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Longevity and healthy aging

Edited by
Tzvi Dwolatzky and Gil Atzmon

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Longevity and healthy aging

Topic editors

Tzvi Dwolatzky — Technion Israel Institute of Technology, Israel

Gil Atzmon — University of Haifa, Israel

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OPINION
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Is Life Extension Today a Faustian Bargain?

*S. Jay Olshansky**

University of Illinois at Chicago, Chicago, IL, United States

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The legend of Faust is a thought-provoking tale from the middle ages, which has a surprising connection to the world of aging today. The story is both inspiring and tragic at the same time. In one famous version of the tale from Goethe, Faust is an idealist scholar that becomes disillusioned with his limits to knowledge. Bored and suicidal, Faust becomes the target of the devil Mephistopheles who says he can satisfy Faust's desire for unlimited knowledge and also promises him youth, pleasures of the flesh, and magical powers—for a predetermined period. In exchange, after the allotted time, the devil will claim Faust's soul and forever be enslaved. The story of Faust has become a metaphor for a promise or tradeoff that at first seems appealing, but with time is revealed to be a bad bargain.

The story of human aging and the modern rise in longevity has remarkable correlates to the story of Faust, but with some interesting twists. Here's the connection. The first longevity revolution that began in the middle of the nineteenth century occurred primarily because of gains made against infant and child mortality resulting from advances in basic public health. This was followed by reductions in death rates from cardiovascular disease late in the twentieth century. A quantum leap in life expectancy of 30 years ensued at lightning speed. Humanity displayed a collective sigh of relief as infectious diseases waned—our children had finally been saved. Nothing in history has ever come close to the magnitude of this benefit to humanity. While there is no disputing the value of life and health extension from the first longevity revolution, rarely does something so desirable come without a Faustian-like price.

Along with 30 years of additional life and the opportunity to see almost all our children live long enough to have families of their own, humanity also witnessed a subsequent dramatic escalation in the prevalence of age-related chronic, fatal and disabling diseases and their attendant costs and heartache. That is, we now live long enough to experience the aging of our bodies. If Mephistopheles had been by our side in 1850 to explain what humanity would receive in exchange for longer lives, he would have simply said look around—the tradeoff is visible now among the handful of people fortunate enough to escape the usual scourges of childhood. The price humanity had to pay for 30 years of additional life was the rise in heart disease, cancer, stroke, Alzheimer's disease, Parkinson's, diabetes, and a long list of non-fatal disabling conditions. In retrospect, it was worth every part of the bargain.

But Mephistopheles isn't done with us. Like a street magician that lets you win the first game, and then sucks you into a bigger con with larger stakes, or a drug dealer that gets you hooked with free samples, the next much costlier offer is before us now. We've had our taste of longevity, but now we want more—much more at any cost, and Mephistopheles knows this.

With life itself as the most precious commodity there is, it's easy to see the next con. The first is the rise of what has become known as the antiaging industry—a multibillion dollar enterprise designed to convince us that the secret to the fountain of youth is already within our grasp (1, 2). Pay dearly for their elixirs now and wait for the promise of a longer life to appear decades later. What do you think the chances are that your investment will pay off? The catch is that the alleged benefits don't appear, if at all, until after the longevity salesmen has left the scene and pocketed your cash. This longevity racket has been around from the beginning of time, and I refer to this industry as the second oldest profession. What's different today from the alchemists of the middle ages, or the gland grafters of the early twentieth century, or the hormone vendors

Edited by:
Tzvi Dwolatzky, Rambam Health Care Campus, Israel

Reviewed by:
Alex Zhavoronkov, Biogerontology Research Foundation, United Kingdom
Aleksei G. Golubev, N.N. Petrov Research Institute of Oncology, Russia

***Correspondence:**
S. Jay Olshansky
sjayo@uic.edu

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now peddling their wares, is the rise of the scientific study of aging and genuine opportunity offered for healthy life extension. The modern practitioners of anti-aging medicine try and sell the public what appear to be genuine scientific interventions based on real science, before they're proven to be safe and efficacious. This idea is best personified in an early twentieth century quote from Alan Valentine: "*whenever science makes a discovery, the devil grabs it while the angels are debating the best way to use it*."

The second response to an insatiable desire for more life is also predictable, but the danger could be an even worse Faustian bargain than that posed by the antiaging industry. The method used to manufacture the first longevity revolution is known as the "infectious disease model"—that is, as soon as a disease appears, attack it with everything in the medical arsenal. Beat the disease down, and once you succeed, push the patient out the door until they face their next challenge; then beat that one down. The formula is simple—repeat until failure. This model was perfect for infectious diseases and effective at first for chronic degenerative diseases, and no doubt there is still progress to be made, but evidence has emerged that this approach is likely to run out of steam (3–7). The application of an infectious disease model to chronic fatal and disabling diseases associated with aging is Mephistopheles latest "bargain." The irony behind this new bargain (otherwise known as the current medical model of disease) is that the medical community advocating for disease eradication doesn't even recognize the health consequences of success.

The bargain today is crystal clear—we're being offered incrementally smaller amounts of survival time at a very high cost, and the prospect that most of the additional months and years of life will be riddled with frailty and disability. Keep in mind that the human body has no designer; it was not constructed for long-term use; and our Achilles heels are already visible—neurological conditions such as Alzheimer's disease and related conditions are associated with non-replicating neurons; and muscles and joints have a difficult time navigating the ravages of biological time. *The Faustian bargain before us now is, in exchange for small doses of additional life, humanity will experience a suite of fatal and disabling conditions expressed at later ages that rob us of what we hold most precious – our mental and physical functioning.*

What's the solution? Don't sign the contract! A clue about what we should do instead was presented to us decades ago. In the mid-1950s, gerontologist McKay et al. suggested that attacking aging itself rather than the diseases associated with it offered the greatest hope in warding off the infirmities of old age (8). Some 20 years later, Bernie Strehler coined the term "gerontogeny" to convey the same message (9). The first formal discussion of delayed aging among scientists appeared in Extending the Human Life Span: Social Policy and Social Ethics, published by Neugarten and Havighurst (10). That book was a product of a three-year project on the future of aging funded by the National Science Foundation, culminating in a conference in 1976.

Conference participants were asked to discuss several questions: should the science of biogerontology be devoted to improving older people's quality of life? Or should it extend the lifespan of the human species? If lifespan is extended, what would be its deleterious and beneficial effects on society? How much money should be allocated to research addressed directly to extending the human lifespan? What social and ethical implications would follow from a "magic elixir" that would extend active life expectancy by 15 to 20 years?

At the conference, it was noted that the longevity revolution in the twentieth century brought decades of healthy life, and contributed substantially to our nation's economic growth. But all was not rosy. Conference participants were acutely aware that extended lives came at a price—rapid increases in chronic fatal and disabling diseases. Some scientists there argued we should not pursue life extension as a national goal because the result would be an increase in the number and proportion of people requiring acute nursing care. Gerontological Society president George Sacher expressed concern that extending life without extending health would result in a disproportionate number of years of disease and disability for the 10% of the population living the longest. But most conference participants agreed with James Goddard (former Commissioner of the US Food and Drug Administration), who argued that healthy life extension should be a national goal requiring political support and strong vested interests. Although the National Institute on Aging (NIA) was formed just before the Neugarten conference, the focus of modern medicine (and most the NIA budget since its beginning) has been centered on the disease model rather than the delayed-aging model. Advice from Neugarten conference participants to escalate the attack on aging, as well as to battle against major diseases, was not followed.

In 2006, my colleagues and I extended this line of reasoning by coining the phrase "the Longevity Dividend" to describe the economic and health benefits that would accrue to individuals and societies if we extend healthy life by slowing the biological processes of aging (11–13). This idea was distinctive because we proposed to extend healthy life by shifting our emphasis from disease management to delayed aging. Four factors led to this proposal: rapid increases in life expectancy since the late 1970s; accelerated population aging; and rapid increases in chronic fatal and disabling diseases. These three occurred rapidly in developed nations, and developing nations are catching up. The fourth factor was the most important—recent advances in biogerontology suggested that it is plausible to delay aging in people. (For a summary of this line of reasoning, see asaging.org/blog/delay_aging_further_reading.) A recent article in *Nature* suggests that "senolytics" may offer a unique opportunity to forestall the ravages of aging through the systematic elimination of cells that are still alive, but which no longer function normally (14).

The Longevity Dividend is an approach to public health based on a broader strategy of fostering health for all generations by developing a new horizontal model to health promotion and disease prevention. Unlike the current vertical approach to disease that targets individual disorders as they arise, the Longevity Dividend model seeks to prevent or delay the root causes of disease and disability by attacking the one main risk factor for them all—biological aging. Evidence in models ranging from invertebrates to mammals suggests that all living things have biochemical mechanisms influencing how quickly they age, and these mechanisms are adjustable.

Slowing down the processes of aging—even by a moderate amount—will yield dramatic improvements in health for current

and future generations (12). Advances in the scientific knowledge of aging may thus create new opportunities that allow us, and generations to follow, to live healthier and longer lives than our predecessors. Bernice Neugarten and her colleagues had their finger on the right pulse decades ago—it just took 35 years for the scientific study of aging to catch up. By embracing a new model for health promotion and disease prevention in the twenty-first century, we can give the gift of extended health and economic wellbeing to current and all future generations. What is the cost of this new more effective model of primary prevention that will save the world trillions in health care costs? A fraction of the basic research cost required to create sixth generation fighter jets; or the salary from just one quarterback in the National Football League.

The case can now be made that delayed aging could be the most efficient method of achieving primary prevention available to us in this century. A large-scale, concerted, and coordinated effort is now underway to translate the science behind the Longevity Dividend Initiative, also known as Geroscience, into real-world clinical trials and a suite of therapeutic interventions (15–18).

So, are we giving up too much to satisfy our insatiable appetite for more life? Is today's Faustian bargain worth it?

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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GENERAL COMMENTARY
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Commentary: Is Life Extension Today a Faustian Bargain?

*Aleksei G. Golubev**

N.N. Petrov Research Institute of Oncology, Saint Petersburg, Russia

Keywords: aging, mortality, longevity, health, biology

A commentary on

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> "Such conclusions are always disappointing, but they have the desirable consequences of channeling research in directions that are likely to be fruitful." Williams G.C. Pleiotropy, natural selection and the evolution of senescence. Evolution. 1957; 11:398–411.

Imagine that in a research field, which flourishes on funds allocated for getting an answer to a pressing question, the answer is eventually found. There will be no need to support the field any further. Specialists who sacrificed their lives to developing it will be uncompetitive in other fields, which are being developed by other scholars. That is, science, unlike practice, needs questions, not answers, which may have value for science only as far as they provoke further questions. In this regard, the value of the commented opinion paper (1) is unquestionable.

Questionable is the practice of extracting quotations out of their full contexts. However, how else can one justify comments on it?

> "We're being offered incrementally smaller amounts of survival time at a very high cost…" (1).

"Smaller" and "very high" are quantitative categories. Is there a way to estimate them by numbers? One way is suggested by the Preston curve, which shows cross-country relationships between per capita gross national product (GNP) and life expectancy (LE) (2). Transforming the plot from its usual appearance, which shows how longevity increases with incomes, into showing the price for increasing longevity (**Figure 1**), makes it easy to see that increasing the mean age-at-death above ca. 85 years comes at price rocketing to infinity. A similar trick with data about per capita health-care spending will show the same. The hard cold facts reflected by **Figure 1** suggest that the results of investing ever-increasing available resources into human life are limited with regard to human life span.

> "A clue about what we should do instead…: … attacking aging itself rather than the diseases associated with it…" (1).

How can one know that aging itself rather than something else is attacked? In populations, aging is manifested as a gradually increasing risk of death with increasing age. This relationship is captured by the Gompertz–Makeham law (GML):

$$\mu(t) = C + \mu_0 \times \exp(\gamma \times t),$$

where μ captures the probability of death per unit time, C is a population-specific parameter, which does not depend on age (t), μ_0 captures the mean initial vulnerability to the causes of death, and γ captures the mean rate of the age-dependent increase in vulnerability, i.e., the demographic rate of aging.

Edited by:
Tzvi Dwolatzky, Rambam Health Care Campus, Israel

Reviewed by:
Marios Kyriazis, ELPIs Foundation for Indefinite Lifespans, Cyprus

***Correspondence:**
Aleksei G. Golubev
lxglbv@rambler.ru

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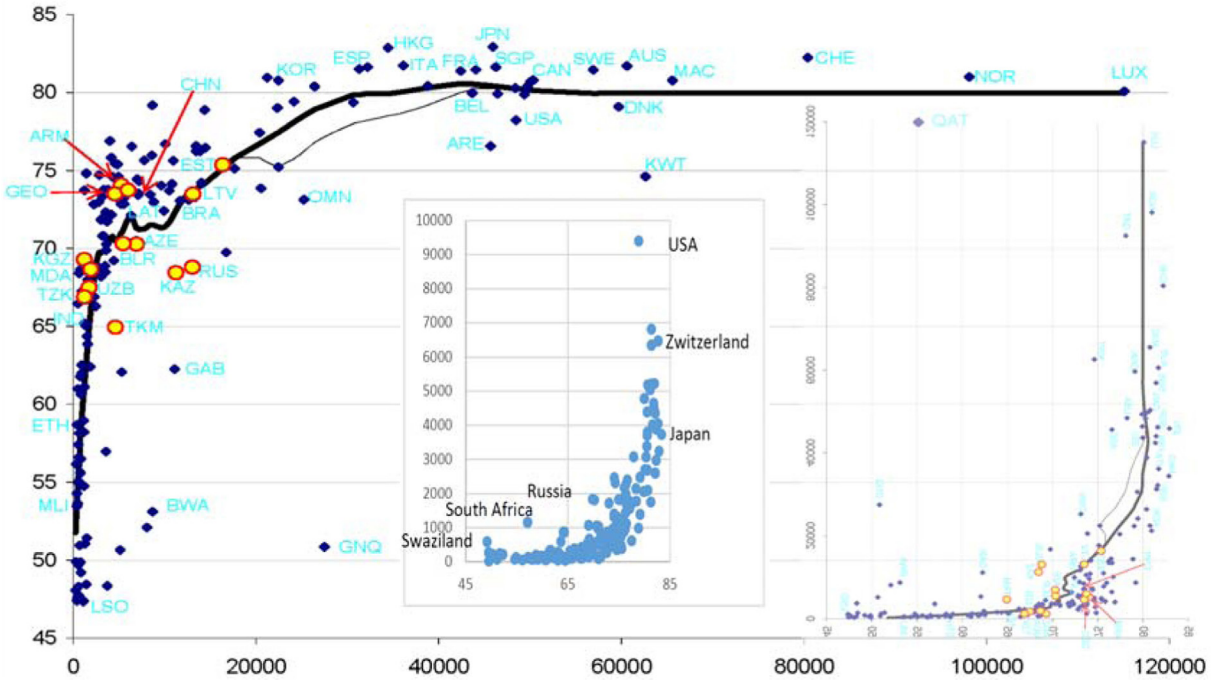

FIGURE 1 | Conventional Preston curve [life expectancy (LE) vs. GDP (in US$)] as of 2010 supplemented with insets showing (right) its transformation into a GDP vs. LE plot and (left) a plot of per capita health-care expenditures vs. LE. The Preston curve is reproduced from Ref. (3). The thick line is obtained by LOESS smoothing. The left inset is based on data available at https://en.wikipedia.org/wiki/List_of_countries_by_total_health_expenditure_per_capita and https://en.wikipedia.org/wiki/List_of_countries_by_life_expectancy.

Attitudes to GML range from considering it as a manifestation of some natural laws (4) to regarding it as merely a handy tool for describing a current situation (5). The latter attitude implies that the situation can be changed qualitatively without violating any law of nature, provided we can devise a means to do that. The former attitude implies that, because of the exponentially increasing mortality, any finite generation, which overlaps with others to constitute a population, will be inevitably exhausted within a finite time. GML imposes significant constrains on the freedom of thought within the scope of its applicability, as any law does. The respective mortality patterns generate characteristically left-skewed age-at-death distributions and allow calculating GML parameters. Only interventions that influence γ may be regarded as targeting "aging itself." Treating human mortality and survival patterns according to GML suggests that changes in C rather than in γ are responsible for historical advances in human lifespan (6, 7). Notably, the best ever review on GML and its implications (8) is coauthored by the author of the opinion paper (1) under discussion. Why then GML is not mentioned in the opinion?

> "Most important—recent advances in biogerontology suggested that it is plausible to delay aging in people… The Longevity Dividend model seeks to prevent or delay the root causes of disease and disability by attacking the one main risk factor for them all—biological aging" (1).

How can one know that the ability to extend lifespan by influencing aging in nematodes may be expanded to nothing else but aging in humans? In the range from less to more advanced organisms, such as from nematodes through flies to mice, the magnitude of lifespan-modifying effects and their relevance to aging decline, making their projections to human aging uncertain. Rapamycin is an example of this uncertainty (9, 10). Therefore, the relevance of recent advances in experimental life/health span-extending drugs to attacking specifically aging in humans is disputable.

> "The modern practitioners of anti-aging medicine try and sell the public what appear to be genuine scientific interventions based on real science, before they're proven to be safe and efficacious. …" (1).

If paying for anti-aging elixirs offered by anti-aging pharma without due testing is a "Faustian bargain" (which it surely is), how one should esteem testing numerous putative anti-aging drugs for their applicability to humans? Is not it another way of making people pay for the anti-aging agenda?—This time for research (which is supported by taxpayers in the final account) aimed to check whether prospective products are useful, rather than for ready-to-use products having unproved usefulness. Thus, we have another Faustian bargain, albeit more intricate.

