacalini).

## ARTICLE INFO

## Keywords:

Parkinson's disease  
Inflammaging  
Neurodegeneration  
Omic

## ABSTRACT

Advanced age is the major risk factor for idiopathic Parkinson's disease (PD), but to date the biological relationship between PD and ageing remains elusive. Here we describe the rationale and the design of the H2020 funded project "PROPAG-AGEING", whose aim is to characterize the contribution of the ageing process to PD development. We summarize current evidences that support the existence of a continuum between ageing and PD and justify the use of a Geroscience approach to study PD. We focus in particular on the role of inflammaging, the chronic, low-grade inflammation characteristic of elderly physiology, which can propagate and transmit both locally and systemically. We then describe PROPAG-AGEING design, which is based on the multi-omic characterization of peripheral samples from clinically characterized drug-naïve and advanced PD, PD discordant twins, healthy controls and "super-controls", i.e. centenarians, who never showed clinical signs of motor disability, and their offspring. Omic results are then validated in a large number of samples, including in vitro models of dopaminergic neurons and healthy siblings of PD patients, who are at higher risk of developing PD, with the final aim of identifying the molecular perturbations that can deviate the trajectories of healthy ageing towards PD development.

## 1. Introduction

In 2016, 6.1 million people suffered from Parkinson's Disease (PD) worldwide (Dorsey et al., 2018). Among neurodegenerative diseases, PD is the second most common (after Alzheimer's Disease) and the one that displayed the largest increase in prevalence, which more than doubled from 1990 to 2016 (Dorsey et al., 2018). This increase in the number of PD patients is largely, although not exclusively, sustained by the ageing of the population, as advanced age is acknowledged to be the major risk factor for developing PD (Reeve et al., 2014). Accordingly, PD is uncommon before 50 years and its prevalence steeply increases after 65 years, peaking between 85 and 89 years of age (Bennett et al., 1996; Dorsey et al., 2018; Pringsheim et al., 2014).

In spite of this epidemiological evidence, to date, the biological relationship between PD and ageing remains elusive. This is at least in part due the paucity of experimental settings specifically aimed at investigating PD in the framework of the ageing process, in particular when studies performed on humans are considered (Pang et al., 2019).

With the aim of filling this gap, the European Consortium PROPAG-AGEING ("The continuum between healthy ageing and idiopathic Parkinson Disease within a propagation perspective of inflammation and damage: the search for new diagnostic, prognostic and therapeutic targets"; grant agreement 634821) has been established in the framework of the European call PHC-01-2014 (Understanding health, ageing and disease: determinants, risk factors and pathways). PROPAG-AGEING Consortium is highly interdisciplinary and gathers together 9 participants from high ranking academic and non-academic institutions throughout Europe, with a well-established expertise at both clinical and molecular level (Table 1).

PROPAG-AGEING implements a Geroscience approach for the study

**Table 1**  
PROPAG-AGEING Consortium.

Partner	Acronym	Country
AZIENDA UNITA' SANITARIA LOCALE DI BOLOGNA	AUSL- ISNB	Italy
UNIVERSITY COLLEGE LONDON	UCL	United Kingdom
UNIVERSITAETSMEZIN GOETTINGEN - GEORG- AUGUST-UNIVERSITAET GOETTINGEN - STIFTUNG OEFFENTLICHEN RECHTS	UMG- GOE	Germany
SERVICIO ANDALUZ DE SALUD	SAS	Spain
PERSONAL GENOMICS SRL	PG	Italy
THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF UCAM CAMBRIDGE	UCAM	United Kingdom
CONSORZIO INTERUNIVERSITARIO RISONANZE MAGNETICHE DI METALLOPROTEINE	CIRMMMP	Italy
KAROLINSKA INSTITUTET	KI	Sweden
ALMA MATER STUDIORUM - UNIVERSITA DI BOLOGNA	UNIBO	Italy

of PD. Geroscience is an interdisciplinary field that emphasizes the common mechanisms (operating at the level of molecules, cells, organs, systems and ecosystems) shared by physiological ageing and age-related diseases (ARDs) (Kennedy et al., 2014). According to Geroscience ARDs, including PD, are interpreted as the result of a local or systemic accelerated ageing process. This approach can contribute to the study of PD at different levels: i) at a mechanistic level, by investigating whether the molecular/cellular perturbations characteristic of the ageing process occur, possibly in a more pronounced or accelerated fashion, also in PD; ii) at a diagnostic/prognostic level, by investigating whether the biomarkers used to track ageing are also able to detect the onset or the progression of PD; iii) at the therapeutic level, by investigating whether anti-ageing drugs and interventions are potentially effective also in the treatment and prevention of PD.

In this framework, the main goal of PROPAG-AGEING is to identify the molecular/cellular perturbations deviating the phenotype of elderly subjects from a physiological decline to clinically overt PD. The design of the project is thus specifically implemented in order to track the trajectories of healthy ageing and of PD, using the same analytical approaches.

In the next paragraphs we will summarize the rationale at the basis of PROPAG-AGEING, which justifies the use of a Geroscience approach to study PD, and we will describe the design of the project and its implementation by the members of the Consortium.

## 2. PROPAG-AGEING rationale: the continuum between ageing and PD and the propagation hypothesis

Ageing is the result of a complex interplay between ontogenetic programs, genetic influences, life course environmental exposures and stochastic events (Cevenini et al., 2010; Dorsey et al., 2018; Franceschi et al., 2020) that concur to the high heterogeneity of phenotypes observed among the elderly (Franceschi et al., 2017b). Indeed, if on the one hand some persons develop age-related disease like PD, on the other some persons can reach the extreme limit of life in good health, i.e. the centenarians. Amongst these two extremes, there is a continuum of intermediate phenotypes including persons with subclinical manifestations of diseases more or less pronounced. This implies that, for a determined age range, it is difficult to classify an individual as absolutely healthy and that each individual follows his specific ageing trajectory (Fig. 1).

Different authors have identified a limited but highly interconnected set of hallmarks of ageing that is also shared with ARDs and therefore contributes to their risk (Kennedy et al., 2014; López-Otín et al., 2013). More specifically, features shared between ageing and PD include neuroanatomical changes, accumulation of  $\alpha$ -synuclein, cell senescence and changes in glial environment, mitochondrial dysfunction, oxidative and nitrate stress, impairment in proteasome and lysosome functions, gut microbiome deregulation and alteration of glial environment,

among others (Calabrese et al., 2018).

Histopathologically, PD is characterized by a patterned, preferential loss of dopaminergic neurons (DA) in the *pars compacta* region in the *substantia nigra* (SN) and the presence of intracellular inclusions, called Lewy bodies, containing  $\alpha$ -synuclein aggregates (Braak et al., 2003). A decrease in the number of DA neurons, together with other pathological changes in this brain region, is also observed during physiological ageing, without clinical symptoms suggesting PD. The course of neurodegeneration is gradual and slow and from the initiation of the neuronal damage and death to the PD diagnosis there is a long-time lag. Buchman et al. considered a large cohort of 744 healthy elderly (average age 88.5 years) without a clinical diagnosis of PD at death and found that about 1/3 showed a mild to severe neuronal loss within the *substantia nigra*, 17 % showed Lewy bodies and 10 % showed both these pathological features (Buchman et al., 2012). This study plasticly exemplifies the linear relationship between the concomitant increase of life expectancy and PD case prevalence. A PD-like pathology is likely much more common in the apparently healthy elderly population, but the vast majority of cases do not survive enough to pass the quantitative cutoff of neurodegeneration and experience the onset of clinically overt disease (Burke and O'Malley, 2013; Cheng et al., 2010). Other studies reported an age-dependent increase in  $\alpha$ -synuclein in the brains of healthy aged humans (Chu and Kordower, 2007; Xuan et al., 2011) as well as in the brain and in the enteric nervous system of animal models (Chu and Kordower, 2007; Li et al., 2018; Phillips et al., 2009), supporting the Braak hypothesis of gut-to-brain spreading of  $\alpha$ -synuclein (Braak et al., 2003; Kim et al., 2019). Experiments in Rhesus monkeys also reported a selective age-dependent decline in DA neurons in the ventral tier of SN, similarly to what happens in PD (Kanaan et al., 2007).

$\alpha$ -synuclein clearance from the cytosol is performed by the ubiquitin-proteasome and lysosome-autophagy system – advancing age and the PD disease being processes both associated with decreased activity in these systems (Collier et al., 2011). Accordingly, histological analyses in post-mortem PD tissues identified impaired proteasomes and lysosomes (McNaught et al., 2003).