Ironically, the most praised "anti-aging" drugs, such as resveratrol, rapamycin, and metformin, are believed to mimic the

effects of shifting body energy balance from storage, growth, and self-reproduction to self-maintenance (11, 12). Then what is the reason to use mimetics instead of real things, such as proper calorie intake and adequate physical activity supplemented with moderate alcohol (13–15)? Is it true that the most important bottleneck in increasing health span is the inadequate support of research in anti-aging pharmacology rather than inadequate human attitudes to health? May it be that healthy habits promotion is more cost-effective than anti-aging pills development?

This is not to say that aging research has turned into scholastic exercises performed for their own sake. Delving into the basic mechanisms of aging does help to find novel therapies, which are likely to be overlooked in studies focused on a specific malady. An example is the story of resveratrol, which apparently fails to culminate in a pill to attack human aging, yet continues by patenting new drugs to attack human diseases (16).

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ORIGINAL RESEARCH
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Association between Sleep Patterns and Health in Families with Exceptional Longevity

Lavy Klein[1†], Tina Gao[2†], Nir Barzilai[2,3] and Sofiya Milman[2]*

[1] Department of Geriatrics, Shoham Medical Center, Pardes-Hanna, The Technion-Israel Institute of Technology, Haifa, Israel,
[2] Institute for Aging Research, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, United States,
[3] Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, United States

Background: Sleep patterns such as longer sleep duration or napping are associated with poor health outcomes. Although centenarians and their offspring demonstrate a delayed onset of age-related diseases, it is not known whether they have healthier sleep patterns or are protected against the negative effects of sleep disturbances.

Methods: Data on sleep patterns and health history were collected from Ashkenazi Jewish subjects of the Longevity Genes Project using standardized questionnaires. Participants included individuals with exceptional longevity (centenarians) with preserved cognition ($n = 348$, median age 97 years), their offspring ($n = 513$, median age 69 years), and controls ($n = 199$) age-matched to the offspring. Centenarians reported on their sleep patterns at age 70, while the offspring and controls on their current sleep patterns. Biochemical parameters were measured at baseline. Models were adjusted for age, sex, BMI, and use of sleep medication.

Results: The offspring and controls reported similar sleep patterns, with 33% sleeping ≥8 h and 17% napping in each group. At age 70, centenarians were more likely to have slept ≥8 h (55%) and to have napped (28%) compared with offspring and controls, $p < 0.01$. Among centenarians, no association was noted between sleep patterns and health outcomes. Sleeping for ≥8 h was associated with lower high-density lipoprotein cholesterol levels in the offspring and controls, and with insulin resistance in the offspring, but not with diabetes. Napping was associated with insulin resistance among the controls ($p < 0.01$), but not the offspring. Controls, but not offspring, who napped were 2.79 times more likely to have one or more of the following diseases: hypertension, myocardial infarction, stroke, or diabetes (OR 2.79, 95% CI 1.08–7.21, $p = 0.04$).

Conclusion: Despite being more likely to exhibit risky sleep patterns at age 70 compared with the offspring and controls, the centenarians were protected from age-related morbidities. The offspring of centenarians did exhibit metabolic disturbances in association with less healthy sleep patterns; however, unlike the controls, they were much less likely to manifest age-related diseases. This suggests that offspring may have inherited resilience genotypes from their centenarian parents that protect them against the harmful effects of sleep disturbances.

Keywords: centenarians, longevity, aging, sleep, nap, age-related diseases

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Tzvi Dwolatzky, Rambam Health Care Campus, Israel

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Doron Garfinkel, Israel Cancer Association, Israel
Graziamaria Corbi, University of Molise, Italy

***Correspondence:**
Sofiya Milman
sofiya.milman@einstein.yu.edu

†These authors have contributed equally to this work.

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INTRODUCTION

Sleep is the restorative phase of the daily arousal-sleep cycle. A circadian clock, which includes the suprachiasmatic nucleus (SCN) and related structures in the brain, is genetically regulated through identified circadian genes (1, 2), and controls sleep patterns (3). The circadian genes also regulate the metabolic and hormonal diurnal and nocturnal fluctuations of the organism (4, 5). Peripheral circadian clocks in organs such as the liver (6) and the kidney (7) are synchronized with the main SCN clock. Aging of the circadian clock system is associated with sleep disorders in the elderly (8–10) and with characteristic age-related changes in glucose and lipid metabolism (4, 11, 12). Thus, sleep disorders and metabolic changes are regulated *via* a common apparatus, the circadian clock. This may explain the link between sleep disorders and metabolic diseases like diabetes and obesity. Like other genetically determined traits, sleep pattern is subjected to changes in circadian-gene expression that may be influenced by epigenetic alterations that result from aging (13, 14).

Sleep patterns change throughout the lifespan. Predictable changes in sleep quality that frequently affect older individuals include reduced hours of nighttime sleep, sleep fragmentation, and daytime napping (15). Metabolic function in humans is linked to sleep duration and sleep quality. Glycemic control is regulated by the sleep-arousal cycle (16) and sleep loss has been associated with insulin resistance and Type 2 diabetes mellitus (T2DM) (17). Experimentally controlled circadian rhythm disruption in diabetes-prone transgenic rats has accelerated development of diabetes through pancreatic cell dysfunction (18). Cortisol, growth hormone, leptin, and ghrelin levels are also modulated by sleep duration and quality (19). Preservation of regular sleep-wake patterns has been associated with higher high-density lipoprotein (HDL) cholesterol and lower triglyceride levels in the elderly (20). The impact of sleep patterns on mortality has also been widely investigated in longitudinal studies. In a recent meta-analysis of 27 cohort studies, long and short sleep duration were associated with increased all-cause mortality in the elderly (21).

Whether sleep pattern is primarily a genetically determined phenotype or a lifestyle habit remains open to debate. However, there are studies which show that sleep disorders are heritable and that genetic factors play a substantial role in the pathophysiology of sleep disorders (22–24). Exceptional longevity is also an inherited trait and first-degree relatives of centenarians are 8–17 times more likely to achieve longevity themselves (25). Centenarians delay the onset of most age-related diseases and exhibit unique biological phenotypes (26–29), which are often inherited by their offspring (30–32). This can explain why centenarian offspring stay healthier and live longer (33–35). With the knowledge that sleep patterns are associated with metabolic conditions, we conducted a study looking at whether reported sleep quality, sleep duration, and daytime napping were associated with health outcomes in individuals with exceptional longevity, their offspring, and offspring of parents with usual life expectancy. The hypothesis underlying our study was that centenarians and their offspring are protected from age-related sleep disturbances or the negative health impact of sleep disturbances.

MATERIALS AND METHODS

Study Population

Participants from the cross-sectional Longevity Genes Project (LGP) that was initiated in 1998 were the subjects of this study. Detailed description of the LGP study is available elsewhere (31, 36). In brief, LGP recruited individuals from the Northeastern United States age 95 and older who were living independently at the age of 95, which served as a reflection of general good health (centenarians). In addition, LGP enrolled the offspring of centenarians and controls, most of who were the spouses of the offspring but did not have a centenarian parent. The ages of the centenarians were verified with government issued identification. All of the study subjects were Ashkenazi Jewish, defined by having all four grandparents who were Ashkenazi Jewish. Participants with cognitive impairment, which was defined by Mini-Mental State Examination (MMSE) score $\leq$22 (37, 38) or blind MMSE <16 (39) were excluded from this analysis because the administered sleep questionnaire relied on self-report. The study was approved by the Institutional Review Board at the Albert Einstein College of Medicine. Written informed consent was obtained from all the study participants in accordance with the Declaration of Helsinki.

Sleep Pattern and Health Outcomes

Self-reported sleep patterns and health outcome data from the LGP were analyzed. Due to several revisions of the sleep questionnaire throughout the duration of the study, three versions of the questionnaires existed. All three versions have addressed the following sleep patterns at age 70 for centenarians: sleep duration in a 24 hour period, regular daily napping behavior, and presence of sleep problems. A subset of centenarians also reported on their current sleep patterns at the age of enrollment ($n = 43$). Offspring and controls reported on their current sleep pattern since not all of them have reached age 70. Sleep medication usage at the time of enrollment was reported by all participants. Participants who reported using sleep medications were considered as having a sleep problem.

History of the following diseases was self-reported by all participants: myocardial infarction (MI), hypertension (HTN), stroke or transient ischemic attack (TIA), and diabetes. Physical assessments included measurements of height and weight. Body mass index (BMI) was calculated according to the following formula: BMI = mass (kg)/height $(m)^2$. The following metabolic parameters were measured at the time of enrollment among the offspring and controls at the Montefiore Medical Center clinical laboratories and the Biomarker Analytic Research Core at the Albert Einstein College of Medicine: insulin (excluding participants using insulin or diabetes medications), glucose, HDL cholesterol, high-sensitivity C-reactive protein (CRP), and creatinine. Insulin resistance was quantified by the homeostatic model assessment, HOMA-IR (40), and the glomerular filtration rate (eGFR) was calculated (41). Insulin-like growth factor 1

(IGF-1) and testosterone (in males only) were measured using liquid chromatography/mass spectrometry in previously frozen serum at Quest Diagnostics Nichols Institute laboratories, San Juan Capistrano, CA, USA.

Statistical Analysis

Descriptive statistics for sleep pattern and health outcomes were reported. Daily sleep duration was dichotomized into <8 and ≥8 h. Napping behavior, sleep problems, and daily sleep medication usage were coded as "yes" or "no." In addition to examining history of MI, HTN, stroke or TIA, and diabetes individually, a binary comorbidity index was also created for the aforementioned conditions. Participants who reported having one or more of these diseases would receive a score of one, whereas a score of zero was recorded if none of these diseases were reported. Mann–Whitney, chi-square, and Student's *t*-tests were applied to evaluate significant differences of sleep pattern and health outcomes between sub-cohorts.

For centenarians, a multivariable logistic regression model adjusted for age and sex was applied to examine the associations between each sleep pattern at age 70 (hours of sleep, napping behavior, and sleep problem) and disease history (MI, HTN, stroke/TIA, diabetes, and the comorbidity index). For offspring and controls, two adjusted multivariable logistic regression models were built to determine the associations between each sleep pattern and aforementioned diseases, and two adjusted linear regression models were used for physical and metabolic parameters. For both logistic and linear regressions, Model 1 was adjusted for age, sex, and BMI, while Model 2 was adjusted for the same parameters as Model 1 and additionally for use of sleep medication. CRP level was log-transformed and analysis involving IGF-1 was stratified by sex due to its sex-dependent characteristics. eGFR was only adjusted for BMI in Model 1 and additionally for sleeping medication usage in Model 2, because age and sex were already incorporated into its calculation. Odds ratios with 95% confidence intervals (CI) and beta coefficients with 95% CI were reported for all logistic regression and linear regression models, respectively. A p-value of <0.05 was considered statistically significant. STATA version 12 (College Station, TX, USA) was used for statistical analyses.

RESULTS

As shown in **Table 1**, the median age of centenarians ($n = 348$) at enrollment was 96.8 [interquartile range (IQR) 95.5–99.3] years, of offspring ($n = 513$) 69.3 (IQR 63.1–74.4) years, and of controls ($n = 199$) 70.2 (IQR 63.2–76.5) years. The majority of centenarians were female (69%). Centenarians were significantly more likely to have slept ≥8 h per day and nap regularly at age 70 compared with offspring and controls ($p < 0.01$). Although there was no significant difference between the hours of sleep at current age and at age 70 for centenarians, centenarians were more likely to nap (45.2 vs. 28.1%, $p < 0.05$) and report having sleeping problems (80.8 vs. 28.5%, $p < 0.01$) at current age than at age 70.

Among centenarians, those who had ≥8 h of sleep at age 70 had 0.27 (95% CI 0.14–0.49, $p < 0.01$) times the odds of reporting sleep problems, whereas those who napped regularly at age 70 had 2.85 (95% CI 1.6–5.05, $p < 0.01$) times the odds of reporting sleep problems, after adjusting for age and sex (**Table 2**). There were no significant associations between sleep patterns at age 70 and any disease history in centenarians assessed at the time of enrollment (**Table 2**).

As shown in **Table 3**, the offspring who had ≥8 h of sleep had 1.96 (95% CI 1.06–3.61, $p = 0.03$) times the odds of napping regularly in a model adjusted for age, sex, BMI and sleep medication use (Model 2), and had 0.39 (95% CI 0.24–0.63, $p < 0.01$) times the odds of reporting sleep problems in a model

TABLE 1 | Demographic characteristics and sleep patterns at enrollment and at age 70.

	Centenarians ($n = 348$)		Offspring ($n = 513$)	Controls ($n = 199$)	p-Value[a*,b*,a,b,c,d]	
Median age at enrollment (interquartile range), years	96.8 (95.5–99.3)		69.3 (63.1–74.4)	70.2 (63.2–76.5)	a*: <0.01 b*: <0.01	c: 0.18
Female %, (*n*)	69 (240)		52.8 (271)	52.8 (105)	a*: <0.01 b*: <0.01	c: 0.98
Sleep patterns	**At age 70**	**At enrollment**	**At enrollment**			
Mean duration of sleep ± SD, h (*n*)	7.5 ± 1.3 (263)	7.7 ± 2.5 (32)	7.1 ± 1.1 (461)	7.1 ± 1.0 (178)	a: <0.01 b: <0.01	c: 0.96 d: 0.67
≥8 h of sleep %, (*n*)	55.1 (145)	50 (16)	32.8 (151)	33.2 (59)	a: <0.01 b: <0.01	c: 0.93 d: 0.58
Napped %, (*n*)	28.1 (83)	45.2 (14)	17.7 (80)	17.1 (30)	a: <0.01 b: <0.01	c: 0.85 d: <0.05
Sleeping problem present %, (*n*)	28.5 (80)	80.8 (59)	32.8 (146)	31 (53)	a: 0.22 b: 0.57	c: 0.67 d: <0.01
Current sleeping pill usage %, (*n*)	–	25.1 (50)	12.2 (41)	15.9 (18)	c: 0.31	

[a]*p-Value obtained from comparison between offspring and centenarians at enrollment.*
[b]*p-Value obtained from comparison between controls and centenarians at enrollment.*
[a]*p-Value obtained from comparison between offspring and centenarians at age 70.*
[b]*p-Value obtained from comparison between controls and centenarians at age 70.*
[c]*p-Value obtained from comparison between offspring and controls.*
[d]*p-Value obtained from comparison between centenarians at age 70 and centenarians at enrollment.*

TABLE 2 | Centenarians' sleep patterns at age 70 and disease history at enrollment.

	Sleep hours				Nap				Sleep problems			
	<8 h (*n* = 118)	**≥8 h (*n* = 145)**	***p*-Value[a]**	**OR (95% CI)[b]**	**Yes (*n* = 83)**	**No (*n* = 212)**	***p*-Value[c]**	**OR (95% CI)[d]**	**Yes (*n* = 80)**	**No (*n* = 201)**	***p*-Value[e]**	**OR (95% CI)[f]**
Median age (IQR), years	97.3 (95.6–100.3)	96.5 (95.5–98.8)	0.11		96.5 (95.7–100.1)	96.9 (95.4–99.2)	0.49		97.1 (95.9–100.2)	96.8 (95.5–99.4)	0.18	
Female %, (*n*)	72 (85)	64.1 (93)	0.17		68.7 (57)	70.8 (150)	0.73		75 (60)	68.2 (137)	0.26	
Sleep patterns at age 70												
Napped %, (*n*)	25.7 (29)	31.2 (44)	0.33	1.37 (0.78–2.4), $p = 0.27$	–	–	–	–	–	–	–	–
Sleep problems %, (*n*)	41.9 (44)	15.8 (22)	<0.01	0.27 (0.14–0.49), $p < 0.01$	42.9 (33)	20.7 (40)	<0.01	2.85 (1.6–5.05), $p < 0.01$	–	–	–	–
Disease history												
MI %, (*n*)	15.2 (16)	14.3 (18)	0.84	0.96 (0.46–2.01), $p = 0.92$	17.1 (13)	15.1 (28)	0.68	1.12 (0.54–2.31), $p = 0.76$	20 (15)	14.9 (26)	0.32	1.42 (0.7–2.89), $p = 0.33$
HTN %, (*n*)	59.3 (67)	59.4 (79)	0.99	1.04 (0.62–1.76), $p = 0.87$	55.1 (43)	60.5 (121)	0.41	0.82 (0.48–1.4), $p = 0.47$	59.7 (46)	56.7 (106)	0.65	1.07 (0.62–1.86), $p = 0.81$
Stroke/TIA %, (*n*)	22.6 (26)	21.1 (30)	0.78	0.92 (0.5–1.67), $p = 0.77$	22.2 (18)	20.8 (43)	0.79	1.13 (0.6–2.11), $p = 0.71$	19 (15)	23 (45)	0.47	0.77 (0.4–1.49), $p = 0.43$
Diabetes %, (*n*)	4.7 (5)	7.3 (9)	0.42	1.38 (0.44–4.36), $p = 0.58$	6.6 (5)	7 (13)	0.91	0.99 (0.34–2.9), $p = 0.98$	4.1 (3)	7.4 (13)	0.32	0.54 (0.15–1.97), $p = 0.35$
Positive comorbidity index %, (*n*)	76.4 (84)	70.2 (92)	0.29	0.74 (0.41–1.35), $p = 0.33$	70.1 (54)	74 (145)	0.52	0.86 (0.47–1.56), $p = 0.62$	70.7 (53)	72.6 (135)	0.76	0.86 (0.47–1.57), $p = 0.62$

[a]*p-Value comparing centenarians with <8 vs. ≥8 h of sleep.*
[b]*Age and sex adjusted OR of disease for those with ≥8 h of sleep compared with those with <8 h of sleep.*
[c]*p-Value comparing centenarians who do and do not nap.*
[d]*Age and sex adjusted OR of disease for those who nap compared with those who do not nap.*
[e]*p-Value comparing centenarians with and without sleep problems.*
[f]*Age and sex adjusted OR of disease for those with sleep problems compared with those without sleep problems.*

TABLE 3 | Associations between sleep duration and disease or biochemical profile for offspring and controls.