Increased oxidative and nitrative damage is a hallmark of PD in brain tissue (Dias et al., 2013). Reactive oxygen and nitrogen species (ROS and RNS) are produced by mitochondria as side-products of aerobic respiration, and their lifelong accumulation substantially contributes to ageing (Harman, 1956). Interestingly, accumulation of  $\alpha$ -synuclein in mitochondria was found in PD, associated to an impairment in the activity of electron transport chain complex I and to an increase in oxidative stress and in ROS production (Devi et al., 2008). The boost in the ROS generation is closely related to the higher inflammation level reported both in ageing and PD (Guo et al., 2018; Vida et al., 2014). The

contribution of peripheral inflammation and neuroinflammation to PD pathogenesis has been extensively summarized elsewhere (Caggiu et al., 2019; Calabrese et al., 2018; Collins et al., 2012; Qin et al., 2016). Here we will underline some general aspects of this phenomenon, relevant in the context of the PROPAG-AGEING project:

### 2.1. A close relationship exists between PD-related inflammation and inflammaging

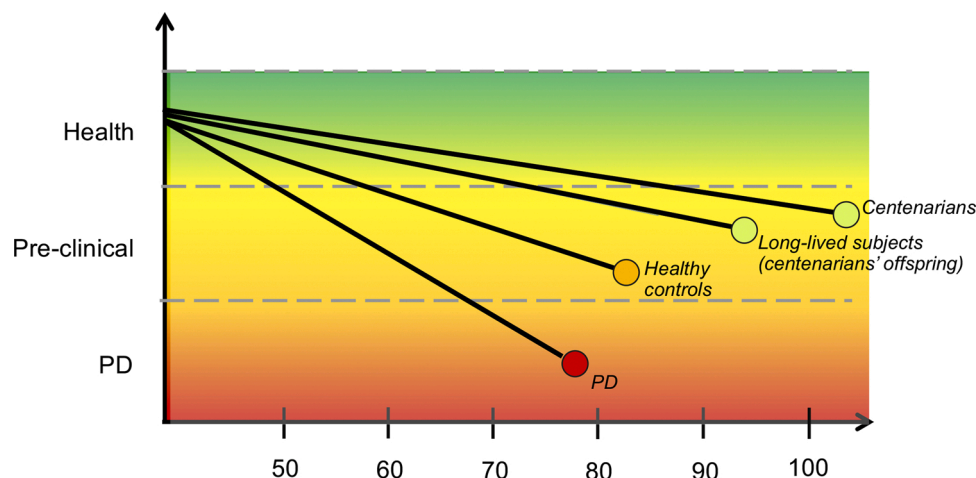
Inflammaging, i.e. the chronic, low-grade inflammation that occurs during ageing (Calabrese et al., 2018), is regarded as one of the main contributors of ARDs, including neurodegenerative diseases (Furman et al., 2019). As recently conceptualized (Franceschi et al., 2018a) inflammaging is triggered by the accumulation of non-self (pathogens), quasi-self (nutrients and microbiota products) and self (damaged and/or misplaced) molecules that converge on the activation of a limited number of sensors. These sensors promote the activation of a pro-inflammatory response, which concomitantly stimulates an adaptive activation of anti-inflammatory processes (anti-inflammaging).

In the brain, neuro-inflammaging is sustained by a complex interplay between different cellular types, including neurons, microglia, astrocytes and leukocytes that can penetrate the damaged blood-brain barrier (Costantini et al., 2018).

### 2.2. Inflammaging can propagate locally (cell-to-cell) and systemically (through the blood and lymphatic stream) (Franceschi et al., 2017a)

For example, damaged/misplaced self-molecules produced within the cell can be released by cell necrosis or actively secreted by extracellular vesicles, like exosomes. These pro-inflammatory compounds can affect the microenvironment of the adjoining cells and/or can enter the circulation, stimulating the inflammatory response in distal tissues and organs. The same propagation process applies also to the complex mixture of cytokines and pro-inflammatory molecules released by senescent cells and termed Senescence Associated Secretory Phenotype (SASP) (Acosta et al., 2013; Tchkonja et al., 2013).

Senescent cells have been detected in brains from elderly subjects and patients with neurodegenerative diseases (Baker and Petersen, 2018; Martínez-Cué and Rueda, 2020). In particular, markers of cell senescence have been reported in astrocytes from PD patients, and they have been reported to accumulate as a consequence of the exposure to environmental compounds like Paraquat (Chinta et al., 2018, 2013). The exact mechanisms by which inflammaging is propagated from the periphery to the brain and vice versa are still elusive, but experiments involving heterochronic parabiosis and plasma administration strongly



**Fig. 1.** The continuum between ageing and PD. The continuum is represented as a shade of color from green to red. Each line corresponds to the ageing trajectories of PD, general population (healthy controls) and long-lived subjects (centenarians and their offspring). The colour of circles corresponds to health status at death.

support the propagation hypothesis of inflammaging and indicate that brain ageing is intimately linked to the presence of pro- and anti-ageing molecules in the circulation (Horowitz and Villeda, 2017). A propagatory model of inflammaging has been presented (Franceschi et al., 2018b; Gordileva et al., 2020), within a conceptualization of the body as a Super-Network (Whitwell et al., 2020).

### 2.3. Inflammaging is a life-course process

The balance between inflammaging and anti-inflammaging is continuously remodelled during the life of the individual and is the result of the complex interaction between their genetic background and the environment to which they are exposed (starting from the very beginning of life and considering also preconception and in utero exposures) (Franceschi et al., 2007). Both genetic and environmental factors substantially contribute to PD, possibly impinging on the balance between inflammaging and anti-inflammaging. Importantly, an interplay between ageing, genetic predisposition to PD and exposure to chemicals has been reported, supporting the hypothesis that the ageing milieu sustains and amplifies the effects of genetic and environmental factors (Liu et al., 2017; Marder et al., 2015; Pang et al., 2019).

In summary, PROPAG-AGEING rationale is based on two main pillars:

- 1) The environment feeding PD onset and progression is the elderly physiology, and there is therefore a continuum between healthy ageing and PD. The project assumes PD as totally embedded within the basic molecular and cellular mechanisms of the ageing process, including inflammaging and neuro-inflammaging, among others.
- 2) Inflammaging, ageing and PD can propagate and transmit both locally and systemically. As a consequence, peripheral biospecimens (like blood, urine and stool) can be investigated not only to identify molecular, cellular and clinical markers of PD, but also to characterize the alterations that trigger the onset and the progression of the disease.

In the next paragraphs, we will discuss the design of PROPAG-AGEING project, which is described in Fig. 2 and Table 2.

### 3. PROPAG-AGEING design: the cohorts included in the study

As mentioned above, few studies have investigated in the same experimental settings the signatures of ageing and those of PD. The

design of PROPAG-AGEING has been specifically implemented in order to fill this gap. The project is based on a large number of human samples deriving from existing multi-center cohorts (that is, collected by the partners before PROPAG-AGEING, in the framework of other national and international projects) and including (Fig. 2):

- *de novo* PD patients, for which clinical characterization and collection of biological specimens have been performed at disease onset, before the dopaminergic therapy, according to the UK Brain Bank Criteria (Gibb and Lees, 1988). The analysis of *de novo* patients is highly informative, as it avoids possible confounding effects associated with the dopaminergic treatment, which is likely to alter the signatures of ageing and PD and to impair the detection of early markers of the disease;
- advanced PD patients;
- monozygotic (MZ) and dizygotic (DZ) twins from the Swedish Twin Registry, overall followed longitudinally for more than 45 years and assessed for lifestyle and place of living, type of work and exposure to potential environmental toxicants. Twin couples discordant for PD have been accurately recorded, and biological samples (blood and sera) have been collected before PD onset (incident cases) and/or after PD onset (prevalent cases);
- healthy control subjects, including sex-, country- and age-matched with PD patients, but also subjects younger and older than PD patients, that allow to track the trajectories of healthy/physiological ageing;
- healthy aged "super-controls", including both thoroughly characterized centenarians who never showed clinical signs of motor disability despite their exceptional lifespan, and their offspring.

In PROPAG-AGEING therefore we will consider a continuum of phenotypes and we will adopt the highly informative strategy of comparing extreme phenotypes (PD patients on one side; centenarians and their offspring, on the other side) (Garagnani et al., 2013; Giuliani et al., 2017) to maximize the possibility to identify PD-specific signatures.