	Offspring					Controls				
	Sleep hours		*p*-Value[a]	Model 1: OR (95% CI)[b]	Model 2: OR (95% CI)[c]	Sleep hours		*p*-Value[a]	Model 1: OR (95% CI)[b]	Model 2: OR (95% CI)[c]
	<8 h (*n* = 310)	≥8 h (*n* = 151)				<8 h (*n* = 119)	≥8 h (*n* = 59)			
Median age (IQR), years	69 (62.2–74.4)	70.3 (64.6–75.8)	0.02			70.3 (63–76.3)	70 (65.2–77.5)	0.34		
Female %, (*n*)	51.9 (161)	58.3 (88)	0.2			50.4 (60)	54.2 (32)	0.63		
Sleep patterns										
Napped %, (*n*)	15.3 (46)	23 (34)	<0.05	1.66 (0.99–2.78), $p = 0.05$	1.96 (1.06–3.61), $p = 0.03$	12.9 (15)	23.7 (14)	0.07	2.1 (0.88–5), $p = 0.09$	2.66 (0.86–8.24), $p = 0.09$
Sleep problem %, (*n*)	38.1 (114)	19.7 (28)	<0.01	0.39 (0.24–0.63), $p < 0.01$	–	37.8 (42)	18.6 (11)	0.01	0.35 (0.16–0.76), $p < 0.01$	–
Sleeping pill usage %, (*n*)	11.8 (27)	11.9 (12)	0.99	0.95 (0.45–1.99), $p = 0.89$	–	17.1 (13)	13.5 (5)	0.62	0.75 (0.24–2.31), $p = 0.62$	–
Disease history										
MI %, (*n*)	4.3 (13)	4.9 (7)	0.78	0.93 (0.34–2.51), $p = 0.88$	0.7 (0.18–2.72), $p = 0.61$	7.8 (9)	8.6 (5)	0.84	1.04 (0.31–3.57), $p = 0.94$	0.63 (0.07–5.43), $p = 0.67$
HTN %, (*n*)	34.8 (103)	44.7 (63)	<0.05	1.48 (0.94–2.32), $p = 0.09$	1.4 (0.79–2.49), $p = 0.25$	37.4 (43)	49.1 (27)	0.15	1.58 (0.8–3.12), $p = 0.19$	2.12 (0.86–5.21), $p = 0.1$
Stroke/TIA %, (*n*)	1 (3)	2.1 (3)	0.33	1.51 (0.27–8.43), $p = 0.64$	1.61 (0.2–13.28), $p = 0.66$	0.9 (1)	7.1 (4)	0.02	8.01 (0.82–78.3), $p = 0.07$	Omitted due to small sample size
Diabetes %, (*n*)	6.9 (21)	7.7 (11)	0.77	1.15 (0.5–2.62), $p = 0.74$	1.44 (0.5–4.17), $p = 0.5$	8.6 (10)	8.5 (5)	0.99	0.84 (0.25–2.84), $p = 0.78$	1.21 (0.27–5.39), $p = 0.8$
Positive comorbidity index %, (*n*)	40.7 (118)	49.3 (69)	0.09	1.41 (0.89–2.23), $p = 0.14$	1.32 (0.74–2.34), $p = 0.35$	47.8 (54)	57.4 (31)	0.25	1.37 (0.69–2.72), $p = 0.38$	2.1 (0.84–5.24), $p = 0.11$
	Offspring					Controls				
	Median (IQR)		*p*-Value[a]	Model 1: β-coef (95% CI)[d]	Model 2: β-coef (95% CI)[e]	Median (IQR)		*p*-Value[a]	Model 1: β-coef (95% CI)[d]	Model 2: β-coef (95% CI)[e]
	<8 h (*n* = 310)	≥8 h (*n* = 151)				<8 h (*n* = 119)	≥8 h (*n* = 59)			
BMI kg/m^2, (*n*)	25.2 (23.2–28.4), (309)	25.3 (23.2–28.2), (149)	0.95	−0.25 (−1.07 to 0.57), $p = 0.55$	−0.44 (−1.44 to 0.56), $p = 0.39$	25.3 (23.5–28.3), (118)	24.9 (22.6–27.9), (58)	0.66	−0.12 (−1.57 to 1.33), $p = 0.87$	−0.08 (−2.16 to 1.99), $p = 0.94$
Biochemical measures										
Insulin mU/L, (*n*)	11.8 (6.9–26.7), (150)	14.2 (8.7–24.9), (68)	0.13	7.53 (0.66–14.4), $p = 0.03$	5.25 (−1.25 to 11.76), $p = 0.11$	16.1 (10.6–27.1), (58)	13.4 (9.4–21.6), (27)	0.32	−3.06 (−11.18 to 5.06), $p = 0.46$	2.81 (−7.76 to 13.39), $p = 0.6$
Glucose mg/dL, (*n*)	84 (74–96), (297)	86 (76–98), (147)	0.32	2.29 (−3.7 to 8.28), $p = 0.45$	6.97 (−0.62 to 14.56), $p = 0.07$	86 (76–96), (115)	85 (76–98), (54)	0.74	3.73 (−5.03 to 12.48), $p = 0.4$	5.21 (−6.19 to 16.62), $p = 0.37$
Insulin resistance HOMA, (*n*)	2.3 (1.3–5.9), (144)	3.1 (1.6–6), (66)	0.06	2.85 (0.48–5.23), $p = 0.02$	2.4 (0.14–4.66), $p = 0.04$	3.5 (2–5.7), (57)	3 (1.9–5), (22)	0.45	−1.3 (−3.48 to 0.88), $p = 0.24$	−0.35 (−2.97 to 2.27), $p = 0.79$
HDL mg/dL, (*n*)	61 (51–74), (305)	59 (47–73), (150)	0.19	−2.71 (−5.94 to 0.52), $p = 0.1$	−3.75 (−7.63 to 0.14), $p = 0.06$	60 (51–72), (117)	60 (49–75), (59)	0.53	−1.66 (−6.23 to 2.9), $p = 0.47$	−5.94 (−11.3 to −0.58), $p = 0.03$
IGF-1 ng/mL, (*n*)	119 (97.5–144), (264)	125 (92–146), (130)	0.86	0.12 (−8.38 to 8.61), $p = 0.98$	4.98 (−5.17 to 15.12), $p = 0.34$	110 (89–145), (79)	123.5 (92.5–146), (44)	0.3	10.31 (−5.79 to 26.41), $p = 0.21$	5.22 (−13.93 to 24.37), $p = 0.59$
CRP[f] mg/L, (*n*)	0.2 (0.1–0.4), (156)	0.25 (0.1–0.4), (85)	0.43	0.06 (−0.16 to 0.28), $p = 0.6$	0.05 (−0.2 to 0.3), $p = 0.69$	0.3 (0.1–0.5), (59)	0.3 (0.2–0.7), (31)	0.17	0.14 (−0.2 to 0.49), $p = 0.42$	0.21 (−0.19 to 0.61), $p = 0.3$

(*Continued*)

TABLE 3 | Continued

	Offspring					Controls				
	Median (IQR)		*p*-Value[a]	Model 1: β-coef (95% CI)[d]	Model 2: β-coef (95% CI)[e]	Median (IQR)		*p*-Value[a]	Model 1: β-coef (95% CI)[d]	Model 2: β-coef (95% CI)[e]
	<8 h (*n* = 310)	≥8 h (*n* = 151)				<8 h (*n* = 119)	≥8 h (*n* = 59)			
eGFR[g] mL/min/1.73 m^2, (*n*)	75.6 (64.4–88.1), (296)	71.7 (59.4–86.1), (145)	0.04	−3.63 (−6.93 to −0.32), *p* = 0.03	−5 (−8.91 to −1.09), *p* = 0.01	71.4 (62.1–82.9), (116)	73 (60.1–86.2), (55)	0.87	−0.22 (−5.81 to 5.37), *p* = 0.94	0.41 (−6.83 to 7.66), *p* = 0.91
Testosterone[h] ng/dL, (*n*)	391 (310–500), (129)	362 (302–503), (54)	0.46	−9.93 (−61.64 to 41.78), *p* = 0.71	−20.71 (−81.8 to 40.39), *p* = 0.5	383 (298–500), (37)	323 (290–405), (20)	0.19	−61.57 (−129.63 to 6.49), *p* = 0.08	−63.24 (−149.16 to 22.69), *p* = 0.14

[a]*p-Value comparing those with <8 vs. ≥8 h of sleep.*

[b]*Age, sex, and BMI adjusted OR of disease for those with ≥8 h of sleep compared with those with <8 h of sleep.*

[c]*Age, sex, BMI, and sleep pill adjusted OR of disease for those with ≥8 h of sleep compared with those with <8 h of sleep.*

[d]*Age, sex, and BMI adjusted beta coefficient for ≥8 h of sleep compared with those with <8 h of sleep.*

[e]*Age, sex, BMI, and sleep pill adjusted beta coefficient for ≥8 h of sleep compared with those with <8 h of sleep.*

[f]*Beta coefficients are log transformed.*

[g]*Model 1 is adjusted only for BMI and Model 2 is adjusted only for BMI and sleeping pill usage.*

[h]*Testosterone is reported for males only.*

adjusted for age, sex, and BMI (Model 1). Offspring who slept ≥8 h also had statistically significantly higher levels of insulin, insulin resistance (HOMA), as well as marginally higher glucose level. Furthermore, they had lower eGFR and marginally lower HDL cholesterol level than their counterparts who slept <8 h. Similar to offspring, controls who slept ≥8 h were less likely to report sleep problems, OR = 0.35 (95% CI 0.16–0.76, $p < 0.01$) and were found to have significantly lower level of HDL cholesterol than their counterparts who had <8 h of sleep (**Table 3**) (Model 2). However, no statistically significant associations were noted between sleep duration and any of the diseases among the offspring or controls.

Regular napping was not associated with self-reported sleep problems, sleeping pill usage, or any of the individual diseases among offspring and controls (**Table 4**). However, controls who napped regularly had significantly higher odds of having one or more conditions in the comorbidity index after adjusting for age, sex, and BMI (OR = 2.79, 95% CI 1.08–7.21, $p = 0.04$). Offspring who napped regularly had higher level of glucose, but not insulin or HOMA. Regular nappers among the offspring also had lower IGF-1 level than their counterparts who did not nap. However, the IGF-1 level was only significantly lower in male offspring (sex-stratified data not shown in **Table 4**). On the other hand, controls who napped had higher levels of insulin and insulin resistance, and had lower eGFR. Presence of self-reported sleep problems was not significantly associated with any diseases, physical parameters, or biochemical markers (data not shown).

DISCUSSION

Favorable sleep patterns have been associated with decreased risk of disease and mortality in the general population. Interestingly, in this study individuals with exceptional longevity did not report more favorable sleep patterns at age 70 compared with similarly aged controls, who were not genetically enriched for longevity. Furthermore, the sleep patterns between the offspring of centenarians and controls did not differ, suggesting that genetic predisposition to longevity is not dependent on healthy sleep patterns. Despite demonstrating similar sleep patterns, the offspring of centenarians with unhealthy sleep patterns were significantly less likely to manifest age-related diseases compared with controls with unhealthy sleep patterns. This finding suggests that centenarians and their offspring possess protective genes that make them resilient to the adverse effects of unfavorable sleep patterns.

Other clinical studies on sleep quality among centenarians have reported various results, ranging from reports of overall good sleep (42) to reports of sleeping problems among the majority of the participants (43). The majority of centenarians in our study reported sleeping problems at enrollment, but had a similar prevalence of sleep problems at age 70 compared with offspring and controls. However, there were more centenarians who slept ≥8 h and who had taken daytime naps at age 70 than offspring and controls. Although the centenarians did not report healthy sleep patterns at age 70, prolonged sleep and daytime napping

TABLE 4 | Associations between napping behavior and disease or biochemical profile for offspring and controls.

	Offspring					Controls				
	Nap		*p*-Value[a]	Model 1: OR (95% CI)[b]	Model 2: OR (95% CI)[c]	Nap		*p*-Value[a]	Model 1: OR (95% CI)[b]	Model 2: OR (95% CI)[c]
	Yes (n = 80)	No (n = 372)				Yes (n = 30)	No (n = 146)			
Median age (IQR), years	72.9 (65.7–77.6)	69.3 (62.9–74.2)	<0.01			75.4 (69.1–81.1)	69.1 (63–75)	<0.01		
Female %, (n)	40 (32)	57 (212)	<0.01			43.3 (13)	54.8 (80)	0.25		
Sleep patterns										
Sleep problem %, (n)	31.6 (24)	32.9 (118)	0.83	0.98 (0.57–1.69), p = 0.94	–	26.7 (8)	31.9 (44)	0.58	0.67 (0.27–1.71), p = 0.41	–
Sleeping pill usage %, (n)	12.3 (7)	12 (32)	0.95	1.08 (0.44–2.65), p = 0.87	–	25 (4)	14.6 (14)	0.29	1.93 (0.52–7.18), p = 0.33	–
Disease history										
MI %, (n)	6.4 (5)	4.2 (15)	0.39	1.11 (0.38–3.26), p = 0.85	0.82 (0.2–3.35), p = 0.78	16.7 (5)	6.3 (9)	0.06	1.49 (0.4–5.52), p = 0.55	1.13 (0.13–9.81), p = 0.91
HTN %, (n)	39.5 (30)	38 (134)	0.81	0.72 (0.41–1.25), p = 0.24	0.89 (0.45–1.76), p = 0.74	62.1 (18)	36 (50)	<0.01	2.16 (0.91–5.12), p = 0.08	2.4 (0.78–7.43), p = 0.13
Stroke/TIA %, (n)	3.9 (3)	0.8 (3)	0.04	3.22 (0.61–16.88), p = 0.17	3.11 (0.38–25.36), p = 0.29	10.3 (3)	1.4 (2)	0.01	9.44 (0.85–104.26), p = 0.07	Omitted due to small sample size
Diabetes %, (n)	11.5 (9)	6.1 (22)	0.09	1.37 (0.58–3.25), p = 0.48	2.6 (0.93–7.24), p = 0.07	17.2 (5)	6.2 (9)	<0.05	3.55 (0.98–12.8), p = 0.05	5.02 (0.9–27.97), p = 0.07
Positive comorbidity index %, (n)	49.3 (37)	42.7 (148)	0.29	0.85 (0.48–1.48), p = 0.56	1.06 (0.54–2.07), p = 0.87	75.9 (22)	44.9 (61)	<0.01	2.79 (1.08–7.21), p = 0.04	2.8 (0.83–9.37), p = 0.1
	Offspring					Controls				
	Median (IQR)		*p*-Value[a]	Model 1: β coef (95% CI)[d]	Model 2: β coef (95% CI)[e]	Median (IQR)		*p*-Value[a]	Model 1: β coef (95% CI)[d]	Model 2: β coef (95% CI)[e]
	Yes (n = 80)	No (n = 372)				Yes (n = 30)	No (n = 146)			
BMI kg/m^2, (n)	26.5 (23.7–29.1), (80)	25.1 (23.1–28.2), (369)	0.07	0.81 (–0.21 to 1.83), p = 0.12	0.4 (–0.82 to 1.61), p = 0.52	25.9 (24.1–28.6), (30)	25.1 (22.7–28.2), (144)	0.13	1.28 (–0.58 to 3.15), p = 0.18	2.1 (–0.7 to 4.9), p = 0.14
Biochemical measures										
Insulin mU/L, (n)	16.1 (8.7–38.5), (38)	12 (7–23), (176)	0.16	2.64 (–6.07 to 11.36), p = 0.55	–0.19 (–8.32 to 7.94), p = 0.96	19 (14.6–49.8), (13)	15.3 (9–22.7), (72)	0.03	15.8 (5.81–25.78), p < 0.01	16.8 (–1.19 to 34.78), p = 0.07
Glucose mg/dL, (n)	87 (75–111), (77)	84 (75–94), (357)	0.08	6.56 (–0.93 to 14.05), p = 0.09	11.91 (2.73–21.09), p = 0.01	88 (79–102), (30)	83 (75–95), (137)	0.16	0.32 (–10.7 to 11.34), p = 0.95	–1.39 (–16.76 to 13.97), p = 0.86
Insulin resistance HOMA, (n)	3.6 (1.5–8.7), (37)	2.4 (1.3–5.4), (168)	0.09	0.7 (–2.3 to 3.69), p = 0.65	0.36 (–2.48 to 3.2), p = 0.8	3.7 (2.9–12.1), (13)	3.3 (1.7–4.8), (66)	<0.05	3.72 (1.18–6.27), p < 0.01	6.61 (2.67–10.55), p < 0.01
HDL mg/dL, (n)	54 (46–66), (79)	62 (51–76), (367)	<0.01	–2.75 (–6.79 to 1.28), p = 0.18	–3.49 (–8.22 to 1.24), p = 0.15	54 (47–66), (30)	61 (52–75), (144)	<0.05	–3.79 (–9.68 to 2.09), p = 0.21	–4.26 (–11.71 to 3.2), p = 0.26
IGF-1 ng/mL, (n)	110 (82–138), (67)	124 (98–145.5), (316)	0.03	–13.58 (–24.13 to –3.03), p = 0.01	–13.47 (–26.09 to –0.84), p = 0.04	105 (74–136), (20)	118 (94–145), (101)	0.28	–9.31 (–30.52 to 11.9), p = 0.39	4.13 (–23.09 to 31.34), p = 0.76
CRP[f] mg/L, (n)	0.3 (0.1–0.5), (44)	0.2 (0.1–0.4), (193)	0.37	0.06 (–0.21 to 0.34), p = 0.66	0.09 (–0.21 to 0.39), p = 0.57	0.5 (0.2–0.6), (17)	0.3 (0.2–0.5), (71)	0.21	–0.004 (–0.44 to 0.43), p = 0.99	0.12 (–0.38 to 0.62), p = 0.64

(*Continued*)

TABLE 4 | Continued

	Offspring					Controls				
	Median (IQR)		*p*-Value[a]	Model 1: β coef (95% CI)[d]	Model 2: β coef (95% CI)[e]	Median (IQR)		*p*-Value[a]	Model 1: β coef (95% CI)[d]	Model 2: β coef (95% CI)[e]
	Yes (n = 80)	No (n = 372)				Yes (n = 30)	No (n = 146)			
eGFR[b] mL/min/1.73 m^2, (n)	73 (59.6–85.8), (77)	75.6 (62.7–88.5), (354)	0.20	−1.95 (−6.04 to 2.15), p = 0.35	−3.04 (−7.72 to 1.64), p = 0.2	68.1 (55.9–76), (30)	73.3 (61.8–86.9), (139)	0.05	−7.08 (−13.83 to −0.33), p = 0.04	−9.74 (−19.18 to −0.31), p = 0.04
Testosterone[b] ng/dL, (n)	403.5 (325–532), (40)	371.5 (300–491), (138)	0.40	46.89 (−8.76 to 102.55), p = 0.1	26.01 (−37.47 to 89.49), p = 0.42	349 (277–378), (10)	358 (298–500), (45)	0.43	−20.41 (−113.12 to 72.3), p = 0.66	−52.64 (−168.58 to 63.3), p = 0.36

[a]*p-Value comparing those who nap and do not nap.*
[b]*Age, sex, and BMI adjusted OR of disease for those who nap compared with those who do not nap.*
[c]*Age, sex, BMI, and sleep pill adjusted OR of disease for those who nap compared with those who do not nap.*
[d]*Age, sex, and BMI adjusted beta coefficient for those who nap compared with those who do not nap.*
[e]*Age, sex, BMI, and sleep pill adjusted beta coefficient for those who nap compared with those who do not nap.*
[f]*Beta coefficients are log transformed.*
[g]*Model 1 is adjusted only for BMI and Model 2 is adjusted only for BMI and sleeping pill usage.*
[h]*Testosterone is reported for males only.*

at that age were not associated with diabetes or cardiovascular diseases later in life in this group. Furthermore, all the centenarians in this study had preserved cognition. These findings suggest that among individuals with genetic predisposition to exceptional longevity, sleep disturbances do not negatively impact health outcomes or survival.

The association between sleep duration and survival has been widely investigated. The National Health and Nutrition Examination Survey I (NHANES I) found a U-shaped relationship between sleep duration and mortality in elderly subjects (44). In a subsequent meta-analysis of 13 independent cohort studies, a U-shaped relationship between sleep duration and mortality has been confirmed (45). The Bambui Health and Aging Study, on the other hand, found a linear correlation between sleep duration and mortality (46). The largest report with more than 1 million participants aged 30–102 years concluded that 7 h of sleep was associated with the lowest mortality risk (47). In fact, prolonged, rather than short sleep duration, was more consistently associated with higher mortality in older populations (48–50). Longer duration of sleep was associated not only with overall mortality but also with mortality from cardiovascular diseases (21) and from dementia (51, 52). A number of possible mechanisms have been suggested to explain this relationship, including fatigue, cardiorespiratory and underlying diseases, as well as impaired immune function (53). However, frailty and chronic inflammation have been found to be insignificant factors in long-sleep-associated mortality in Taiwan (48). Considering the epidemiological evidence for association of longer sleep duration and mortality, some have even recommended sleep restriction for the elderly (54). However, there is no evidence that sleep restriction would be helpful, both because there is a lack of randomized clinical trials investigating this question and because longer sleep duration may not be the causative factor for disease and mortality but rather the result of the underlying condition. Even among older adults with good health status, sleeping >8 h has been associated with higher mortality risk and the risk increased with longer sleep duration (55).

Both offspring and controls with longer duration of sleep demonstrated overall riskier metabolic profiles. Offspring and controls who slept ≥8 h had higher insulin levels and HOMA-IR and lower HDL cholesterol levels compared with their counterparts who slept less than 8 h. The definitions of short and long duration of sleep vary between studies, with short sleep duration often defined as <5 h and long sleep duration as >8 h (45). Several studies have shown a U-shape relationship between the duration of sleep and risk of T2DM (56–58), with the lowest risk among those who slept 7–8 h per night (59). Compared with 7 h of sleep, the risk for developing diabetes ranged between 1.47 and 1.95 for shorter sleep duration and between 1.4 and 3.12 for longer sleep duration (60). Other studies have shown positive association between long, but not short, sleep duration and diabetes (61, 62). A meta-analysis of seven studies found that HOMA-IR did not differ between individuals with short and long sleep durations, but what defined long and short sleep duration varied between studies (63).

Chronic low-grade inflammation has been regarded as a strong risk factor for insulin resistance in longitudinal studies, including elderly cohorts (64–66). The inflammatory

biomarkers CRP and interleukin-6 (IL-6) were found to be elevated in sleep apnea and excessive sleepiness (67, 68). Therefore, the mechanism provoking diabetes in long sleepers has been hypothesized to be induced by these proinflammatory cytokines, which are elevated in chronic low-grade inflammation. A meta-analysis that included 27 studies on sleep duration concluded that long, but not short duration of sleep was associated with increased levels of CRP and IL-6 (69). Of note, the participants in these studies were mainly women and younger. In our study, sleeping for ≥8 h had been associated with insulin resistance in the offspring, but not with diabetes. We also did not find a significant difference in CRP concentrations between groups with different sleep durations. Thus, the difference in glucose metabolism in offspring and controls who were long-sleepers could not be explained by the existence of a chronic inflammatory condition.

The association of daytime napping and mortality is controversial, with studies having reported contradictory results. A large cohort study conducted in Greece has found an inverse relationship between short daytime napping and increased mortality (70). Another study conducted in Great Britain has reported an association between daytime napping and all-cause mortality, independent of preexisting health conditions (71). In a meta-analysis of 16 cohort studies, nine studies have shown an association between daytime napping and all-cause mortality (72). The largest cohort study that addressed the relationship between cardiovascular risk and napping has found a strong association between daytime napping and risk of cardiovascular mortality (73). Daytime napping for ≥30 min was associated not only with coronary artery disease but also with cancer in both genders (74). A potential explanation for the increased risk in CVD among daytime nappers may be based on the same biological mechanisms that were related to a higher incidence of MI and stroke after arousal from night sleep, which included elevated blood pressure, acute change in posture, and hypercoagulability (75, 76). We found that controls who napped regularly had higher levels of insulin and HOMA-IR. They were also more likely to have one or more of the age-related diseases compared with those who did not nap. On the other hand, offspring who napped were not found to be at increased risk for age-related diseases, suggesting that longevity genes that they have inherited from their centenarian parent may protect them from the negative impact of napping.

Melatonin, the pineal secreted hormone, has an important role in regulating the circadian rhythm and its levels have been found to decline over the lifespan (77). However, melatonin was not measured in our study. Melatonin secretion is high at night and very low during the day. Thus, an effective evaluation of melatonin secretion requires the collection of several blood samples or urine samples for measurements of its metabolite, 6-hydroxymelatonin sulfate, over a 24-h period (78, 79). Our study was not designed for repeated blood draws and all samples were collected in the morning. Future studies should consider investigating whether melatonin secretion impacts longevity.

Although our study has a number of strengths, including a cohort of relative genetic and socioeconomic homogeneity that is comprised of centenarians, offspring, and controls, it also has a number of limitations. Since all blood samples were collected at the time of study enrollment, but only a small subgroup of centenarians were questioned about their current sleep patterns, we could not associate current sleep patterns with present diseases and biochemical parameters in the centenarian group. We used a brief self-reported sleep questionnaire, as have many other published epidemiological studies (80). A correlation of 0.45 has been found between self-reported and measured sleep duration (81). However, large epidemiological studies cannot always use objectively measured techniques such as actigraphy recording. In addition, our questionnaire addressed habitual sleep patterns and did not rely on a single-day report, thereby potentially providing a more global view of sleep patterns in an individual rather than an episodic one. Since we relied on self-report of sleep patterns at age 70 in centenarians, their responses may have been subject to recall bias. Recall bias is also a consideration in offspring and controls, although to a lesser extent than in the centenarian group since the offspring and controls were asked to recall current, rather than past, sleep patterns. However, because both offspring and controls are subject to the same recall bias, the bias is non-differential among these groups and thus is unlikely to meaningfully affect the final results. Another consideration is that not all offspring may have inherited the protective longevity genes from their centenarian parent; thus, even though the offspring group is enriched for longevity genes it is likely that not everyone in that group actually possesses them. However, as a group, the offspring are enriched for longevity genes. It is also plausible that some of the controls may carry longevity genes, but that possibility is quite low given the rare phenotype of centenarians in the general population. Although our study was conducted in an Ashkenazi Jewish sample, our prior published work demonstrates that Ashkenazi Jewish centenarians are very similar to centenarians of other ethnic backgrounds (29) and our findings have been validated in several other populations. Therefore, the findings from this study may be generalizable to other ethnic groups (82, 83), but additional validation will be required.

Strong evidence exists for the association between unfavorable sleep patterns and risky metabolic profiles and cardiovascular complications in humans. However, this study demonstrated that although centenarian offspring and controls have similar sleep patterns that are associated with unfavorable metabolic profiles in both groups, only the controls were found to have higher odds of age-related diseases in the setting of napping. These findings suggest that offspring may inherit longevity genes from their long-lived parents, which protect them from the hazardous effects of unfavorable sleep patterns. Thus, the offspring and centenarians appear to be resistant to risky sleep patterns. Further prospective studies are needed to verify whether offspring of parents with exceptional longevity, who report unfavorable sleep patterns, share their parents' protective genome.

ETHICS STATEMENT

The study was approved by the Institutional Review Board at the Albert Einstein College of Medicine. Written informed consent

was obtained from all the study participants in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

LK, TG, NB, and SM contributed to the design of the study and interpretation of the data. LK and TG contributed to the acquisition of data and writing of the manuscript. TG and SM contributed to the analysis of the data. NB and SM contributed to the critical revisions of the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The Effects of Combinations of Cognitive Impairment and Pre-frailty on Adverse Outcomes from a Prospective Community-Based Cohort Study of Older Chinese People

Ruby Yu[1,2], John E. Morley[3], Timothy Kwok[1,2], Jason Leung[4], Osbert Cheung[1] and Jean Woo[1,2]*

[1] Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, [2] The Chinese University of Hong Kong Jockey Club Institute of Ageing, Shatin, Hong Kong, [3] Division of Geriatric Medicine, Saint Louis University School of Medicine, St Louis, MO, United States, [4] The Chinese University of Hong Kong Jockey Club Centre for Osteoporosis Care and Control, Shatin, Hong Kong

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****Correspondence:***
Ruby Yu
rubyyu@cuhk.edu.hk

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Objectives: To examine how various combinations of cognitive impairment (overall performance and specific domains) and pre-frailty predict risks of adverse outcomes; and to determine whether cognitive frailty may be defined as the combination of cognitive impairment and the presence of pre-frailty.

Design: Community-based cohort study.

Participants: Chinese men and women (n = 3,491) aged 65+ without dementia, Parkinson's disease and/or frailty at baseline.

Measurements: Frailty was characterized using the Cardiovascular Health Study criteria. Overall cognitive impairment was defined by a Cantonese Mini-Mental Status Examination (CMMSE) total score (<21/24/27, depending on participants' educational levels); delayed recall impairment by a CMMSE delayed recall score (<3); and language and praxis impairment by a CMMSE language and praxis score (<9). Adverse outcomes included poor quality of life, physical limitation, increased cumulative hospital stay, and mortality.

Results: Compared to those who were robust and cognitively intact at baseline, those who were robust but cognitively impaired were more likely to develop pre-frailty/frailty after 4 years (P < 0.01). Compared to participants who were robust and cognitively intact at baseline, those who were pre-frail and with overall cognitive impairment had lower grip strength (P < 0.05), lower gait speed (P < 0.01), poorer lower limb strength (P < 0.01), and poorer delayed recall at year 4 [OR, 1.6; 95% confidence interval (CI), 1.2–2.3]. They were also associated with increased risks of poor quality of life (OR, 1.5; 95% CI, 1.1–2.2) and incident physical limitation at year 4 (OR, 1.8; 95% CI, 1.3–2.5), increased cumulative hospital stay at year 7 (OR, 1.5; 95% CI, 1.1–2.1), and mortality

over an average of 12 years (OR, 1.5; 95% CI, 1.0–2.1) after adjustment for covariates. There was no significant difference in risks of adverse outcomes between participants who were pre-frail, with/without cognitive impairment at baseline. Similar results were obtained with delayed recall and language and praxis impairments.

Conclusion: Robust and cognitively impaired participants had higher risks of becoming pre-frail/frail over 4 years compared with those with normal cognition. Cognitive impairment characterized by the CMMSE overall score or its individual domain score improved the predictive power of pre-frailty for poor quality of life, incident physical limitation, increased cumulative hospital stay, and mortality. Our findings support to the concept that cognitive frailty may be defined as the occurrence of both cognitive impairment and pre-frailty, not necessarily progressing to dementia.

Keywords: cognitive frailty, cognitive impairment, frailty, length of hospital stay, mortality, physical limitation

INTRODUCTION

Frailty represents a state of decline in functional reserves, which increases the risk of adverse health outcomes such as morbidity, disability, and institutionalization, after a stressor event (1). It can be preceded by, but also occurs in the absence of chronic disease (2) and has been suggested as a better predictor of health and well-being than the presence or absence of disease. Although the term frailty is commonly used in clinical practice, there is no consensus on the definition of frailty. A popular approach to the assessment of frailty as proposed by Fried et al. (1) (i.e., the phenotype approach) encompasses the assessment of five criteria-based primarily on physical attributes and capabilities including poor grip strength, slow walking speed, low levels of physical activity, exhaustion, and unintentional weight loss, whereas an individual is considered to be frail if they present with three or more of five criteria. Another notable approach to the assessment of frailty is that of Rockwood and Mitnitski (3, 4) (i.e., the deficit accumulation model) in which frailty is viewed in terms of the number of health deficits (i.e., integration with measures of physical frailty and other domains) that are manifest in the individual, leading to a continuous measure of frailty (frailty index).

More recently, there is general consensus that measures of cognitive function should be added to physical performance for the definition of frailty, in that there is a bidirectional relationship between physical frailty and cognitive impairment. There is also a parallel pathway among frailty discourse, that cognitive vulnerability (or impairment) may be a precursor of mild neurodegenerative disorder [akin to pre-dementia state of Mild Cognitive Impairment (MCI)] (5) and subsequently major neurodegenerative disorder (dementia) (6). Numerous studies have demonstrated that cognitive impairment may lead to increased risk of acquiring individual components of frailty syndrome (e.g., faster gait speed decline)/future frailty (7–9). The reciprocal relationship, which frailty predicts cognitive decline/incident dementia, has also been reported (10–14). Both frailty and cognitive impairment share many common risk factors and underlying mechanisms (6, 15, 16). Although many studies demonstrate close relationship between frailty and cognitive impairment, most of them have characterized frailty and cognitive impairment as two different entities, and the term "cognitive frailty" has been proposed, to characterize the co-existence of both frailty and cognitive impairment. An international consensus group organized by the International Academy on Nutrition and Aging (IANA) and the International Association of Gerontology and Geriatrics (IAGG) proposed the definition as a clinical condition characterized by the simultaneous presence of both physical frailty and MCI (Clinical Dementia Rating = 0.5) (17). Recent studies have reported that cognitive frailty conferred additional greater risk of adverse outcomes including disability, hospitalization, and mortality (6, 18–20). Understanding the temporal relationship between cognitive impairment and frailty is important, in predicting the onset of the other, with implications for screening and intervention programs. For example, in the Baltimore longitudinal study of aging, a bidirectional relationship was noted for usual gait speed and executive function, with each predicting change in the other, while poor fast walking performance predicted future executive function and memory changes but not *vice versa* (14). Although there is no universal consensus regarding the entity of cognitive frailty and its definition, there is general consensus of the importance of recognizing cognitive impairment, as differentiated from screening for dementia (21).

According to the IANA/IAGG, the primary criterion of cognitive frailty is the presence of physical frailty and MCI, without dementia. However, different states of cognitive vulnerability and frailty may be relevant to identify older persons with cognitive frailty. Furthermore, it is likely that MCI may represent a later stage of cognitive impairment at which multiple domains of cognition have already occurred. Early detection of abnormalities in specific domains of cognitive function (e.g., memory problems, difficulties in word finding) together with identification of the pre-frail state (an intermediate stage between non-frail and frail) may allow opportunities for reversibility through intervention strategies, which is supported by the findings from a home-based program to prevent functional decline in physically frail elderly persons in which the benefit of the program was observed among those with moderate frailty, but not those with severe frailty (22). Using the Mr and MsOs study of older

Chinese men and women who were free of dementia and/or Parkinson's disease and who were non-frail at baseline, we examined how various combinations of cognitive impairment (overall performance as well as two selected *a priori* domains) and pre-frailty predict risks of adverse outcomes (poor quality of life, physical limitation, increased cumulative hospital stay, and mortality), and to determine whether cognitive frailty may be defined as the combination of cognitive impairment (overall or domain specific) and the presence of pre-frailty.