Centenarians can be considered a paradigm of healthy ageing, as they largely avoided or postponed most of ARDs. Interestingly, while dementia is present in a minority of centenarians, PD is not (Arosio et al., 2017; Marcon et al., 2020), suggesting that PD is not an unavoidable result of the ageing process. We and others previously demonstrated that centenarians are characterized by specific clinical, cellular and molecular signatures associated to a healthy phenotype (Collino et al., 2013;

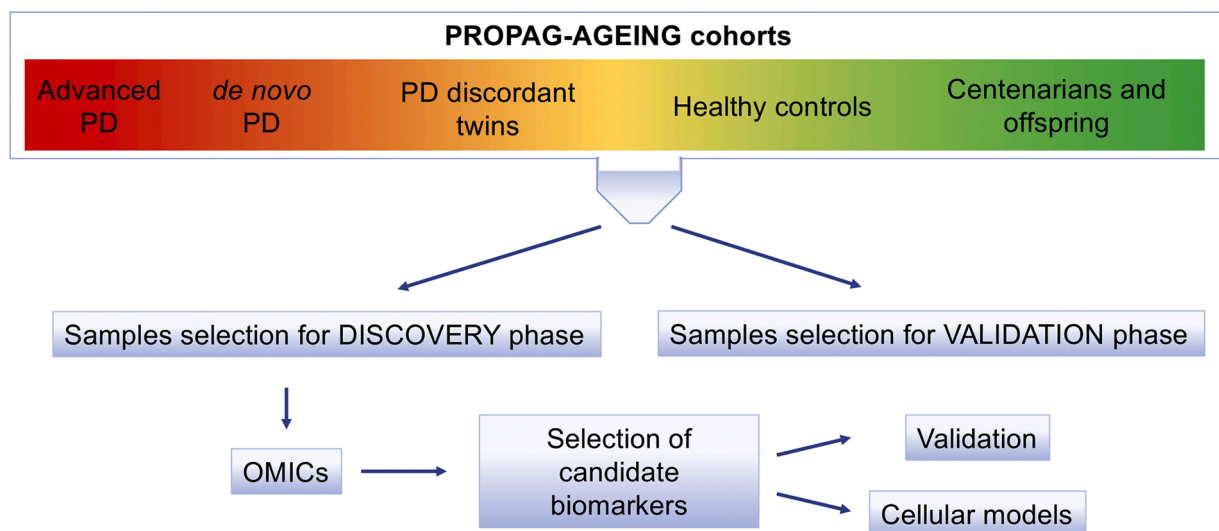


Fig. 2. PROPAG-AGEING design. The cohorts included in the project and the envisaged workflow are reported. The continuum of phenotypes is represented as a shade of colour from red (PD) to green (long-lived subjects).



**Table 2**

Overview of PROPAG-AGEING analyses. The table reports the analyses foreseen in the project (considering the discovery and the validation phases), the techniques applied, the cohorts available for each analysis and the biospecimen. Finally, it indicates the comparisons and the scientific questions that will be addressed.

Analysis	Techniques	Cohorts	Biospecimens	Comparisons	Scientific questions/Expected results
<b>Genetics discovery</b> (UCL)	Whole genome sequencing	de novo PD, controls (UMG-GOE)	Whole blood	PD (de novo and advanced) vs controls, taking into account the recruitment center	Genetic variants associated to PD
		advanced PD (AUSL-ISNB) centenarians, controls (UNIBO)		PD vs centenarians, taking into account the recruitment center	Genetic variants associated to PD and not associated to successful ageing (comparison between extreme phenotypes)
<b>Genetics validation</b> (UNIBO)	iPLEX MassARRAY	de novo PD, controls (UMG-GOE)	Whole blood	PD (de novo and advanced) vs controls, taking into account the recruitment center	Genetic variants associated to PD
		advanced PD (AUSL-ISNB) centenarians, controls (SAS) PD siblings (UMG-GOE, AUSL-ISNB, SAS)		PD vs centenarians, taking into account the recruitment center	Genetic variants associated to PD and not associated to successful ageing (comparison between extreme phenotypes)
<b>Epigenetics discovery</b> (AUSL-ISNB)	Infinium MethylationEPIC (Illumina)	de novo PD, controls (UMG-GOE)	Whole blood	Association with risk factors in PD siblings	Genetic variants associated with risk of prodromal PD
		advanced PD (AUSL-ISNB) centenarians, centenarians' offspring, controls of different age (UNIBO)		de novo PD vs controls	DNAm changes in early phases of PD not under treatment
<b>Epigenetics validation</b> (UNIBO)	EpiTYPER MassARRAY	de novo PD, controls (UMG-GOE)	Whole blood	Advanced PD vs controls	DNAm changes in PD under treatment
		advanced PD (AUSL-ISNB) centenarians, centenarians' offspring, controls of different age (UNIBO)		PD (de novo and advanced) vs centenarians' offspring	DNAm changes associated to PD and not associated to successful ageing
<b>Epigenetics validation</b> (UNIBO)	EpiTYPER MassARRAY	MZ and DZ twins discordant for PD (KI)	Whole blood	Association with age (controls of different age) and with successful ageing (centenarians)	Comparison of DNAm trajectories in healthy/successful ageing respect to PD; epigenetic clocks (accelerated ageing in PD?)
		de novo PD, controls (UMG-GOE) advanced PD (AUSL-ISNB) centenarians, centenarians' offspring, controls of different age (UNIBO)		Intra-couple analysis in discordant twins	DNAm changes associated to PD, taking into account the genetic background/environmental exposures
<b>Transcriptomics discovery</b> (SAS)	RNA-seq	MZ and DZ twins discordant for PD (KI)	Whole blood	As in the discovery phase of epigenetic analysis	As in the discovery phase of epigenetic analysis
		de novo PD, controls (UMG-GOE) centenarians, controls (UNIBO)		Association with risk factors in PD siblings	DNAm changes associated with risk of prodromal PD
<b>Transcriptomics validation</b> (SAS)	Open Array Real-Time PCR system	de novo PD, controls (UMG-GOE)	Whole blood	de novo PD vs controls	Differentially expressed genes in early phases of PD not under treatment
		advanced PD, controls (SAS)		de novo PD vs centenarians controls vs centenarians	Differentially expressed genes associated to PD, ageing and successful ageing
<b>miRNomics discovery</b> (PG)	miRNA-Seq	de novo PD, controls (UMG-GOE)	Serum	de novo PD vs controls	Differentially expressed genes in early phases of PD not under treatment
		centenarians, controls (UNIBO)		de novo PD vs controls advanced PD vs controls	Differentially expressed genes associated with risk of prodromal PD
<b>miRNomics validation</b> (PG)	qPCR	PD siblings (UMG-GOE, AUSL-ISNB, SAS)	Serum	Association with risk factors in PD siblings	Differentially expressed genes associated with risk of prodromal PD
		de novo PD, controls (UMG-GOE) centenarians, controls (UNIBO)		de novo PD vs controls	Differentially expressed circulating miRNA in early phases of PD not under treatment
<b>miRNomics validation</b> (PG)	qPCR	MZ and DZ twins discordant for PD (KI)	Serum	de novo PD vs centenarians controls vs centenarians	Differentially expressed circulating miRNA associated to PD, ageing and successful ageing
		de novo PD, advanced PD, controls (UMG-GOE) centenarians, controls (UNIBO)		Intra-couple analysis in discordant twins	Differentially expressed circulating miRNA associated to PD, taking into account the genetic background/environmental exposures
<b>miRNomics validation</b> (PG)	qPCR	de novo PD, advanced PD, controls (UMG-GOE) centenarians, controls (UNIBO)	Serum	de novo PD vs controls advanced PD vs controls	Differentially expressed circulating miRNA in early phases of PD not under treatment and in PD under treatment

(continued on next page)

Table 2 (continued)

Analysis	Techniques	Cohorts	Biospecimens	Comparisons	Scientific questions/Expected results
<b>Metabolomics discovery</b> (CIRMMP)	NMR	PD siblings (UMG-GOE, AUSL-ISBNB, SAS)		de novo PD vs centenarians advanced PD vs centenarians controls vs centenarians Association with risk factors in PD siblings	Differentially expressed circulating miRNA associated to PD, ageing and successful ageing Differentially expressed circulating miRNA associated with risk of prodromal PD Metabolic profiles in early phases of PD not under treatment
		de novo PD, controls (UMG-GOE) centenarians, controls (UNIBO)	Serum and urine from UMG-GOE Serum from KI and UNIBO	de novo PD vs controls de novo PD vs centenarians controls vs centenarians	Metabolic profiles associated to PD, ageing and successful ageing Metabolic profiles associated to PD, taking into account the genetic background/ environmental exposures Metabolic profiles in early phases of PD not under treatment
		MZ and DZ twins discordant for PD (KI)		Intra-couple analysis in discordant twins	Metabolic profiles in PD under treatment
<b>Metabolomics validation</b> (CIRMMP)	NMR	de novo PD, advanced PD, controls (UMG-GOE) advanced PD, controls (SAS) centenarians, centenarians' offspring, controls of different age (UNIBO)	Serum from UMG-GOE and UNIBO Plasma from SAS	Advanced PD vs controls PD (de novo and advanced) vs centenarians' offspring Association with age (controls of different age) and with successful ageing (centenarians)	Metabolic profiles associated to PD and not associated to successful ageing Comparison of metabolomic profiles in healthy/successful ageing respect to PD; metabolomic clock (accelerated ageing in PD?)
		PD siblings (UMG-GOE, AUSL-ISBNB, SAS) de novo PD, controls (UMG-GOE) centenarians, controls (UNIBO)	Serum Plasma	Association with risk factors in PD siblings de novo PD vs controls de novo PD vs centenarians controls vs centenarians	Metabolic profiles associated with risk of prodromal PD Proteomic profiles in early phases of PD not under treatment Proteomic profiles associated to PD, ageing and successful ageing Proteomic profiles associated to PD, taking into account the genetic background/ environmental exposures
<b>Proteomics discovery</b> (UCL)	Deep phenotyping by label-free proteomics and nano 2D-LC QTOF MSE	MZ and DZ twins discordant for PD (KI)	Serum from KI	Intra-couple analysis in discordant twins	Proteomic profiles in early phases of PD not under treatment Proteomic profiles in PD under treatment
		de novo PD, advanced PD, controls (UMG-GOE) advanced PD, controls (SAS) centenarians, centenarians' offspring, controls of different age (UNIBO)	Plasma and urine from UMG-GOE Plasma from SAS and UNIBO	de novo PD vs controls Advanced PD vs controls PD (de novo and advanced) vs centenarians' offspring Association with age (controls of different age) and with successful ageing (centenarians)	Proteomic profiles associated to PD and not associated to successful ageing Comparison of proteomic profiles in healthy/successful ageing respect to PD; proteomic clock (accelerated ageing in PD?)
<b>Proteomics validation</b> (UCL)	UPLC-MS/MS targeted proteomics	PD siblings (UMG-GOE, AUSL-ISBNB, SAS) de novo PD, controls (UMG-GOE) advanced PD, controls (SAS)	Plasma	Association with risk factors in PD siblings	Proteomic profiles associated with risk of prodromal PD Glycomic profiles in early phases of PD not under treatment; GlycoAge score Glycomic profiles in PD under treatment
		PD siblings (UMG-GOE, AUSL-ISBNB, SAS) PD siblings (UMG-GOE, AUSL-ISBNB, SAS)	Plasma Stool	Association with risk factors in PD siblings Association with risk factors in PD siblings	Glycomic profiles associated with risk of prodromal PD Metagenomic profiles associated with risk of prodromal PD
<b>Glycomics</b> (UNIBO)	DSA-FACE	de novo PD, controls (UMG-GOE) advanced PD, controls (SAS)	Serum from UMG-GOE Plasma from SAS	de novo PD vs controls Advanced PD vs controls	
<b>Metagenomics</b> (AUSL-ISBNB)	16S sequencing	PD siblings (UMG-GOE, AUSL-ISBNB, SAS) PD siblings (UMG-GOE, AUSL-ISBNB, SAS)	Stool	Association with risk factors in PD siblings	
<b>Imaging</b> (SAS)	<sup>123</sup> I]FP-CIT SPECT T1 3D MRI	de novo PD, controls (UMG-GOE) advanced PD (SAS)	Neuroimaging	de novo PD vs controls de novo PD vs advanced PD Association with other biomarkers in PD	Brain aging in PD using neuroimaging data

Guo et al., 2018; Horvath et al., 2015; Montoliu et al., 2014; Rampelli et al., 2020; Santoro et al., 2018; Sayed et al., 2019). However, centenarians are unavoidably very old people, and it is therefore difficult to disentangle longevity from ageing. For this reason, PROPAG-AGEING envisages the inclusion of centenarians' offspring, a well-established model of healthy ageing, characterized by decelerated ageing (Bucci et al., 2016; Conte et al., 2020; Gentilini et al., 2013, 2012; Horvath et al., 2015; Ostan et al., 2013; Vitale et al., 2012). Centenarians offspring population age range is very similar to those of the major

ARs, including PD, thus it is a priceless instrument to distinguish between healthy versus unhealthy ageing trajectories.

Taken together, these cohorts represent an unprecedented league of datasets and bio-materials to grasp the molecular pathophysiology of PD. All PD patients involved in PROPAG-AGEING have undergone deep phenotyping, including international standards of motor classification (Hoehn and Yahr stages), Unified Parkinson's Disease Rating Scale (MDS-UPDRS) scores, MRI imaging data and the assessment of non-motor symptoms.

#### 4. PROPAG-AGEING design: the envisaged characterizations

In recent years, a growing number of studies has attempted to unveil the molecular basis of PD using omic approaches, offering an in-depth characterization of potentially pathological alterations in specific biological layers like the genome, the epigenome, the transcriptome, the proteome, the metabolome or the metagenome (Redenšek et al., 2018). Results of these studies are usually not performed on the same PD patients, and are not always overlapping nor concordant, possibly because they largely differ in size, type of analyzed samples (genetic or idiopathic PD; de novo or advanced PD patients), type of biospecimen (for example, brain, blood, plasma/serum, stool, urine, CSF) and/or analytical approaches. Most importantly, in the vast majority of cases these studies are disjointed, i.e. a certain PD cohort has been characterized using only one or few omic approaches (Hertel et al., 2019).

PROPAG-AGEING aims at overcoming the fragmentation of data and interpretation inherent in previous studies by characterizing the cohorts described in the previous paragraph using a comprehensive set of advanced omics (whole genome and mtDNA sequencing, RNA-Seq, genome-wide DNA methylation, circulating microRNA, proteomics, metabolomics and glycomics), that whenever possible are applied to the same subjects (Table 2). All the analyses are performed on peripheral biospecimen (whole blood for genetic, epigenetic and transcriptomic analysis; plasma/serum/urine for circulating microRNA, proteomic, metabolomic and glycomic analyses), in accordance with the propagation hypothesis at the basis of PROPAG-AGEING. Furthermore, the use of easily accessible biospecimens allows to identify potential diagnostic and prognostic biomarkers assessable in clinical practice.

The analytical approach envisaged in PROPAG-AGEING allows to identify omic-specific markers, i.e. signatures neatly characterized by a single omic layer, as well as signatures that are better described as "multi-omic" covariates, i.e. "compound signatures" constituted by a cluster of different omics. Several approaches will be applied for the multi-omics integration of PROPAG-AGEING data. One of them consists in the application of multilayer networks (Boccaletti et al., 2014). Multilayer networks have been successfully applied to the integration (and interpretation) of several data types. A multilayer network can be defined as  $M=(G,C)$  where  $G$  is a set of graphs and  $C$  is the interconnection between them. For example in the framework of PROPAG-AGEING project we will implement a multilayer network in which each layer represents a type of omic data. In this way we can both analyse each layer independently (for example analysing communities) and in a fused fashion with the others, obtaining, comparing and evaluating single omic signatures, as well as integrated ones.

In parallel to the multilayer networks approach, we will also use an integration of omics data to be used as input features in a deep learning framework. In particular, we will design experiments to estimate biological age using a deep learning approach. The biological age estimation will be first performed in each omic independently as well as in the imaging data (MRI brain scans available for the cohort). Subsequently, omics will be combined and the integrated dataset will be used in order to undercover integrated and combined effects of omic and imaging integration towards biological age estimation. Furthermore, the available epidemiological, lifestyle and clinical data can be incorporated with omic results and newly generated biochemical data, providing a comprehensive *systems biology* view of the disease.