MATERIALS AND METHODS

Participants

Four thousand community-dwelling Chinese men and women aged 65 years and older were recruited for a cohort study on osteoporosis and general health (Mr and MsOs study) in Hong Kong between August 2001 and December 2003 by placing recruitment notices in housing estates and community centers for older adults. Several talks were also given at these centers explaining the purpose, procedures, and investigations to be carried out. Participants were volunteers, and the aim was to recruit a stratified sample so that approximately 33% would each be aged 65–69, 70–74, and 75 years and older. Those who were unable to walk independently, had bilateral hip replacement, or were not competent to give informed consent were excluded. Eligible persons were invited to attend a health check at the School of Public Health, The Chinese University of Hong Kong. A team of trained research assistants administered the study questionnaire and took physical measurements for each participant on the same day. In the present study, we excluded 352 participants who had reported a history of dementia/probable dementia [Cantonese Mini-Mental Status Examination (CMMSE) total score <18 (no education), <21 (primary school), or <25 (secondary school and above)] and/or Parkinson's disease and 157 participants who were frail at baseline, yielding a study of 3,491 participants for the descriptive analysis. Participants (n = 662) who did not assess for frailty at the 4-year follow-up were further excluded from the analyses for the risk prediction of adverse outcomes. The valid study population included in the respective analysis is shown in **Figure 1**. Details of the study population have been reported elsewhere (23). All participants gave written consent in accordance with the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong (CRE-2003.102).

Questionnaire

The information from the questionnaire used in this study included demographics, educational levels, socioeconomic status, smoking habits, alcohol intake, physical activity, quality of diet, quality of life, and daily functioning. Socioeconomic status was assessed by asking the participants to mark their self-perceived position on a ladder with 10 rungs, with the lowest and highest

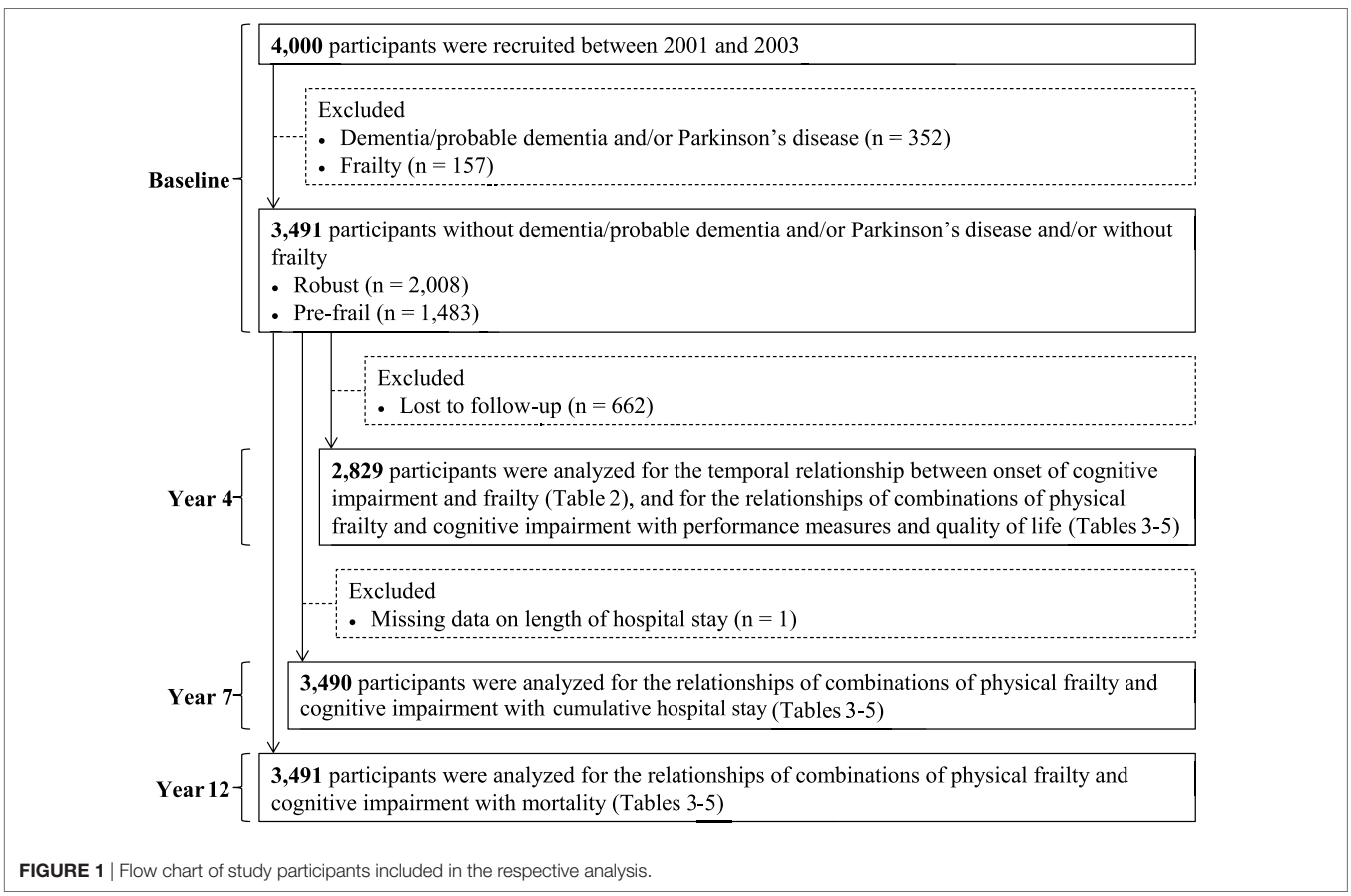

FIGURE 1 | Flow chart of study participants included in the respective analysis.

rungs representing the lowest and highest socioeconomic status in society (Hong Kong ladder). Smoking habits were categorized as non-current smoker and current smoker. Alcohol intake was categorized as non-drinker (≤12 alcoholic drinks in the past 12 months) and drinker (>12 alcoholic drinks in the past 12 months). Physical activity levels were assessed using the Physical Activity Scale of the Elderly (PASE) (24). The quality of diet was assessed using the Diet Quality Index-International (DQI-I) (25). Quality of life was assessed using the 12-Item Short Form Health Survey (SF-12) (26). Information on daily functioning was obtained regarding impairment in walking two to three blocks outside on level ground, climbing up 10 steps without resting, preparing own meals, doing heavy housework, such as scrubbing floors or washing windows, and doing own shopping for groceries or clothes.

Physical Measurements

Body weight was measured with the Physician Balance Beam Scale (Health-O-Meter, Arlington Heights, IL, USA). Height was measured with the Holtain Harpenden stadiometer (Holtain, Crosswell, UK). Body mass index (BMI) was calculated by dividing the weight in kilogram by height in meter squared. Grip strength was measured using a dynamometer (JAMAR Hand Dynamometer 5030JO; Sammons Preston, Bolingbrook, IL, USA). Two readings were taken from each side and the maximum value of the right or left was used for analysis. The intra-class correlation coefficients for right and left handgrip strength were 0.921 [95% confidence interval (CI), 0.914–0.927] and 0.916 (95% CI, 0.909–0.923), respectively. Gait speed was measured using the best time in seconds to complete a walk along a straight line 6 m long in distance. A warm up period of less than 5 min was followed by two walks, and the best time was recorded. The intra-class correlation coefficient for the two walking trials was 0.752 (95% CI, 0.732–0.770). Chair stand was measured by asking the participant to rise from a chair (seat height 45 cm), with arms folded across the chest, five times as quickly as possible. The time taken was recorded.

Frailty Assessment

Frailty was assessed using the five-item Cardiovascular Health Study (CHS) frailty phenotype, with total score ranging from 0 to 5 (1). The five items are unintentional weight loss, self-rated exhaustion, weakness (grip strength), slow walking speed, and low physical activity. The equivalent variables used in this study for the construction of the CHS score were BMI less than 18.5 kg/m^2, having no energy, grip strength measurement in the lowest quartile, walking speed measurement in the lowest quartile, and PASE score in the lowest quartile. The total scores were used to categorize participants as robust (score = 0), pre-frail (score = 1–2), and frail (score = 3–5).

Cognitive Function Assessment

Cognitive function was assessed using the CMMSE (27). CMMSE is a validated Cantonese version of Mini-Mental Status Examination (28), which is composed of 30 items that assess multiple domains of cognitive function, including tests of orientation to time (max score: 5) and place (max score: 5), registration (max score: 3), attention and calculation (max score: 5), recall (max score: 3) and language and praxis (max score: 9). Score by the CMMSE is ranged from 0 to 30; a lower CMMSE score reflects more dementia-related cognitive impairment. A score of less than 21 in individuals with no education, a score of less than 24 in individuals with primary education, or a score of less than 27 in well-educated individuals with secondary or tertiary education are identified as overall cognitive impairment. Alternatively, individual who failed to recall any of the three words during the CMMSE delayed recall (i.e., a CMMSE delayed recall score of less than 3) or were unable to complete one or more language and praxis tasks on the CMMSE (i.e., a CMMSE language and praxis score of less than 9) were classified as cognitive impairment. These two domains (one amnestic and one non-amnestic) were selected *a priori*.

Adverse Outcomes at Follow-up

Participants were invited to return for re-assessments after 4 years. Quality of life was assessed using the SF-12. Physical limitation was assessed using the following two questions: do you have any difficulty in climbing stairs (possible answers: no, a little, a lot) and do you have any difficulty in carrying out the following household activities such as moving chairs or tables (possible answers: no, a little, a lot). Participants were categorized as having physical limitation if the answer to either question was "a little" or "a lot," while those who answered "no" to both questions were categorized as having no physical limitation. Incident physical limitation was defined as progression from those without limitation at baseline to having limitation at follow-up. Cumulative length of hospital stay from baseline to year 7 was obtained from the Hong Kong Hospital Authority records, which covered more than 93% of the hospitalizations in the Hong Kong population. The cutoff date for determining length of hospital stay was 30 September 2008. Increased cumulative hospital stay refers to the highest quintile (i.e., 20 days). Mortality was documented through a search of the Hong Kong Death Registry. The cutoff date for determining mortality was 31 March 2014.

Data Analysis

Data were summarized as means (SDs) for continuous variables and as percentages for categorical data. Chi-square tests were used to compare the differences in the development of pre-frailty/frailty between robust/pre-frail participants with and without cognitive impairment at baseline. Analysis of covariance or logistic regression were performed to estimate the performance measures and the risk of adverse outcomes (poor quality of life, incident physical limitation, increased cumulative hospital stay, and mortality) after 4–12 years across groups of participants with different frailty (as per CHS criteria) and cognitive (as per CMMSE criteria) status at baseline, including (1) robust and cognitively intact, (2) robust and cognitively impaired, (3) pre-frail and cognitively intact, and (4) pre-frail and cognitively impaired. Covariates including age, sex, educational level, socioeconomic status, smoking habit, alcohol intake, physical activity, DQI-I, BMI, and baseline values of respective outcome variable were adjusted. The above analyses were repeated, substituting the CMMSE individual domain scores (delayed recall score, language and praxis score) in place of the CMMSE

total score. All analyses were carried out using the Window-based SPSS Statistical Package (v23.0; SPSS, Inc., Chicago, IL, USA), and *P* values less than 0.05 were considered statistically significant.

RESULTS

At baseline, the mean age of the study sample was 72.0 (4.9) years, 48.4% were female, and 72.0% had primary or lower education. In total, 57.5% were robust, 42.5% were pre-frail, and 17.4% had overall cognitive impairment, with their CMMSE total score <21–27 (depending on participants' educational levels). Of those who were pre-frail ($n = 1,483$), 20.4% had overall cognitive impairment (CMMSE total score < 21–27), 55.3% had delayed recall impairment (delayed recall score < 3), and 51.4% had language and praxis impairment (language and praxis score < 9) (**Table 1**).

The prevalence of overall cognitive impairment was higher in the pre-frail group (17.8%) than in the robust group (14.4%). Compared to participants who were robust and cognitively intact at baseline, those who were robust but cognitively impaired were more likely to develop pre-frailty/frailty after 4 years ($P < 0.01$). Participants who were pre-frail but cognitively intact at baseline were also more likely to develop frailty at the 4-year follow-up than their cognitively impaired counterparts. However, the association was not significant ($P = 0.056$) (**Table 2**).

Compared to participants who were robust and cognitively intact at baseline, those who were pre-frail and with overall cognitive impairment had lower grip strength ($P < 0.05$), lower gait speed ($P < 0.01$), poorer lower limb strength ($P < 0.01$), and poorer performance in delayed recall at year 4 (OR, 1.6; 95% CI 1.2–2.3). They were also associated with increased risks of poor quality of life (OR, 1.5; 95% CI, 1.1–2.2) and incident physical limitation at year 4 (OR, 1.8; 95% CI, 1.3–2.5), increased cumulative hospital stay at year 7 (OR, 1.5; 95% CI, 1.1–2.1), and mortality over an average of 12 years (OR, 1.5; 95% CI, 1.0–2.1) after adjustment for covariates. Participants who were pre-frail and cognitively impaired at baseline were also associated with a higher risk of incident physical limitation at year 4 (OR, 1.8; 95% CI, 1.1–2.8) as compared to the robust but cognitively impaired participants, and had poorer cognitive performance at year 4 as compared to their cognitively intact counterparts ($P < 0.01$). However, there was no significant difference in risks of adverse outcomes between participants who were pre-frail, with or without cognitive impairment at baseline (**Table 3**).

When a single *a priori* selected cognitive domain was used to define cognitive impairment, participants with pre-frailty and a delayed recall score <3 at baseline had lower gait speed ($P < 0.001$), poorer lower limb strength ($P < 0.05$), poorer cognitive performance in terms of time orientation (OR, 1.7; 95% CI 1.3–2.3), place orientation (OR, 1.7; 95% CI, 1.3–2.2), attention/calculation (OR, 1.5; 95% CI, 1.1–1.9), as well as language and praxis at year 4 (OR, 1.5; 95% CI, 1.1–1.9). They were also associated with increased risks of poor quality of life (OR, 1.7; 95% CI, 1.3–2.3), and incident physical limitation at year 4 (OR, 1.8;

TABLE 1 | Descriptive characteristic of participants at baseline.

Baseline characteristic	Mean ± SD/*n*(%)
All participants	
Age, years	72.03 ± 4.91
Sex	
Male	1,800 (51.56)
Female	1,691 (48.44)
Educational levels	
No education	721 (20.65)
Primary school	1,793 (51.36)
Secondary school or above	977 (27.99)
Social economic status ladder—Hong Kong[a]	
≤4	1,411 (42.51)
>4	1,908 (57.49)
Smoking habits	
Current smokers	243 (6.95)
Non-current smokers	3,248 (93.04)
Alcohol intake[a]	
>12 alcoholic drinks in past 12 months	489 (14.01)
≤12 alcoholic drinks in past 12 months	3,001 (85.99)
Physical activity (PASE total score)	94.80 ± 42.87
Dietary intakes (DQI-I)[a]	64.71 ± 9.36
BMI, kg/m^2	23.77 ± 3.22
Frailty	
Robust	2,008 (57.52)
Pre-frailty	1,483 (42.48)
Cognitive impairment	
Defined by CMMSE total score <21/24/27[b]	
No cognitive impairment	2,884 (82.61)
Cognitive impairment	607 (17.39)
Defined by CMMSE delayed recall score <3	
No cognitive impairment	1,592 (45.60)
Cognitive impairment	1,899 (54.40)
Defined by CMMSE language, repetition and commands score <9	
No cognitive impairment	1,811 (51.88)
Cognitive impairment	1,680 (48.12)
Participants with pre-frailty	
Among participants with pre-frailty	
W/o cognitive impairment (CMMSE total score ≥ 21/24/27)	1,181 (79.64)
W cognitive impairment (CMMSE total score < 21/24/27)	302 (20.36)
Among participants with pre-frailty	
W/o cognitive impairment (CMMSE delayed recall score = 3)	663 (44.71)
W cognitive impairment (CMMSE delayed recall score < 3)	820 (55.29)
Among participants with pre-frailty	
W/o cognitive impairment (CMMSE language and praxis score = 9)	721 (48.62)
W cognitive impairment (CMMSE language and praxis score < 9)	762 (51.38)

[a]Missing observations (social economic status ladder—Hong Kong, n = 172; alcohol intake, n = 1; DQI-I, n = 4).

[b]Cognitive impairment was defined by CMMSE total score <21 (no education), <24 (primary school), or <27 (secondary school and above).

CMMSE, Cantonese Mini-Mental Status Examination; DQI-I, Diet Quality Index-International; PASE, Physical Activity Scale of the Elderly; BMI, body mass index.

TABLE 2 | Transitions in frailty status over 4 years by cognitive status according to baseline CMMSE total score.

	Cognitive impairment at baseline[a]		
	No (n = 2,383)	Yes (n = 446)	P
Participants who were robust at baseline and were reassessed at year 4	1,460	246	
Robust at baseline and follow-up	818 (56.03)	115 (46.75)	
Robust at baseline and pre-frail at follow-up	604 (41.37)	120 (48.78)	
Robust at baseline and frail at follow-up	38 (2.60)	11 (4.47)	0.007[†]
Participants who were pre-frailty at baseline and were reassessed at year 4	923	200	
Pre-frail at baseline and robust at follow-up	274 (29.69)	48 (24.00)	
Pre-frail at baseline and follow-up	535 (57.96)	135 (67.50)	
Pre-frail at baseline and frail at follow-up	114 (12.35)	17 (8.50)	0.056[‡]

Data are reported as either number (percentage).
[a]Cognitive impairment was defined by Cantonese Mini-Mental Status Examination total score <21 (no education), <24 (primary school), or <27 (secondary school and above).
[†]P-value was obtained from Chi-square test comparing the differences in the development of frailty (with pre-frail and frail participants collapsed into one group) between robust participants with and without cognitive impairment at baseline.
[‡]P-value was obtained from Chi-square test comparing the differences in the development of frailty between pre-frail participants with and without cognitive impairment at baseline. Participants who were pre-frail at baseline and robust at follow-up were excluded.