Given the conceptual assumptions at the basis of PROPAG-AGEING, particular attention is placed to the study of biomarkers of ageing and of inflammaging in the PD cohort. Ageing biomarkers can have different natures and are predictive not only of the chronological age of individuals, but also of their biological age, i.e. a proxy of their healthy status (Cole et al., 2019; Jylhävä et al., 2017). Biomarkers of ageing can therefore be informative regarding age acceleration processes associated to the onset or the progression of PD. The epigenetic clock, a predictor of age based on DNA methylation data, showed an accelerated ageing in whole blood from advanced PD patients respect to controls (Horvath and

Ritz, 2015), supporting the role of ageing in PD. Within PROPAG-AGEING, besides epigenetic clocks (Bell et al., 2019), we will consider GlycoAge (Vanhooren et al., 2008), based on glycomic signatures, and brainAge (Cole and Franke, 2017), based on brain imaging data. We will also refer to recently published papers that tracked metabolomic and proteomic changes with age (Johnson et al., 2020; Robinson et al., 2020). Furthermore, omics data will be specifically interrogated in order to evaluate inflammaging, considering pro- and anti-inflammatory molecules in the available datasets, like proteomic data (for example CRP, IL6, TGF beta, TNF alpha, IL10 and IL18) or miRNA data (for example miR-21, miR-155, miR-146).

Importantly, other national and international studies (*in primis* the Parkinson's Progression Marker Initiative – PPMI) are designed to establish a comprehensive set of clinical, imaging and bio-sample data (characterized by a multi-omic approach) that can be used to define biomarkers of PD onset and progression. PROPAG-AGEING originally contributes to this cooperative network of research studies, because the systems biology approach for the analysis of PD and healthy samples is contextualized in the framework of the ageing process.

#### 5. PROPAG-AGEING design: the discovery and the validation phases

To achieve an optimal balance between in depth characterization of the samples and cost-effectiveness of the analyses, PROPAG-AGEING envisages two main blocks of activities:

- i) A DISCOVERY PHASE, where a limited number of highly informative samples from the existing cohorts (*de novo* PD patients, twins discordant for the disease, healthy subjects of different ages, centenarians and centenarians' offspring as super-controls) are analyzed in depth by the above-described omics;
- ii) A VALIDATION PHASE, where a selection of the most informative molecules (genetic variants, epigenetic and transcriptomic signatures, proteins, metabolites and glycomic markers) emerging from the discovery phase and integrated by the environmental and clinical datasets, are tested in larger existing cohorts of PD patients (*de novo* and advanced), healthy and super-healthy subjects. This phase allows therefore the technical validation of potential biomarkers, possibly by high-throughput techniques alternative or complementary to the omic approaches that have been used in the discovery phase. In addition, the validation phase allows to further investigate the relationship between specific molecular alterations and clinical characteristics of PD.

The validation phase takes advantage of two additional models:

- 1) Dopaminergic neurons (DAn) obtained from human induced pluripotent stem cells (iPSC) deriving from PD patients, controls and super-healthy controls. Appropriate manipulations of this model (in vitro ageing, exposure to stressors related to neuro-inflammaging, etc) allow to functionally validate the molecular alterations emerging in the discovery phase and to evaluate the propagation hypothesis of ageing and PD (Mohamed et al., 2019; Ravaoli et al., 2018).
- 2) a multi-center cohort of siblings of PD patients, not affected by PD at the time of recruitment but possibly showing more risk factors for PD compared to the general population, specifically recruited in the framework of PROPAG-AGEING. Indeed, a genetic (risk) component is present in the sporadic PD, supported by GWAS studies and observational studies reporting an increased risk of PD associated with a family history of the disease (Berg et al., 2015; Delamarre and Meissner, 2017; Kalia and Lang, 2015). Within PROPAG-AGEING, the molecular alterations emerging in the discovery phase are tested in the cohort of high-risk PD

siblings in order to evaluate their potential as early biomarkers of the disease. The PD siblings' cohort is deeply characterized for several clinical parameters, with particular regard for non-motor symptoms that usually precede the motor dysfunction (premotor or prodromal phase of the disease) by more than a decade (Kalia and Lang, 2015). These non-motor symptoms include sleep disorders (Sateia, 2014), in particular REM Sleep Behavior Disorder, olfactory dysfunction, cognitive impairment, constipation and autonomic dysfunction (Kalia and Lang, 2015). Accurate evaluation of these and other parameters allows estimating the risk of developing PD and to correlate it with the levels of biomarkers identified in the framework of PROPAG-AGEING. Multiple aliquots of different biospecimen (whole blood, plasma/serum, urine and stool) are collected from PD siblings. The collection of stool samples is of particular interest given the emerging role of the gut microbiota and of the gut-brain-axis alterations in ageing and neurodegenerative diseases (Baizabal-Carvalho and Alonso-Juarez, 2020; Elfil et al., 2020; Quercia et al., 2014; Santoro et al., 2018).

## 6. Conclusions

The conceptual assumption of PROPAG-AGEING is that there is a continuum between healthy ageing and neurodegenerative age-related motor disorders. In this framework, the project has the ambitious aim to identify specific cellular and molecular patterns that can deviate the trajectories of healthy ageing towards the development of PD, or alternatively can protect centenarians and their offspring from developing this and other neurodegenerative disorders. These molecular signatures hopefully will represent reliable early markers of PD and new potentially druggable targets.

## Acknowledgements

This work was supported by the Horizon 2020 Framework Programme (grant number 634821, PROPAG-AGEING).