95% CI, 1.4–2.3), and increased cumulative hospital stay at year 7 (OR, 1.4; 95% CI, 1.1–1.9) as compared to participants who were robust and had a delayed recall score = 3 (**Table 4**). Similar results were obtained when cognitive impairment was redefined by language and praxis score (**Table 5**).

The risks of having adverse outcomes at follow-up were also compared between those who were robust and cognitively intact and the rest of the groups. As expected, participants who were pre-frail but cognitively intact at baseline were associated with increased risk of poor quality of life (OR, 1.4; 95% CI, 1.1–1.7) and incident physical limitation at year 4 (OR, 1.5; 95% CI, 1.2–1.8) as well as increased cumulative hospital stay at year 7 (OR, 1.4; 95% CI, 1.2–1.8) as compared to participants who were robust and cognitively intact at baseline. However, there was no significant difference in risks of adverse outcomes between participants who were robust, with or without cognitive impairment at baseline (Table S1 in Supplementary Material).

DISCUSSION

In a cohort of older people free of dementia and/or Parkinson's disease and/or frailty at baseline, we showed that robust and cognitively impaired participants were more likely to develop pre-frailty/frailty after 4 years than the robust and cognitively

TABLE 3 | Performance measures, quality of life, and risk of adverse outcomes of participants in different frailty and cognitive status according to baseline CMMSE total score.[a]

Outcome	Robust		Pre-frailty		P/OR(95% CI)[†]		
	No cognitive impairment[(1)] (n = 1,703)	Cognitive impairment[(2)] (n = 305)	No cognitive impairment[(3)] (n = 1,181)	Cognitive impairment[(4)] (n = 302)	(1 vs. 4)	(2 vs. 4)	(3 vs. 4)
Physical performance at year 4[b]							
Grip strength, kg	28.55 ± 7.99	24.96 ± 7.28	25.20 ± 7.69	22.35 ± 7.03	0.013	0.749	0.286
Gait speed, m/s	1.00 ± 0.21	0.95 ± 0.21	0.88 ± 0.23	0.84 ± 0.22	0.002	0.719	0.138
Five chair stand, s	9.75 ± 3.56	10.16 ± 3.80	11.60 ± 5.87	12.20 ± 6.27	0.001	0.118	0.729
Cognitive performance at year 4[b]							
Global cognitive functioning							
CMMSE total score	26.89 ± 2.95	24.61 ± 3.95	26.31 ± 3.20	24.93 ± 4.06	0.063	0.437	0.006
Domain-specific cognition							
CMMSE time orientation score <5	251 (17.19)	72 (29.27)	203 (21.99)	56 (28.00)	1.09 (0.72, 1.64)	0.88 (0.53, 1.45)	1.04 (0.69, 1.56)
CMMSE place orientation score <5	333 (22.81)	80 (32.52)	280 (30.34)	69 (34.50)	1.11 (0.76, 1.62)	1.08 (0.68, 1.71)	0.99 (0.68, 1.43)
CMMSE registration score <3	35 (2.40)	14 (5.69)	37 (4.01)	9 (4.50)	1.55 (0.65, 3.68)	0.91 (0.34, 2.44)	1.35 (0.62, 2.96)
CMMSE attention/calculation score <5	572 (39.18)	156 (63.41)	401 (43.45)	115 (57.50)	1.04 (0.71, 1.54)	0.70 (0.44, 1.13)	0.91 (0.61, 1.36)
CMMSE delayed recall score <3	537 (36.78)	117 (47.56)	373 (40.41)	104 (52.00)	1.63 (1.17, 2.28)	1.39 (0.91, 2.14)	1.54 (1.10, 2.17)
CMMSE language and praxis score <9	780 (53.42)	166 (67.48)	573 (62.08)	134 (67.00)	1.33 (0.92, 1.92)	1.05 (0.66, 1.66)	1.08 (0.75, 1.57)
Adverse outcomes at year 4–12[b]							
Poor quality of life (SF-12 PCS) at year 4	328 (22.47)	60 (24.39)	302 (32.72)	70 (35.00)	1.53 (1.06, 2.22)	1.39 (0.84, 2.29)	1.09 (0.76, 1.57)
Poor quality of life (SF-12 MCS) at year 4	279 (19.11)	46 (18.70)	207 (22.43)	58 (29.00)	1.28 (0.86, 1.91)	1.29 (0.75, 2.21)	1.33 (0.90, 1.95)
Incident physical limitation at year 4	374 (25.62)	76 (30.89)	332 (35.97)	86 (43.00)	1.78 (1.26, 2.51)	1.78 (1.13, 2.82)	1.23 (0.87, 1.72)
Increased cumulative hospital stay at year 7	278 (16.32)	49 (16.12)	306 (25.91)	75 (24.83)	1.48 (1.06, 2.06)	1.53 (0.96, 2.44)	1.06 (0.77, 1.46)
Mortality over an average of 12 years	232 (13.62)	44 (14.43)	261 (22.10)	71 (23.51)	1.46 (1.02, 2.07)	1.55 (0.94, 2.54)	1.19 (0.85, 1.67)

Data are reported as either number (percentage) or mean ± SD.
[a]Cognitive impairment was defined by CMMSE total score <21 (no education), <24 (primary school), or <27 (secondary school and above).
[b]Analyses were based on valid cases observed for grip strength (n = 2,798), gait speed (n = 2,821), five chair stand (n = 2,789), CMMSE, SF-12 and incident physical limitation (n = 2,829), increased cumulative hospital stay (n = 3,490), and mortality (n = 3,491).
[†]P-values/ORs (95% CI) were obtained from multivariate linear regression/logistic regression adjusting for age, sex, education (below secondary vs. secondary or above), social economic status ladder—Hong Kong (≤4 vs. >4), smoking (current smokers vs. non-current smokers), alcohol intake (>12 vs. ≤12 alcoholic drinks in past 12 m), physical activity (PASE total score), dietary intakes (DQI-I), BMI, and baseline value of respective outcome variable (when appropriate).
CMMSE, Cantonese Mini-Mental Status Examination; DQI-I, Diet Quality Index-International; PASE, Physical Activity Scale of the Elderly; BMI, body mass index.

TABLE 4 | Performance measures, quality of life, and risk of adverse outcomes of participants in different frailty and cognitive status according to baseline CMMSE delayed recall score.[a]

Outcome	Robust		Pre-frailty		*P*/OR(95% CI)[†]		
	No cognitive impairment(1) (*n* = 929)	Cognitive impairment(2) (*n* = 1,079)	No cognitive impairment(3) (*n* = 663)	Cognitive impairment(4) (*n* = 820)	(1 vs. 4)	(2 vs. 4)	(3 vs. 4)
Physical performance at year 4[b]							
Grip strength, kg	28.47 ± 7.92	27.65 ± 8.03	25.33 ± 7.92	24.17 ± 7.39	0.098	0.010	0.681
Gait speed, m/s	1.01 ± 0.21	0.97 ± 0.21	0.89 ± 0.23	0.86 ± 0.22	<0.001	0.085	0.543
Five chair stand, s	9.61 ± 3.51	9.98 ± 3.67	11.50 ± 5.65	11.88 ± 6.18	0.027	0.015	0.323
Cognitive performance at year 4[b]							
Global cognitive functioning							
CMMSE total score	27.06 ± 2.84	26.13 ± 3.45	26.57 ± 3.08	25.66 ± 3.60	0.114	0.255	0.536
Domain-specific cognition							
CMMSE time orientation score <5	128 (16.10)	195 (21.41)	94 (18.58)	165 (26.74)	1.71 (1.26, 2.33)	1.40 (1.06, 1.86)	1.48 (1.09, 2.01)
CMMSE place orientation score <5	154 (19.37)	259 (28.43)	145 (28.66)	204 (33.06)	1.66 (1.25, 2.21)	1.07 (0.83, 1.38)	1.08 (0.82, 1.42)
CMMSE registration score <3	20 (2.52)	29 (3.18)	20 (3.95)	26 (4.21)	1.45 (0.69, 3.04)	1.15 (0.61, 2.15)	1.02 (0.54, 1.93)
CMMSE attention/calculation score <5	317 (39.87)	411 (45.12)	213 (42.09)	303 (49.11)	1.46 (1.12, 1.90)	1.24 (0.96, 1.59)	1.39 (1.07, 1.81)
CMMSE delayed recall score <3	235 (29.56)	419 (45.99)	163 (32.21)	314 (50.89)	1.21 (0.75, 1.96)	1.05 (0.83, 1.32)	1.11 (0.68, 1.79)
CMMSE language and praxis score <9	414 (52.08)	532 (58.40)	303 (59.88)	404 (65.48)	1.46 (1.13, 1.89)	1.31 (1.02, 1.67)	1.22 (0.94, 1.58)
Adverse outcomes at year 4–12[b]							
Poor quality of life (SF-12 PCS) at year 4	162 (20.38)	226 (24.81)	159 (31.42)	213 (34.52)	1.68 (1.25, 2.26)	1.34 (1.03, 1.75)	1.12 (0.85, 1.47)
Poor quality of life (SF-12 MCS) at year 4	132 (16.60)	193 (21.19)	116 (22.92)	149 (24.15)	1.35 (0.98, 1.85)	0.89 (0.66, 1.19)	1.05 (0.78, 1.42)
Incident physical limitation at year 4	187 (23.52)	263 (28.87)	181 (35.77)	237 (38.41)	1.78 (1.36, 2.34)	1.48 (1.15, 1.91)	1.10 (0.85, 1.43)
Increased cumulative hospital stay at year 7	136 (14.66)	191 (17.70)	174 (26.24)	207 (25.24)	1.43 (1.08, 1.91)	1.20 (0.93, 1.55)	0.83 (0.65, 1.08)
Mortality over an average of 12 years	125 (13.46)	151 (13.99)	138 (20.81)	194 (23.66)	1.27 (0.94, 1.72)	1.17 (0.88, 1.55)	1.01 (0.76, 1.32)

Data are reported as either number (percentage) or mean ± SD.

[a]Cognitive impairment was defined by a CMMSE delayed recall score of <3.

[b]Analyses were based on valid cases observed for grip strength (n = 2,798), gait speed (n = 2,821), five chair stand (n = 2,789), CMMSE, SF-12 and incident physical limitation (n = 2,829), increased cumulative hospital stay (n = 3,490), and mortality (n = 3,491).

[†]P-values/ORs (95% CI) were obtained from multivariate linear regression/logistic regression adjusting for age, sex, education (below secondary vs. secondary or above), social economic status ladder—Hong Kong (≤4 vs. >4), smoking (current smokers vs. non-current smokers), alcohol intake (>12 vs. ≤12 alcoholic drinks in past 12 m), physical activity (PASE total score), dietary intakes (DQI-I), BMI, and baseline value of respective outcome variable (when appropriate).

CMMSE, Cantonese Mini-Mental Status Examination; DQI-I, Diet Quality Index-International; PASE, Physical Activity Scale of the Elderly; BMI, body mass index.

intact participants. Furthermore, participants with both pre-frailty and cognitive impairment at baseline had poorer physical and cognitive performances, higher risks of poor quality of life, incident physical limitation, increased cumulative hospital stay, and mortality over follow-up than those with none of these conditions. These findings support a concept of the combination of cognitive impairment (overall or specific domains) and pre-frailty representing cognitive frailty, with subsequent adverse consequences. In view of the reversibility of the frailty continuum (29) and non-pharmacological strategies to improve frailty status and cognitive impairment (30–34), early detection of cognitive frailty has public health implications since participation in group exercises that combines aerobic and resistance elements with or without cognitive training may retard decline or even lead to some improvement (35). This concept of earlier detection of abnormalities is similar to the current thinking in dementia research, where intervention may be more effective if applied at an early stage.

Our finding is in close agreement with some previous studies which consistently show a higher prevalence of cognitive impairment among physically pre-frail/frail elderly (14, 36), supporting the notion that physical and cognitive impairment are closely related and are integral components of frailty. Our findings also extend a previous study examining the association of impaired cognition with frailty (8, 9) by showing the longitudinal relationship between low cognitive scores and higher risk of incident pre-frailty/frailty, which support results of previous studies proposing the inclusion of cognitive function in the assessment of frailty (3, 37, 38). Several mechanisms might explain the association between cognitive impairment and increased risk of frailty. First, poor cognition in robust individuals may be associated with underlying risk factors (e.g., poor nutritional status, physical inactivity) known to affect the development of frailty. Second, the association could reflect the existence of shared factors (e.g., increased inflammatory markers) that may be causing cognitive decline and the onset of frailty (39, 40).

Given the demonstrated increased risk of developing frailty associated with cognitive impairment at baseline, we further examined the physical and cognitive profile at the 4-year follow-up of participants with both cognitive impairment and pre-frailty at baseline. These participants had lower grip strength, lower gait speed, and poorer performance in the chair stand test as compared to robust and cognitively intact participants; and had poorer cognitive performance in the CMMSE test (in terms of CMMSE total score) compared to their cognitively intact counterparts. Furthermore, they had significantly lower

TABLE 5 | Performance measures, quality of life, and risk of adverse outcomes of participants in different frailty and cognitive status according to baseline CMMSE language and praxis score.[a]

Outcome	Robust		Pre-frailty		*P*/OR(95% CI)[†]		
	No cognitive impairment(1) (*n* = 1,090)	Cognitive impairment(2) (*n* = 918)	No cognitive impairment(3) (*n* = 721)	Cognitive impairment(4) (*n* = 762)	(1 vs. 4)	(2 vs. 4)	(3 vs. 4)
Physical performance at year 4[b]							
Grip strength, kg	29.51 ± 8.22	26.20 ± 7.28	26.05 ± 7.80	23.36 ± 7.27	0.094	0.069	0.944
Gait speed, m/s	1.02 ± 0.21	0.95 ± 0.21	0.91 ± 0.23	0.83 ± 0.21	<0.001	0.037	0.038
Five chair stand, s	9.42 ± 3.19	10.29 ± 3.99	11.06 ± 4.95	12.35 ± 6.72	0.001	0.246	0.901
Cognitive performance at year 4[b]							
Global cognitive functioning							
CMMSE total score	27.32 ± 2.74	25.63 ± 3.50	26.83 ± 3.11	25.32 ± 3.52	0.012	0.532	0.851
Domain-specific cognition							
CMMSE time orientation score <5	131 (13.88)	192 (25.20)	98 (17.66)	161 (28.35)	2.08 (1.51, 2.88)	1.22 (0.91, 1.64)	1.28 (0.94, 1.76)
CMMSE place orientation score <5	211 (22.35)	202 (26.51)	159 (28.65)	190 (33.45)	1.30 (0.98, 1.73)	1.17 (0.88, 1.54)	0.94 (0.71, 1.25)
CMMSE registration score <3	13 (1.38)	36 (4.72)	22 (3.96)	24 (4.23)	3.51 (1.49, 8.27)	0.83 (0.44, 1.55)	0.88 (0.46, 1.68)
CMMSE attention/calculation score <5	325 (34.43)	403 (52.89)	214 (38.56)	302 (53.17)	1.63 (1.24, 2.12)	1.08 (0.82, 1.41)	1.28 (0.98, 1.68)
CMMSE delayed recall score <3	326 (34.53)	328 (43.04)	211 (39.82)	256 (45.07)	1.55 (1.19, 2.01)	1.02 (0.79, 1.32)	1.21 (0.93, 1.56)
CMMSE language and praxis score <9	434 (45.97)	512 (67.19)	296 (53.33)	411 (72.36)	2.41 (1.53, 3.80)	1.14 (0.87, 1.50)	1.70 (1.07, 2.69)
Adverse outcomes at year 4–12[b]							
Poor quality of life (SF-12 PCS) at year 4	204 (21.61)	184 (24.15)	152 (27.39)	220 (38.73)	1.82 (1.36, 2.42)	1.69 (1.28, 2.24)	1.65 (1.24, 2.19)
Poor quality of life (SF-12 MCS) at year 4	175 (18.54)	150 (19.69)	118 (21.26)	147 (25.88)	1.08 (0.79, 1.49)	1.36 (1.00, 1.86)	1.19 (0.87, 1.63)
Incident physical limitation at year 4	215 (22.78)	235 (30.84)	178 (32.07)	240 (42.25)	1.91 (1.46, 2.51)	1.65 (1.27, 2.15)	1.30 (0.99, 1.71)
Increased cumulative hospital stay at year 7	166 (15.23)	161 (17.56)	184 (25.52)	197 (25.85)	1.46 (1.09, 1.94)	1.32 (1.00, 1.74)	0.89 (0.68, 1.16)
Mortality over an average of 12 years	153 (14.04)	123 (13.40)	161 (22.33)	171 (22.44)	1.14 (0.83, 1.55)	1.29 (0.95, 1.76)	0.83 (0.62, 1.10)

Data are reported as either number (percentage) or mean ± SD.

[a]Cognitive impairment was defined by a CMMSE language and praxis score of <9.

[b]Analyses were based on valid cases observed for grip strength (n = 2,798), gait speed (n = 2,821), five chair stand (n = 2,789), CMMSE, SF-12 and incident physical limitation (n = 2,829), increased cumulative hospital stay (n = 3,490), and mortality (n = 3,491).

[†]P-values/ORs (95% CI) were obtained from multivariate linear regression/logistic regression adjusting for age, sex, education (below secondary vs. secondary or above), social economic status ladder—Hong Kong (≤4 vs. >4), smoking (current smokers vs. non-current smokers), alcohol intake (>12 vs. ≤12 alcoholic drinks in past 12 m), physical activity (PASE total score), dietary intakes (DQI-I), BMI, and baseline value of respective outcome variable (when appropriate).