## References

- Acosta, J.C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J.P., Athineos, D., Kang, T.-W., Lasitschka, F., Andrulis, M., Pascual, G., Morris, K.J., Khan, S., Jin, H., Dharmalingam, G., Snijders, A.P., Carroll, T., Capper, D., Pritchard, C., Inman, G.J., Longrich, T., Sansom, O.J., Benitah, S.A., Zender, L., Gil, J., 2013. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15, 978–990. <https://doi.org/10.1038/ncb2784>.
- Arosio, B., Ostan, R., Mari, D., Damanti, S., Ronchetti, F., Arcudi, S., Scurti, M., Franceschi, C., Monti, D., 2017. Cognitive status in the oldest old and centenarians: a condition crucial for quality of life methodologically difficult to assess. *Mech. Ageing Dev.* 165, 185–194. <https://doi.org/10.1016/j.mad.2017.02.010>.
- Baizabal-Carvalho, J.F., Alonso-Juarez, M., 2020. The Link between Gut Dysbiosis and Neuroinflammation in Parkinson's Disease. *Neuroscience* 432, 160–173. <https://doi.org/10.1016/j.neuroscience.2020.02.030>.
- Baker, D.J., Petersen, R.C., 2018. Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. *J. Clin. Invest.* 128, 1208–1216. <https://doi.org/10.1172/JCI95145>.
- Bell, C.G., Lowe, R., Adams, P.D., Baccarelli, A.A., Beck, S., Bell, J.T., Christensen, B.C., Gladyshev, V.N., Heijmans, B.T., Horvath, S., Ideker, T., Issa, J.-P.J., Kelsey, K.T., Marioni, R.E., Reik, W., Relton, C.L., Schalkwyk, L.C., Teschendorff, A.E., Wagner, W., Zhang, K., Rakyant, V.K., 2019. DNA methylation aging clocks: challenges and recommendations. *Genome Biol.* 20, 249. <https://doi.org/10.1186/s13059-019-1824-y>.
- Bennett, D.A., Beckett, L.A., Murray, A.M., Shannon, K.M., Goetz, C.G., Pilgrim, D.M., Evans, D.A., 1996. Prevalence of parkinsonian signs and associated mortality in a community population of older people. *N. Engl. J. Med.* 334, 71–76. <https://doi.org/10.1056/NEJM199601113340202>.
- Berg, D., Postuma, R.B., Adler, C.H., Bloem, B.R., Chan, P., Dubois, B., Gasser, T., Goetz, C.G., Halliday, G., Joseph, L., Lang, A.E., Liepelt-Scarfone, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C.W., Poewe, W., Stern, M., Deuschl, G., 2015. MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* 30, 1600–1611. <https://doi.org/10.1002/mds.26431>.
- Boccalletti, S., Bianconi, G., Criado, R., Del Genio, C.I., Gómez-Gardeñes, J., Romance, M., Sendiña-Nadal, I., Wang, Z., Zanin, M., 2014. The structure and dynamics of multilayer networks. *Phys. Rep.* 544, 1–122. <https://doi.org/10.1016/j.physrep.2014.07.001>.
- Braak, H., Tredici, K.D., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. [https://doi.org/10.1016/S0197-4580\(02\)00065-9](https://doi.org/10.1016/S0197-4580(02)00065-9).
- Bucci, L., Ostan, R., Cevenini, E., Pini, E., Scurti, M., Vitale, G., Mari, D., Caruso, C., Sansoni, P., Fanelli, F., Pasquali, R., Guerresi, P., Franceschi, C., Monti, D., 2016. Centenarians' offspring as a model of healthy aging: a reappraisal of the data on Italian subjects and a comprehensive overview. *Aging (Albany NY)* 8, 510–519. <https://doi.org/10.18632/aging.100912>.
- Buchman, A.S., Shulman, J.M., Nag, S., Leurigans, S.E., Arnold, S.E., Morris, M.C., Schneider, J.A., Bennett, D.A., 2012. Nigral pathology and parkinsonian signs in elders without Parkinson disease. *Ann. Neurol.* 71, 258–266. <https://doi.org/10.1002/ana.22588>.
- Burke, R.E., O'Malley, K., 2013. Axon degeneration in Parkinson's disease. *Exp. Neurol.* 246, 72–83. <https://doi.org/10.1016/j.expneurol.2012.01.011>.
- Caggiu, E., Arru, G., Hosseini, S., Niegowska, M., Sechi, G., Zarbo, I.R., Sechi, L.A., 2019. Inflammation, infectious triggers, and Parkinson's disease. *Front. Neurol.* 10, 122. <https://doi.org/10.3389/fneur.2019.00122>.
- Calabrese, V., Santoro, A., Monti, D., Crupi, R., Di Paola, R., Latteri, S., Cuzzocrea, S., Zappia, M., Giordano, J., Calabrese, E.J., Franceschi, C., 2018. Aging and Parkinson's disease: inflammaging, neuroinflammation and biological remodeling as key factors in pathogenesis. *Free Radic. Biol. Med.* 115, 80–91. <https://doi.org/10.1016/j.freeradbiomed.2017.10.379>.
- Cevenini, E., Caruso, C., Candore, G., Capri, M., Nuzzo, D., Duro, G., Rizzo, C., Colonna-Romano, G., Lio, D., Di Carlo, D., Palmas, M.G., Scurti, M., Pini, E., Franceschi, C., Vasto, S., 2010. Age-related inflammation: the contribution of different organs, tissues and systems. How to face it for therapeutic approaches. *Curr. Pharm. Des.* 16, 609–618. <https://doi.org/10.2174/138161210790883840>.
- Cheng, H.-C., Ulane, C.M., Burke, R.E., 2010. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann. Neurol.* 67, 715–725. <https://doi.org/10.1002/ana.21995>.
- Chinta, S.J., Lieu, C.A., Demaria, M., Laberge, R.-M., Campisi, J., Andersen, J.K., 2013. Environmental stress, ageing and glial cell senescence: a novel mechanistic link to Parkinson's disease? *J. Intern. Med.* 273, 429–436. <https://doi.org/10.1111/joim.12029>.
- Chinta, S.J., Woods, G., Demaria, M., Rane, A., Zou, Y., McQuade, A., Rajagopalan, S., Limbad, C., Madden, D.T., Campisi, J., Andersen, J.K., 2018. Cellular senescence is induced by the environmental neurotoxin paraquat and contributes to neuropathology linked to Parkinson's disease. *Cell Rep.* 22, 930–940. <https://doi.org/10.1016/j.celrep.2017.12.092>.
- Chu, Y., Kordower, J.H., 2007. Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? *Neurobiol. Dis.* 25, 134–149. <https://doi.org/10.1016/j.nbd.2006.08.021>.
- Cole, J.H., Franke, K., 2017. Predicting age using neuroimaging: innovative brain ageing biomarkers. *Trends Neurosci.* 40, 681–690. <https://doi.org/10.1016/j.tins.2017.10.001>.
- Cole, J.H., Marioni, R.E., Harris, S.E., Deary, I.J., 2019. Brain age and other bodily 'ages': implications for neuropsychiatry. *Mol. Psychiatry* 24, 266–281. <https://doi.org/10.1038/s41380-018-0098-1>.
- Collier, T.J., Kanaan, N.M., Kordower, J.H., 2011. Ageing as a primary risk factor for Parkinson's disease: evidence from studies of non-human primates. *Nat. Rev. Neurosci.* 12, 359–366. <https://doi.org/10.1038/nrn3039>.
- Collino, S., Montoliu, I., Martin, F.-P.J., Scherer, M., Mari, D., Salvioli, S., Bucci, L., Ostan, R., Monti, D., Biagi, E., Brigidi, P., Franceschi, C., Rezzi, S., 2013. Metabolic signatures of extreme longevity in northern Italian centenarians reveal a complex remodeling of lipids, amino acids, and gut microbiota metabolism. *PLoS One* 8, e56564. <https://doi.org/10.1371/journal.pone.0056564>.
- Collins, L.M., Toulouse, A., Connor, T.J., Nolan, Y.M., 2012. Contributions of central and systemic inflammation to the pathophysiology of Parkinson's disease. *Neuropharmacology* 62, 2154–2168. <https://doi.org/10.1016/j.neuropharm.2012.01.028>.
- Conte, M., Conte, G., Martucci, M., Monti, D., Casarosa, L., Serra, A., Mele, M., Franceschi, C., Salvioli, S., 2020. The smell of longevity: a combination of Volatile Organic Compounds (VOCs) can discriminate centenarians and their offspring from age-matched subjects and young controls. *Geroscience* 42, 201–216. <https://doi.org/10.1007/s11357-019-00143-6>.
- Costantini, E., D'Angelo, C., Reale, M., 2018. The role of immunosenescence in neurodegenerative diseases. *Mediators Inflamm.* 2018, 6039171. <https://doi.org/10.1155/2018/6039171>.
- Delamarre, A., Meissner, W.G., 2017. Epidemiology, environmental risk factors and genetics of Parkinson's disease. *Presse Med.* 46, 175–181. <https://doi.org/10.1016/j.jlpm.2017.01.001>.
- Devi, L., Raghavendran, V., Prabhu, B.M., Avadhani, N.G., Anandatheerthavarada, H.K., 2008. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J. Biol. Chem.* 283, 9089–9100. <https://doi.org/10.1074/jbc.M710012200>.
- Dias, V., Junn, E., Mouradian, M.M., 2013. The role of oxidative stress in Parkinson's disease. *J. Parkinsons Dis.* 3, 461–491. <https://doi.org/10.3233/JPD-130230>.
- Dorsey, E.R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J.C., Ansha, M. G., Brayne, C., Choi, J.-Y.J., Collado-Mateo, D., Dahodwala, N., Do, H.P., Edessa, D., Endres, M., Fereshtehnejad, S.-M., Foreman, K.J., Ganke, F.G., Gupta, R., Hankey, G.J., Hay, S.I., Hegazy, M.I., Hibstu, D.T., Jabcseanian, A., Khader, Y., Khalil, I., Khang, Y.-H., Kim, Y.J., Kokubo, Y., Logroscino, G., Massano, J., Ibrahim, N.M., Mohammed, M.A., Mohammadi, A., Moradi-Lakeh, M., Naghavi, M.,