CMMSE, Cantonese Mini-Mental Status Examination; DQI-I, Diet Quality Index-International; PASE, Physical Activity Scale of the Elderly; BMI, body mass index.

delayed recall domain score. These findings concur with findings from a recent study, which demonstrated that individuals with cognitive frailty showed worse performance in cognitive function, as assessed by a battery of neuropsychological tests than their cognitively normal peers (41). However, these participants did not have poorer performance in non-memory function, suggesting that memory function may decline first in the pre-frail state, while non-memory cognitive function such as executive function and attention may be more closely associated with frailty, but not pre-frailty (42).

In the present study, participants with pre-frailty and cognitive impairment at baseline had increased risks of poor quality of life, incident physical limitation, increased cumulative hospital stay, and mortality over follow-up, independent of age, sex, educational levels, and other potential cofounders. These findings are compatible with previous findings that a measure of frailty that combines a range of diverse deficits, including cognitive functioning, is a better predictor of adverse health outcomes. For example, in the Three-City Study and the Singapore Longitudinal Ageing Studies (18, 20), including cognitive impairment to the operational criteria defining the frailty phenotype could increase its predictive validity with regard to adverse health outcomes. In a sample of community-dwelling Koreans aged 65 years and older, frail persons with cognitive impairment had a lower survival rate as compared to those non-frail and not cognitively impaired (43).

Although cognitive impairment improves predictive validity of frailty, there is no consensus on how cognitive impairment should be defined, and numerous different criteria exist (e.g., amnestic and non-amnestic cognitive impairment; single-domain and multiple-domain impairment). Cognitive impairment is a transitional state between normal cognition and dementia; thus, varying the threshold used for defining impairment would results in different rates of cognitive impairment. To capture cognitive impairment at a point at which the decline in multiple systems is still occurring in its earliest stages, the early symptoms of cognitive impairment (e.g., memory problems, difficulties in word finding) were tested against multiple-domain cognitive impairment to be used in the criteria for cognitive impairment in terms of their predictive value of adverse outcomes. Our findings demonstrated that lower scores on the two selected *a priori* domains (delayed recall as well as language and praxis) in combinations with pre-frailty at baseline were associated with higher risks of incident physical limitation and increased cumulative hospital stay over follow-up, suggesting that single-domain cognitive impairment may be useful in risk prediction.

Although evidence has shown that multiple-domain amnestic cognitive impairment may be a better predictor of dementia than single-domain amnestic or non-amnestic cognitive impairment (44, 45), those with single-domain cognitive impairment have a relatively high rate of reversion to normal cognition (46). Furthermore, multiple-domain cognitive impairment possibly represents a heterogeneous group of individuals with different neuropsychological profiles; hence subtyping cognitive impairment according to number and types of domains impaired may improve the characterization of the cognitive impairment construct and be useful for risk prediction in relation to different outcomes (47). From the clinical practice point of view, a short screening tool would be important, followed by interventions. Our findings suggest that the use of single cognitive domain may be effective in characterizing cognitive impairment groups; and the use of pre-frailty also identifies a subset of individuals at risk of progressing to frailty. Taken together, the findings of this study together with current available literature of cross-sectional and longitudinal studies lend support to the concept that cognitive frailty may be defined as the existence of overall cognitive impairment (or an individual domain) together with pre-frailty. This definition obviates the need for a psychiatric diagnosis such as the concomitant diagnosis of MCI (as proposed by Kelaiditi et al.) (17), or the need to consider cognitive frailty as a precursor condition of dementia.

There were some limitations in this study. First, the study participation was voluntary which could result in selection bias. Compared to the general elderly population in Hong Kong, the participants may represent those who are more robust, as they tended to be more health conscious, had a higher educational level and more physical active. The other limitation relates to the use of CMMSE for delineation of cognitive status. Due to ceiling effect, it may under-diagnose individuals with early dementia such that these individuals were included in the sample. Similarly, it may under-diagnose cognitive impairment such that some individuals, in particular highly educated individuals, are classified as "no cognitive impairment," (48) albeit the expected effect would be a bias toward the null. Another limitation is the use of *a priori* selected domains and the arbitrary domain scores from CMMSE, which would be expected to be less psychometrically robust compared with domain scores derived from a neuropsychological battery, and may potentially lead to more false-positives among older people with lower educational levels. Another limitation could be represented by the collection of length of hospital stay using the Hong Kong Hospital Authority records that do not cover the 100% of hospitalization in the Hong Kong population. Finally, the incidence of dementia was not available. However, data regarding incident dementia is being collected in an ongoing follow-up study, which allows incident dementia to be related to baseline cognitive frailty.

CONCLUSION

In conclusion, our results showed that robust and cognitively impaired participants had higher risks of becoming pre-frail/frail over a period of 4 years than their counterparts with normal cognition. Furthermore, cognitive impairment improved the predictive power of pre-frailty for poor quality of life, incident physical limitation, increased cumulative hospital stay, and mortality. The findings of this study support to the concept that cognitive frailty may be defined as the occurrence of both cognitive impairment and pre-frailty [as opposed to established frailty as per the IANA/IAGG definition by Kelaiditi et al. (17)], not necessarily progressing to dementia. Our results also showed that lower scores in delayed recall as well as language and praxis, in combinations with pre-frailty, may also be used as criteria for cognitive impairment in terms of their predictive value of adverse outcomes.

ETHICS STATEMENT

All participants gave written consent in accordance with the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

AUTHOR CONTRIBUTIONS

RY, JM, TK, and JW conceived and designed the study and wrote the paper; RY, JL, and OC carried out the analysis.

SUPPLEMENTARY MATERIAL

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REVIEW
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The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates

Claudio Franceschi[1], Paolo Garagnani[2,3,4,5], Cristina Morsiani[2], Maria Conte[2], Aurelia Santoro[2,6], Andrea Grignolio[7], Daniela Monti[8], Miriam Capri[2,6†] and Stefano Salvioli[2,6†]*

[1] Institute of Neurological Sciences, University of Bologna, Bellaria Hospital, Bologna, Italy, [2] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy, [3] Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden, [4] Applied Biomedical Research Center (CRBA), S. Orsola-Malpighi Polyclinic, Bologna, Italy, [5] CNR Institute of Molecular Genetics, Unit of Bologna, Bologna, Italy, [6] Interdepartmental Center "L. Galvani" (CIG), University of Bologna, Bologna, Italy, [7] Unit and Museum of History of Medicine, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy, [8] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

Geroscience, the new interdisciplinary field that aims to understand the relationship between aging and chronic age-related diseases (ARDs) and geriatric syndromes (GSs), is based on epidemiological evidence and experimental data that aging is the major risk factor for such pathologies and assumes that aging and ARDs/GSs share a common set of basic biological mechanisms. A consequence is that the primary target of medicine is to combat aging instead of any single ARD/GSs one by one, as favored by the fragmentation into hundreds of specialties and sub-specialties. If the same molecular and cellular mechanisms underpin both aging and ARDs/GSs, a major question emerges: which is the difference, if any, between aging and ARDs/GSs? The hypothesis that ARDs and GSs such as frailty can be conceptualized as accelerated aging will be discussed by analyzing in particular frailty, sarcopenia, chronic obstructive pulmonary disease, cancer, neurodegenerative diseases such as Alzheimer and Parkinson as well as Down syndrome as an example of progeroid syndrome. According to this integrated view, aging and ARDs/GSs become part of a continuum where precise boundaries do not exist and the two extremes are represented by centenarians, who largely avoided or postponed most ARDs/GSs and are characterized by decelerated aging, and patients who suffered one or more severe ARDs in their 60s, 70s, and 80s and show signs of accelerated aging, respectively. In between these two extremes, there is a continuum of intermediate trajectories representing a sort of gray area. Thus, clinically different, classical ARDs/GSs are, indeed, the result of peculiar combinations of alterations regarding the same, limited set of basic mechanisms shared with the aging process. Whether an individual will follow a trajectory of accelerated or decelerated aging will depend on his/her genetic background interacting lifelong with environmental and lifestyle factors. If ARDs and GSs are manifestations of accelerated aging, it is urgent to identify markers capable of distinguishing between biological and chronological age to identify subjects at higher risk of developing ARDs and GSs. To this aim, we propose the use of DNA methylation, N-glycans profiling, and gut microbiota composition to complement the available disease-specific markers.

Keywords: aging, longevity, age-related diseases, inflammaging, biomarkers, geroscience

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***Correspondence:**
Aurelia Santoro
aurelia.santoro@unibo.it

†*Senior coauthorship.*

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INTRODUCTION: AGING AND PATHOLOGIES SHARE THE SAME COMMON MECHANISMS

The longstanding question if old age is itself a disease has been addressed since ancient times, starting from the Roman playwright Terentius, who claimed "*senectus ipsa est morbus*" (old age itself is a disease), and Cicero who some decades later argued in *De Senectute:* "*pugnandum, tamquam contra morbum sic contra senectutem*" (we have to fight against aging, as we do against a disease). These quotations elegantly summarize a long-held view of aging and old age addressed by several scholars (see Appendix for further details). Notwithstanding, with the birth of modern medicine in the nineteenth century, this old tenet has been somehow put apart, as the main interest at that time was to define precise medical entities (diseases and syndromes) and their causes (infections, genetics, degenerative processes, inflammation, etc.). This process ended up in considering aging and diseases as separate phenomena that could eventually interact but that are essentially different in nature. In this review, we will reappraise and challenge the old tenet that aging and age-related diseases (ARDs) and geriatric syndromes (GSs) are separate entities, and we will suggest instead that both should be considered as parts of a continuum. To support this hypothesis, we will highlight that aging and ARDs/GSs share the same basic molecular and cellular mechanisms.

Aging is the predominant risk factor for most diseases and conditions that limit healthspan. Accordingly, interventions in animal models that end up in an extension of lifespan prevent or delay many chronic diseases. Why? For many years the explanation was that aging *per se* is a physiological condition, which favors the onset of many diseases. However, their relationship is likely much more complex, and a major reason is because they share the basic mechanisms. Assuming that aging and ARDs/GSs share the same mechanisms, which are commonalities and differences? In this review, we will argue that an integrated hypothesis, fitting most epidemiological and experimental data, is to consider ARDs/GSs as an acceleration of the aging process. The conceptualization of accelerated aging started from the observation of rare genetic disorders (1), including Hutchinson–Gilford progeria (2), mandibuloacral dysplasia (3), Werner's syndrome (4), and aneuploidies such as Down syndrome (DS) (5). Here, we extend the concept of acceleration of aging to those members of the general population undergoing ARDs and GSs, in comparison with a small minority of people, such as centenarians, who reach extreme age largely avoiding or postponing most ARDs/GSs. This consideration is reinforced by the observation that among centenarians there are few subjects who never suffered of any overt ARDs. These exceptional individuals can be taken as a proof of principle that "healthy" aging and diseases can occur separately, as phenotypes at the extreme of a continuum, which is fueled by a common set of molecular and cellular mechanisms.

Which are the basic mechanisms shared by aging and ARDs/GSs? A group of international experts identified "seven pillars" which actually include adaptation to stress, loss of proteostasis, stem cell exhaustion, metabolism derangement, macromolecular damage, epigenetic modifications, and inflammation (6). Many chronic diseases and pathological conditions (listed in **Table 1**) are at least in part determined by (some of) these mechanisms, as it will be detailed in the next paragraphs, lending support to this hypothesis.

Following this idea, the very difference between aging and diseases would relay on the rate/speed and intensity of aging cellular and molecular processes, combined with specific organ/systems genetic and lifestyle/habit predisposition. Thus, on the long run, all the functional domains undergo a physiological decline that eventually can lead to overt clinical diseases, favored by organ/system-specific genetic and environmental factors. This progressive path generates a continuum between the healthy juvenile status and the impaired unhealthy elderly one. Accordingly, all major ARDs/GSs are characterized by a long subclinical incubation period, where the diagnostic signs of diseases are largely unobservable due to the high operational redundancy of biological systems. This redundancy, together with the progressive capacity of cells and systems to adapt ("remodeling theory of aging") (31, 32) is capable to buffer the progressive accumulation of molecular damages, thus hampering the availability of objective early diagnostic signs/tools. As an extreme example in neurodegenerative diseases such as Parkinson's disease (PD), it is possible to ascertain advanced anatomopathological alterations in the absence of any specific clinical symptoms in patients died of other diseases.

Accordingly, aging on one side and ARDs/GSs on the other have to be considered different trajectories of the same process but with a different rate depending on diverse genetic background and lifestyle (33–35). Some considerations can help the reasoning on this topic: (i) aging has not been selected during evolution, and no gerontogene has been identified so far, i.e., no gene has been apparently selected with the precise purpose to trigger/cause the aging phenomenon, thus leaving a large space for stochasticity (36); (ii) genetics and environment interact with each other to determine the eventual phenotype. These two considerations can explain (a large part of) the heterogeneity of phenotypes observed in aged persons. Actually, the primary aim of a gene (or group of genes) is always devoted to increase the survival or reproductive fitness of the organism, and aging could be an unpredicted byproduct of its basic function. Following this idea, some years ago Mikhail Blagosklonny and Michael Hall proposed that aging could be conceptualized as a sort of dysregulated continuation of the normal developmental process and related cellular "programs," with particular emphasis on mTOR-driven growth (37, 38). According to this theory, overactivation of signal transduction pathways and exacerbation of normal cellular functions such as growth, leading to alteration of homeostasis, malfunction, and organ damage are likely the driving forces of the aging process including the onset of ARDs. This theory complements the "inflammaging" theory of aging (39). Inflammation is among the aforementioned "seven pillars," and inflammaging is defined as the chronic, low-grade (subclinical) and sterile inflammation that is observed in old persons. It is caused by increased stimulation of innate immune system by "non-self" (persistent infections), "self" (cell debris, nucleic acids, glycated proteins, etc.), and "quasi-self" [gut microbiome (GM)] components of our

TABLE 1 | Age-related pathologies and molecular relationship with aging.

Age-related pathology	Mechanisms shared with aging process	Markers	References/ reviews
Alzheimer's disease	Inflammation Oxidative stress Mitochondrial dysfunction Decreased proteasome activity Cellular senescence Gut microbiota alterations	IL-6, TNF-α, IL-1β, TGFβ, IL-12, IL-18, and INFγ 8-hydroxyguanosine, 8-hydroxy-2′-deoxyguanosine, oxidized proteins, and lipid peroxidation 20S core reduced activity Presence of senescent cells Activation of pro-inflammatory cytokines, increased intestinal permeability	(7) (8) (9) (10) (11) (12)
Cancer	Inflammation Cellular senescence	IL-6; presence of senescent cells	(13–16)
Chronic obstructive pulmonary disease	Telomere shortening Oxidative stress Cellular senescence Inflammation, inflammasome; activation of NLRP3 Activation of PI3 kinase–mTOR signal Dysregulated nutrients sensing; loss of proteostasis autophagy mitochondrial dysfunction Stem cell exhaustion	p21CIP1/WAF1, p16INK4a, β galactosidase activity, and senescence-associated secretory phenotype IL-1β, IL-6, IL-18, chemokines (CXCL8 and CCL2), metalloproteinases Stress markers such as Parkin and phosphatase and tensin homolog-induced protein kinase 1	(17, 18)
Maculopathy	Chronic retinal inflammation, dysregulation of autophagy, accumulation of oxidative stress-induced damage, protein aggregation, and lipofuscinogenesis	Heat shock proteins; Abs vs self-epitopes; and inflammasome activation	(19, 20)
Osteoarthritis	Cell disruption; cellular senescence; mitochondrial dysfunction and oxidative stress; and reduced autophagy; inflammation	HGMB1; HGMB2; and IL-8	(21)
Osteopenia/ osteoporosis	Chronic inflammation	TNF-α; IL-6; CRP; and inflammatory markers	(22)
Parkinson's disease	Inflammation Cellular senescence Gut microbiota alterations	Presence of inflammatory cells (astrocytes) and senescent cells Activation of pro-inflammatory cytokines, increased intestinal permeability, and alteration of the serotonin system	(23) (24) (25)
Periodontitis	Inflammation	*Porphyromonas gingivalis* express peptidylarginine deiminase generating citrullinated epitopes Pro-inflammatory cytokines	(26)
Rheumatoid arthritis	Cell death and chronic inflammation	Abs vs modified self-epitopes; HGMB1 Matrix metalloproteinases TNF-α; IL-1β; and IL-6	(27)
Sarcopenia	Inflammation and oxidative stress	Elevated levels of TNF-α, IL-6, IL-1, and CRP	(28–30)

body as a meta-organism, and by accumulation of senescent cells characterized by a pro-inflammatory secretory profile (40, 41). Thus, both the overactivation and inflammaging theories agree that programs selected for development and survival (inflammation) can turn detrimental when continue to be active unabated for a period time longer than that predicted by evolution. The same can apply for other programs of the abovementioned "seven pillars."

AGE-RELATED PATHOLOGIES

In this paragraph, we will discuss the involvement of some molecular mechanisms known to cause aging in a number of ARDs/GSs, in particular frailty and sarcopenia, chronic obstructive pulmonary disease (COPD), cancer, and Alzheimer and Parkinson diseases. We will also discuss the manifestations of premature senescence of a genetic syndrome, such as DS, which are present also in normal aging but occur much earlier at the level of immune and nervous system in DS. Cardiovascular diseases and type 2 diabetes are also very important pathologies that affect millions of patients and do share molecular mechanisms with aging, including inflammation and oxidative stress, but for reasons of space limitations, a detailed discussion of these pathologies will be skipped out.