- Nguyen, B.T., Nirayo, Y.L., Ogbo, F.A., Owolabi, M.O., Pereira, D.M., Postma, M.J., Qorbani, M., Rahman, M.A., Roba, K.T., Safari, H., Safiri, S., Satpathy, M., Sawhney, M., Shafieesabet, A., Shiferaw, M.S., Smith, M., Szoek, C.E.L., Tabarés-Seisdedos, R., Truong, N.T., Ukawja, K.N., Venkatasubramanian, N., Villafaina, S., Weldegewers, Kgidey, Westerman, R., Wijeratne, T., Winkler, A.S., Xuan, B.T., Yonemoto, N., Feigin, V.L., Vos, T., Murray, C.J.L., 2018. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 17, 939–953. [https://doi.org/10.1016/S1474-4422\(18\)30295-3](https://doi.org/10.1016/S1474-4422(18)30295-3).
- Elfil, M., Kamel, S., Kandil, M., Koo, B.B., Schaefer, S.M., 2020. Implications of the Gut Microbiome in Parkinson's Disease. *Mov. Disord.* <https://doi.org/10.1002/mds.28004>.
- Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C., Salvioli, S., 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech. Ageing Dev.* 128, 92–105. <https://doi.org/10.1016/j.mad.2006.11.016>.
- Franceschi, C., Garagnani, P., Vitale, G., Capri, M., Salvioli, S., 2017a. Inflammaging and “Garb-aging”. *Trends Endocrinol. Metab.* 28, 199–212. <https://doi.org/10.1016/j.tem.2016.09.005>.
- Franceschi, C., Salvioli, S., Garagnani, P., de Equileor, M., Monti, D., Capri, M., 2017b. Immunobiography and the heterogeneity of immune responses in the elderly: a focus on inflammaging and trained immunity. *Front. Immunol.* 8, 982. <https://doi.org/10.3389/fimmu.2017.00982>.
- Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018a. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14, 576–590. <https://doi.org/10.1038/s41574-018-0059-4>.
- Franceschi, C., Zaikin, A., Gordleeva, S., Ivanchenko, M., Bonifazi, F., Storci, G., Bonafè, M., 2018b. Inflammaging 2018: an update and a model. *Semin. Immunol.* 40, 1–5. <https://doi.org/10.1016/j.smim.2018.10.008>.
- 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>.
- Furman, D., Campisi, J., Verdine, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D.W., Fasano, A., Miller, G.W., Miller, A.H., Mantovani, A., Weyand, C.M., Barzilai, N., Goronzy, J.J., Rando, T.A., Effros, R.B., Lucia, A., Kleinstreuer, N., Slavich, G.M., 2019. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832. <https://doi.org/10.1038/s41591-019-0675-0>.
- Garagnani, P., Giuliani, C., Pirazzini, C., Olivieri, F., Bacalini, M.G., Ostan, R., Mari, D., Passarino, G., Monti, D., Bonfigli, A.R., Boemi, M., Ceriello, A., Genovese, S., Sevini, F., Luiselli, D., Tieri, P., Capri, M., Salvioli, S., Vijg, J., Suh, Y., DelleDonne, M., Testa, R., Franceschi, C., 2013. Centenarians as super-controls to assess the biological relevance of genetic risk factors for common age-related diseases: a proof of principle on type 2 diabetes. *Ageing (Albany NY)* 5, 373–385. <https://doi.org/10.18632/aging.100562>.
- Gentilini, D., Castaldi, D., Mari, D., Monti, D., Franceschi, C., Di Blasio, A.M., Vitale, G., 2012. Age-dependent skewing of X chromosome inactivation appears delayed in centenarians' offspring. Is there a role for allelic imbalance in healthy aging and longevity? *Ageing Cell* 11, 277–283. <https://doi.org/10.1111/j.1474-9726.2012.00790.x>.
- Gentilini, D., Mari, D., Castaldi, D., Remondini, D., Oglari, G., Ostan, R., Bucci, L., Sircchia, S.M., Tabano, S., Cavagnini, F., Monti, D., Franceschi, C., Di Blasio, A.M., Vitale, G., 2013. Role of epigenetics in human aging and longevity: genome-wide DNA methylation profile in centenarians and centenarians' offspring. *Age (Dordr)* 35, 1961–1973. <https://doi.org/10.1007/s11357-012-9463-1>.
- Gibb, W.R., Lees, A.J., 1988. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 51, 745–752. <https://doi.org/10.1136/jnnp.51.6.745>.
- Giuliani, C., Pirazzini, C., DelleDonne, M., Xumerle, L., Descombes, P., Marquis, J., Mengozzi, G., Monti, D., Bellizzi, D., Passarino, G., Luiselli, D., Franceschi, C., Garagnani, P., 2017. Centenarians as extreme phenotypes: an ecological perspective to get insight into the relationship between the genetics of longevity and age-associated diseases. *Mech. Ageing Dev. SI: Centenarians* 165, 195–201. <https://doi.org/10.1016/j.mad.2017.02.007>.
- Gordleeva, S., Kanakov, O., Ivanchenko, M., Zaikin, A., Franceschi, C., 2020. Brain aging and garbage cleaning : Modelling the role of sleep, glymphatic system, and microglia senescence in the propagation of inflammaging. *Semin. Immunopathol.* 42, 647–665. <https://doi.org/10.1007/s00281-020-00816-x>.
- Guo, J.-D., Zhao, X., Li, Y., Li, G.-R., Liu, X.-L., 2018. Damage to dopaminergic neurons by oxidative stress in Parkinson's disease (Review). *Int. J. Mol. Med.* 41, 1817–1825. <https://doi.org/10.3892/ijmm.2018.3406>.
- Harman, D., 1956. Aging: A Theory Based on Free Radical and Radiation Chemistry. *J. Gerontol.* 11, 298–300. <https://doi.org/10.1093/geronj/11.3.298>.
- Hertel, J., Harms, A.C., Heinken, A., Baldini, F., Thinnies, C.C., Glaab, E., Vasco, D.A., Pietzner, M., Stewart, I.D., Wareham, N.J., Langenberg, C., Trenkwalder, C., Krüger, R., Hankemeier, T., Fleming, R.M.T., Mollenhauer, B., Thiele, I., 2019. Integrated Analyses of Microbiome and Longitudinal Metabolome Data Reveal Microbial-Host Interactions on Sulfur Metabolism in Parkinson's Disease. *Cell Rep.* 29, 1767–1777. <https://doi.org/10.1016/j.celrep.2019.10.035>.
- Horowitz, A.M., Villeda, S.A., 2017. Therapeutic potential of systemic brain rejuvenation strategies for neurodegenerative disease. *F1000Res* 6. <https://doi.org/10.12688/f1000research.11437.1>.
- Horvath, S., Ritz, B.R., 2015. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. *Ageing (Albany NY)* 7, 1130–1142. <https://doi.org/10.18632/aging.100859>.
- Horvath, S., Pirazzini, C., Bacalini, M.G., Gentilini, D., Di Blasio, A.M., DelleDonne, M., Mari, D., Arosio, B., Monti, D., Passarino, G., De Rango, F., D'Aquila, P., Giuliani, C., Marasco, E., Collino, S., Descombes, P., Garagnani, P., Franceschi, C., 2015. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Ageing (Albany NY)* 7, 1159–1170. <https://doi.org/10.18632/aging.100861>.
- Johnson, A.A., Shokhiev, M.N., Wyss-Coray, T., Lehallier, B., 2020. Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. *Ageing Res. Rev.* 60, 101070. <https://doi.org/10.1016/j.arr.2020.101070>.
- Jylhvä, J., Pedersen, N.L., Hägg, S., 2017. Biological age predictors. *EBioMedicine* 21, 29–36. <https://doi.org/10.1016/j.ebiom.2017.03.046>.
- Kalia, L.V., Lang, A.E., 2015. Parkinson's disease. *Lancet* 386, 896–912. [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3).
- Kanaan, N.M., Kordower, J.H., Collier, T.J., 2007. Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: relevance to selective neuronal vulnerability. *J. Comp. Neurol.* 502, 683–700. <https://doi.org/10.1002/cne.21333>.
- 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>.
- Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S.S., Lee, S., Lee, J.H., Kim, W.R., Kook, M., Foss, C.A., Shen, C., Lee, H., Kulkarni, S., Pasricha, P.J., Lee, G., Pomper, M.G., Dawson, V.L., Dawson, T.M., Ko, H.S., 2019. Transneuronal propagation of pathologic  $\alpha$ -synuclein from the gut to the brain models Parkinson's disease. *Neuron* 103, 627–641. <https://doi.org/10.1016/j.neuron.2019.05.035>.
- Li, Xin, Yang, W., Li, Xuran, Chen, M., Liu, C., Yu, S., 2018. Age-dependent elevations of oligomeric and phosphorylated alpha-synuclein synchronously occurs in the brain and gastrointestinal tract of cynomolgus monkeys. *Neurosci. Lett.* 662, 276–282. <https://doi.org/10.1016/j.neulet.2017.10.047>.
- Liu, H.-F., Ho, P.W.-L., Leung, G.C.-T., Lam, C.S.-C., Pang, S.Y.-Y., Li, L., Kung, M.H.-W., Ramsden, D.B., Ho, S.-L., 2017. Combined LRRK2 mutation, aging and chronic low dose oral rotenone as a model of Parkinson's disease. *Sci. Rep.* 7, 40887. <https://doi.org/10.1038/srep40887>.
- López-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. *Cell* 153, 1194–1217. <https://doi.org/10.1016/j.cell.2013.05.039>.
- Marcon, G., Manganotti, P., Tettamanti, M., 2020. Is Parkinson's disease a very rare pathology in centenarians? a clinical study in a cohort of subjects. *J. Alzheimers Dis.* 73, 73–76. <https://doi.org/10.3233/JAD-190717>.
- Marder, K., Wang, Y., Alcalay, R.N., Mejia-Santana, H., Tang, M.-X., Lee, A., Raymond, D., Mirelman, A., Saunders-Pullman, R., Clark, L., Ozelius, L., Orr-Urtreger, A., Giladi, N., Bressman, S., LRRK2 Ashkenazi Jewish Consortium, 2015. Age-specific penetrance of LRRK2 G2019S in the Michael J. Fox ashkenazi jewish LRRK2 consortium. *Neurology* 85, 89–95. <https://doi.org/10.1212/WNL.0000000000001708>.
- Martinez-Cué, C., Rueda, N., 2020. Cellular senescence in neurodegenerative diseases. *Front. Cell. Neurosci.* 14. <https://doi.org/10.3389/fncel.2020.00016>.
- McNaught, K.S.P., Belizaire, R., Isacson, O., Jenner, P., Olanow, C.W., 2003. Altered proteasomal function in sporadic Parkinson's disease. *Exp. Neurol.* 179, 38–46. <https://doi.org/10.1006/exnr.2002.8050>.
- Mohamed, N.-V., Larroquette, F., Beitel, L.K., Fon, E.A., Durcan, T.M., 2019. One step into the future: new iPSC tools to advance research in Parkinson's disease and neurological disorders. *J. Parkinsons Dis.* 9, 265–281. <https://doi.org/10.3233/JPD-181515>.
- Montoliu, I., Scherer, M., Beguelin, F., DaSilva, L., Mari, D., Salvioli, S., Martin, F.-P.J., Capri, M., Bucci, L., Ostan, R., Garagnani, P., Monti, D., Biagi, E., Brigidi, P., Kussmann, M., Rezzi, S., Franceschi, C., Collino, S., 2014. Serum profiling of healthy aging identifies phospho- and sphingolipid species as markers of human longevity. *Ageing (Albany NY)* 6, 9–25. <https://doi.org/10.18632/aging.100630>.
- Ostan, R., Bucci, L., Cevenini, E., Palmas, M.G., Pini, E., Scurti, M., Vescovini, R., Caruso, C., Mari, D., Vitale, G., Franceschi, C., Monti, D., 2013. Metabolic syndrome in the offspring of centenarians: focus on prevalence, components, and adipokines. *Age (Dordr)* 35, 1995–2007. <https://doi.org/10.1007/s11357-012-9483-x>.
- Pang, S.Y.-Y., Ho, P.W.-L., Liu, H.-F., Leung, G.C.-T., Li, L., Chang, E.E.S., Ramsden, D.B., Ho, S.-L., 2019. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. *Transl. Neurodegener.* 8, 23. <https://doi.org/10.1186/s40035-019-0165-9>.
- Phillips, R.J., Walter, G.C., Ringer, B.E., Higgs, K.M., Powley, T.L., 2009. Alpha-synuclein immunopositive aggregates in the myenteric plexus of the aging Fischer 344 rat. *Exp. Neurol.* 220, 109–119. <https://doi.org/10.1016/j.expneurol.2009.07.025>.
- Pringsheim, T., Jette, N., Frolkis, A., Steeves, T.D.L., 2014. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov. Disord.* 29, 1583–1590. <https://doi.org/10.1002/mds.25945>.
- Qin, X.-Y., Zhang, S.-P., Cao, C., Loh, Y.P., Cheng, Y., 2016. Aberrations in peripheral inflammatory cytokine levels in parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* 73, 1316–1324. <https://doi.org/10.1001/jama.2016.2742>.
- Quercia, S., Candela, M., Giuliani, C., Turrioni, S., Luiselli, D., Rampelli, S., Brigidi, P., Franceschi, C., Bacalini, M.G., Garagnani, P., Pirazzini, C., 2014. From lifetime to evolution: timescales of human gut microbiota adaptation. *Front. Microbiol.* 5, 587. <https://doi.org/10.3389/fmicb.2014.00587>.
- Rampelli, S., Soverini, M., D'Amico, F., Barone, M., Tavella, T., Monti, D., Capri, M., Astolfi, A., Brigidi, P., Biagi, E., Franceschi, C., Turrioni, S., Candela, M., 2020.

- Shotgun metagenomics of gut microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation. *mSystems* 5. <https://doi.org/10.1128/mSystems.00124-20>.
- Ravaoli, F., Bacalini, M.G., Franceschi, C., Garagnani, P., 2018. Age-related epigenetic derangement upon reprogramming and differentiation of cells from the elderly. *Genes (Basel)* 9. <https://doi.org/10.3390/genes9010039>.
- Redeňšek, S., Dolžan, V., Kunej, T., 2018. From genomics to omics landscapes of Parkinson's disease: revealing the molecular mechanisms. *OMICS* 22, 1–16. <https://doi.org/10.1089/omi.2017.0181>.
- Reeve, A., Simcox, E., Turnbull, D., 2014. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? *Ageing Res. Rev.* 14, 19–30. <https://doi.org/10.1016/j.arr.2014.01.004>.
- Robinson, O., Chadeau Hyam, M., Karaman, I., Climaco Pinto, R., Ala-Korpela, M., Handakas, E., Fiorito, G., Gao, H., Heard, A., Jarvelin, M.-R., Lewis, M., Pazoki, R., Polidoro, S., Tzoulaki, I., Wielscher, M., Elliott, P., Vineis, P., 2020. Determinants of accelerated metabolomic and epigenetic aging in a UK cohort. *Aging Cell* 19, e13149. <https://doi.org/10.1111/acer.13149>.
- Santoro, A., Ostan, R., Candela, M., Biagi, E., Brigidi, P., Capri, M., Franceschi, C., 2018. Gut microbiota changes in the extreme decades of human life: a focus on centenarians. *Cell. Mol. Life Sci.* 75, 129–148. <https://doi.org/10.1007/s00018-017-2674-y>.
- Sateia, M.J., 2014. International classification of sleep disorders-third edition: highlights and modifications. *Chest* 146, 1387–1394. <https://doi.org/10.1378/chest.14-0970>.
- Sayed, N., Gao, T., Tibshirani, R., Hastie, T., Cui, L., Kuznetsova, T., Rosenberg-Hasson, Y., Ostan, R., Monti, D., Lehallier, B., Shen-Orr, S., Maecker, H.T., Dekker, C. L., Wyss-Coray, T., Franceschi, C., Jovic, V., Haddad, F., Montoya, J.G., Wu, J.C., Furman, D., 2019. An inflammatory clock predicts multi-morbidity, immunosenescence and cardiovascular aging in humans. *bioRxiv* 840363. <https://doi.org/10.1101/840363>.
- Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J., Kirkland, J.L., 2013. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J. Clin. Invest.* 123, 966–972. <https://doi.org/10.1172/JCI64098>.
- Vanhooren, V., Laroy, W., Libert, C., Chen, C., 2008. N-glycan profiling in the study of human aging. *Biogerontology* 9, 351–356. <https://doi.org/10.1007/s10522-008-9140-z>.
- Vida, C., González, E.M., De la Fuente, M., 2014. Increase of oxidation and inflammation in nervous and immune systems with aging and anxiety. *Curr. Pharm. Des.* 20, 4656–4678. <https://doi.org/10.2174/1381612820666140130201734>.
- Vitale, G., Brugts, M.P., Ogliaari, G., Castaldi, D., Fatti, L.M., Varewijck, A.J., Lamberts, S. W., Monti, D., Bucci, L., Cevenini, E., Cavagnini, F., Franceschi, C., Hofland, L.J., Mari, D., Janssen, J., 2012. Low circulating IGF-I bioactivity is associated with human longevity: findings in centenarians' offspring. *Aging (Albany NY)* 4, 580–589. <https://doi.org/10.18632/aging.100484>.
- Whitwell, H.J., Bacalini, M.G., Blyuss, O., Chen, S., Garagnani, P., Gordleeva, S.Y., Jalan, S., Ivanchenko, M., Kanakov, O., Kustikova, V., Mariño, I.P., Meyerov, I., Ullner, E., Franceschi, C., Zaikin, A., 2020. The Human Body as a Super Network: Digital Methods to Analyze the Propagation of Aging. *Front. Aging Neurosci.* 12, 136. <https://doi.org/10.3389/fnagi.2020.00136>.
- Xuan, Q., Xu, S.-L., Lu, D.-H., Yu, S., Zhou, M., Uéda, K., Cui, Y.-Q., Zhang, B.-Y., Chan, P., 2011. Increased expression of  $\alpha$ -synuclein in aged human brain associated with neuromelanin accumulation. *J. Neural Transm. (Vienna)* 118, 1575–1583. <https://doi.org/10.1007/s00702-011-0636-3>.