Frailty Syndrome

Typical GSs include frailty, mild cognitive impairment, and metabolic syndrome. In particular, frailty is described as a multidimensional syndrome of the elderly characterized by a loss of physiological reserves, poor response to, and recovery from (even mild) stress. This condition leads to an increased vulnerability to a wide range of adverse health outcomes and is associated with increased morbidity and mortality. However, frailty is peculiar as it displays a wide spectrum of phenotypes depending on the criteria that are considered for its definition, as well as the age range of the subjects studied. To this regard, both clinicians and researchers are becoming more and more aware of the considerable ambiguity around the concept of frailty. Conflicting ideas

have proliferated on the definition of frailty, what criteria should be used for its recognition, and its molecular relationships with aging, disability, and chronic diseases (42).

In the 2001, Fried et al. elaborated five criteria to define frailty, i.e., unintentional weight loss, poor hand grip, slow speed gate, feelings of exhaustion and low physical activity. Persons (usually older than 65 years) meeting three or more out of these five criteria are classified as frail and have an increased risk of incident falls, worsening mobility or ADL disability, hospitalization, and death in the following 3 years. Intermediate frailty status or pre-frail condition, as indicated by the presence of one or two criteria, showed intermediate risk of these outcomes (43). During the last decade, several frailty-rating scales have been developed to detect and screen the level of frailty, such as the Frailty Risk Index (44–46). An impressive amount of literature has been published suggesting that a complex network of clinical signs produce a large spectrum of frailty conditions and phenotypes (47) with different risk index of mortality after 3–5 years of follow-up. Surprisingly, the condition of frailty may be reverted and subjects can return to a non-frail condition, for example, when specific pathologies are cured, or personalized interventions in terms of physical exercise, with or without nutrition supplementation, are applied (48, 49).

This quite peculiar condition deserves some assumptions, such as that frail is an epiphenomenon and "etiology" may be quite different in the population, also depending on the possible overlapping with sarcopenia, i.e., the age-related loss of muscle mass and strength that will be discussed later. Actually, many signs of frailty are related to sarcopenia, and if both conditions are present in the same individual, they favor the state of vulnerability, increasing the risk of negative health outcomes. Nevertheless, a low number of studies have assessed the coexistence of these two entities in the same cohort of older people.

Recently, the Toledo Study of Healthy Aging (a study of 65+ community-dwelling elderly) including 1,611 participants with frailty and sarcopenia assessments indicated that the prevalence of frailty (assessed by Fried's criteria) among those with sarcopenia was from 8.2 up to 15.7%, depending on the different criteria for sarcopenia assessment. Moreover, among frail people, the prevalence of sarcopenia was from 40.27 up to 72.2%, according to the used criteria. Sarcopenia showed a low sensitivity but high specificity for the diagnosis of frailty thus suggesting that frailty and sarcopenia are distinct but interrelated conditions (50).

Furthermore, the molecular mechanisms underpinning frailty syndrome are still not completely clarified even if many data suggest a tight relationship with inflammatory status and immunosenescence which are also shared in sarcopenia (30), as it will described below. Likely, both frailty and sarcopenia contribute to further development of morbidities. Importantly, the role of inflammaging to the frailty syndrome onset is still an open question (51), and further studies are needed to clarify the causality between chronic low-grade inflammation and development of frailty, as well as the conditions/treatments that make possible the reversibility of the frailty status. It is important to note that Fried and Ferrucci (52) were the first to elaborate the concept of frailty as a syndrome of "accelerated aging" and to note that clinical frailty is associated with the presence of multiple chronic diseases. In turn, the risk to become frail increases with the number of such diseases present, thus reinforcing the idea of a continuum between health, diseases, and comorbidity. To this regard, a multidimensional approach allows a more robust interpretation of the various relationships among the pro- and anti-inflammatory markers and inflammaging (53), and likely an important contribution could be obtained by introducing also frailty risk and mortality indexes in a context of a complex dynamical network (54) to better disentangle those clustering factors that may accelerate aging.

Sarcopenia

One of the most pervasive and macroscopic phenomena occurring with aging is the progressive decline of skeletal muscle mass, strength, and function, leading to a condition indicated as sarcopenia. Sarcopenia is associated with a reduced quality of life in older adults, and it is considered as a key risk factor for negative health outcomes associated with disability, frailty, loss of independence, morbidity, and mortality (55, 56). Several factors are involved in the pathophysiology of sarcopenia; however, its etiology is still unclear. The more recent evidence suggests that the onset and progression of sarcopenia depend on a combination of mechanisms that alter the normal physiology of skeletal muscle, some of them being considered also as key driver of the aging process. Among the mechanisms that participate to the pathogenesis of sarcopenia there are endocrine changes, loss of regenerative capacity, muscle fiber denervation, increased deposition of intermuscular and intramuscular fat, mitochondrial dysfunction, oxidative stress, and inflammation (57, 58). These two latter mechanisms in particular are involved also in the aging process. Loss of regenerative capacity of satellite cells (the stem cells of the muscle) is another feature shared with aging. However, this mechanism, although important *in vivo*, has been put under scrutiny and will not be further discussed here. In fact, satellite cells from old muscles display a proliferative capacity similar to those derived from young muscles, if cultured in an appropriate medium enriched with plasma from young donors (59, 60), therefore casting some doubts on the fact that satellite cells from old muscle are actually defective or exhausted. On the contrary, these data suggest that stemness as well as other features of satellite cells are strongly dependent on the environmental context (namely but not exclusively soluble factors) and are therefore cell independent.

Actually, emerging epidemiological and molecular studies indicate that immunosenescence and inflammaging strongly contribute to the pathophysiology of sarcopenia (30, 61). The age-related changes in the cells of the innate immune system indirectly contribute to sarcopenia by an increase of systemic inflammation. In physiological conditions, in response to damage, neutrophils migrate in skeletal muscle, followed by M1 macrophages that lead to muscle inflammation. This early phase is followed by infiltration of M2 macrophages that produce soluble factors that repair the muscle injury and promote regeneration (62). With aging, the activity of neutrophils decreases, especially in terms of migration capacity. It has been hypothesized that, once in the muscle, neutrophils with impaired migration capacity can contribute to increased inflammation (30).

The incomplete muscle recovery is associated with an increase of pro-inflammatory cytokines and a prolonged inflammatory response to muscle injury that causes muscle atrophy and weakness (28).

Regarding systemic inflammation a possible involvement in sarcopenia has been proposed and depends upon the degree of intensity of inflammation. A comparative analysis of skeletal muscle alteration at different ages from four species, i.e., mice, rats, rhesus monkeys, and humans, revealed not only a conserved age-dependent decrease in mitochondrial content, and a reduction in oxidative phosphorylation complexes in monkeys and humans but also a human-specific age-related increase of phosphorylated NF-κB (63). Actually, a moderate inflammation is beneficial and fundamental to activate a response to a stress, but when the inflammation becomes chronic and more elevated, the response to muscle injury turns detrimental. In other words, a mild level of systemic inflammation present in physiological aging may not affect the loss of muscle mass or strength, but only the metabolic quality of skeletal muscle; conversely a more severe systemic inflammation (often accompanied by a local inflammation) present in a condition of accelerated aging, contributes to the loss of muscle mass and strength and the progression of sarcopenia (30). Although the molecular mechanisms associated with inflammaging and the loss of skeletal muscle mass are not yet totally understood, studies revealed that inflammaging contributes to the genesis of sarcopenia by affecting the balance between muscle anabolic and catabolic processes (64). In particular, elevated levels of TNF-α, IL-6, IL-1, and CRP favor muscle protein breakdown and inhibit protein synthesis through the activation of NF-κB and ubiquitin–proteasome pathway. This shift toward catabolic process then culminates in myofiber proteolysis, atrophy and loss in regenerative ability that leads to skeletal muscle functional decline (29, 30). Emerging evidence indicates that the progression of sarcopenia is also amplified by a self-sustaining loop between immunosenescence, inflammaging, and oxidative stress (58, 61). There is in fact a close interconnection and/or overlapping between the molecular pathways of inflammation and those of oxidative stress in the generation of reactive oxygen species (ROS). These species have pathological consequences for the health of human body not only associated with the development of sarcopenia but also a number of other ARDs, including typical age-related endocrine dysfunctions such as decreased pancreatic β-cell function and thyroid autoimmunity, among others (65). An uncontrolled accumulation of oxidative stress and inflammation may act as a bridge between normal aging and accelerated aging. In conditions of accelerated aging, muscle weakness is often accompanied by other pathophysiological features, such as low bone density and increased fat mass, thus leading to osteoporosis and obesity. All these disorders have been recently indicated with the term "osteosarcopenic obesity" (66), and, as mentioned earlier, they can be listed among the determinants of frailty (30, 67).

Chronic Obstructive Pulmonary Disease

Aging is one of major risk factors for many chronic inflammatory diseases, e.g., diabetes, CVD, atherosclerosis, dementia, cancer, and others including COPD, and can impact differently on organs and tissues affecting their functions and structure (68).

Aging of the lung is characterized by reduction of function, pulmonary inflammation, increased gas trapping, loss of lung elastic recoil and enlargement of the distal air space. These pathological signs are slowly progressive and are also pathognomonic of COPD. In fact, the overall increase in COPD is probably related to the aging of the population, as this disease predominantly affects the elderly, with the peak of prevalence at about 65 years (69, 70). COPD is an obstructive lung disease characterized by long-term breathing problems, poor airflow, and destruction of the lung parenchyma (emphysema) (71). The main cause of COPD in industrialized countries is smoking but is also present in underdeveloped countries as a result of exposure to household air pollution, poor nutrition, and damp housing conditions (72–74). The slowly progressive airway obstruction of COPD and in particular the emphysema could represent an acceleration of the normal decline of lung function with age (75, 76).

Recent and extensive studies (18, 77) have pointed out that in COPD are present many of the hallmark of aging, e.g., telomere shortening, activation of PI3 kinase–mTOR signaling, altered autophagy, mitochondrial dysfunction, stem cell exhaustion, as well as a low-grade inflammation and cellular and immune senescence.

Telomere attrition leading to cellular senescence (replicative senescence) or cell death, have been described in leukocytes from patients with COPD in comparison with control subjects in any age range (78). Moreover, parenchymal lung cells of emphysematous patients display shorter telomeres associated with cell senescence and inflammation (79, 80). In a meta-analysis of 14 studies, a significant negative association between telomere length and COPD has been observed (81). This telomere shortening in COPD could be due to an augmented oxidative stress from cigarette smoke that activates p21, leading to cellular senescence and increased release of pro-inflammatory cytokines (79). Cell senescence in COPD is evident by the enhanced expression of senescence markers such as p21CIP1/WAF1, p16INK4a, and senescence-associated β-galactosidase activity in lung cells (82). Lung macrophages from COPD patients can also express senescence markers (18). Furthermore, in COPD there is an increased expression of components of the secretory profile of senescent cells, defined as senescence-associated secretory phenotype (SASP), including pro-inflammatory cytokines (IL-1 and IL-6), chemokines (CXCL8 and CCL2), and matrix metalloproteinase (MMP) 9 (18). As mentioned, SASP, in association with immunosenescence, is a key determinant of inflammaging that have a negative impact in the neighboring lung tissue and, as discussed, probably also in the whole organism (17).

The immunosenescence of both innate and adaptive immune cells and the consequent inflammaging might play a role in COPD development and progression. Recently, it has been demonstrated in aged mice exposed to chronic cigarette smoke, that activation of immune system and inflammaging contribute to the accelerated pathogenesis of emphysema, the increased chronic lung tissue inflammation due to the increased production of inflammatory mediators and this promotes the onset of COPD (83).

The mTOR pathway has an important role in cellular senescence and aging. In fact, an inhibition of this pathway extends the lifespan of many species (84). The activation of PI3 kinase–mTOR signaling pathway has been demonstrated in epithelial cells from the lungs of patients with COPD. The activation of the IGF-1/AKT/mTOR pathway suppresses autophagy, but it also counteracts activation of FOXO transcription factors, which are central regulators of metabolism, cell-cycle progression and programmed cell death (85). A diminished expression of FOXO3 protein has been demonstrated in the lungs of smokers and patients with COPD suggesting that dysregulated nutrient sensing, together with loss of proteostasis, may contribute to the pathogenesis of COPD (85, 86). Two central mechanisms are involved in proteostasis to degrade and remove the misfolded or damaged proteins, i.e., autophagy–lysosome system and the ubiquitin–proteasome system. The impairment of these pathways characterizes numerous ARDs but also the aging process itself (87, 88). A large amount of data indicate that the mechanisms involved in homeostasis and proteostasis collapse with advancing age, favoring the accumulation of the unfolded, misfolded, or aggregated proteins (89). The decline of ubiquitin–proteasome system during aging may be due to various alterations including decreased expression of proteasome subunits and insufficient or inappropriate assembly; reduction of proteasome function due to decreased ATP availability from mitochondrial malfunction. An increase of inducible subunits has been demonstrated as consequence of the abovementioned alterations in many tissue and organs (e.g., the skeletal muscle). This induction could be a compensatory mechanism altering the balance between constitutive proteasomes and immunoproteasomes and an effect of inflammaging (90).

A decline in proteasome activities has also been reported in human senescent fibroblasts (91). Conversely, the fibroblasts from centenarians, a group of individuals who have gone through the aging process successfully because they maintain their good mental and physical shape, show levels of proteasome activities, oxidized proteins, and RNA and protein expression of several proteasome subunits similar to the levels found in cultures obtained from young donor. Consequently, maintenance of proteasome function in centenarians has been suggested to be an important factor for their successful aging (92). Collapse of the mechanisms that lead to failure of proteostasis may have detrimental consequences for organisms. For example, failure of the proteasomal system has been linked to several pathologies, including neurodegenerative diseases (e.g., Alzheimer's; Parkinson's; and Huntington's), cardiovascular diseases (e.g., atherosclerosis), immune system-associated diseases [e.g., rheumatoid arthritis (RA)], skin aging, cancer, and COPD, among others (93). In COPD, the oxidative stress induced by cigarette smoke can alter the proteins such as histone deacetylases contributing to their inefficient degradation by proteasome system or by autophagy (94, 95). Proteasome activity is decreased in patients with COPD and correlates inversely with the loss of lung function (96). Moreover, alveolar macrophages from cigarette smokers showed defective autophagy that could contribute to the accumulation of damaged proteins, abnormal mitochondrial function, and defective clearance of bacteria (97). There is evidence of increased markers of autophagy in lung tissue from patients with emphysema, suggesting that autophagy may be contributory to the apoptosis and alveolar destruction in emphysema (96). As abovementioned autophagy is also impaired through the activation of phosphoinositide 3-kinase–mTOR signaling in COPD (98) and may contribute to defective phagocytosis of bacteria in COPD (99).

Mitochondrial dysfunction is also present in COPD. In particular, an increased mitochondrial ROS production and a reduced number of mitochondria are typical features of the disease (100). The airway epithelial cells from smokers display an altered mitochondrial structure and function (101), and actually markers of mitochondrial stress such as increased expression of Parkin, phosphatase, and tensin homolog–induced protein kinase 1 are present in epithelial cells from patients with COPD (102). These changes in epithelial cells are accompanied by an augment in pro-inflammatory cytokines secretion such as IL-1β, IL-6, and CXCL8 (101). Mitochondrial alterations and ROS production can induce the NLRP3 inflammasome, which stimulates IL-1β and IL-18 secretion in chronic inflammatory diseases. The transcription factor peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α is a critical regulator of mitochondrial biogenesis and the generation of mitochondrial ROS. It is increased in epithelial cells of mild COPD patients but progressively reduced with increasing COPD severity (103).

Finally, stem cell exhaustion, typical of aging process, is also present in COPD. The basal progenitor cells required for air way epithelial differentiation actually display a reduced regenerative capacity in COPD patients (104).

Cancer

Many types of cancer are essentially ARDs, as their frequency dramatically increases with age, and age represents the single most powerful risk factor for cancer to occur. This phenomenon is likely not linked to a decreased efficiency of DNA mutation checkpoint and repair. Conversely, a growing amount of evidence suggests that the increasing number of transforming mutations occurring in old subjects is fostered by a much more permissive environment that allows DNA damage to occur and, probably most important, allows transformed cells to progress into malignancy and metastatization. The main feature of such a permissive environment is likely the presence of an elevated level of pro-inflammatory stimuli, either related to the immune response to cancer or independent from it. Actually multiple lines of evidence indicate that immune inflammatory cells can actively promote tumor growth, as such cells are capable of fostering angiogenesis, cancer cell proliferation, and invasiveness (16). Therefore, a positive response aimed at counteracting cancer has the paradoxical effect of promoting tumor growth, invasion, and metastasis (105–108). Importantly, inflammation is in some cases evident at the earliest stages of neoplastic progression and is demonstrably capable of fostering the development of incipient neoplasias into full-blown cancers (107, 109), as inflammatory cells can release ROS that are actively mutagenic for nearby cancer cells, accelerating their genetic evolution toward heightened malignancy (106). Stressed or necrotic cells can be the source of molecules that can attract inflammatory cells leading to the abovementioned

promoting effects on the tumor, as seen, for example, in melanoma, where exposure to UV light leads to the release of HMGB1 protein from keratinocytes, that in turn attracts and activates neutrophils and induces the production of angiogenetic factors (110). The same effects can be obtained even in the absence of an infiltration of inflammatory cells, granted that other cells can sustain the production of the same array of pro-inflammatory mediators. This is the case when senescent cells accumulate in a tissue. Cell senescence is an effective mechanism to halt neoplastic transformation, as cells with damaged DNA can enter cell senescence and stop proliferating. However, as mentioned, senescent cells are characterized by a pro-inflammatory secretory phenotype (SASP) (111) that includes metalloproteinases and angiogenetic factors. Many of these factors can contribute to the acquiring of malignant and metastatic features of cancer cells (112). Therefore, the occurrence of an antineoplastic mechanism can paradoxically end up in fostering the neoplastic transformation of premalignant cells through SASP (13, 14). Actually, it is known that aging is characterized by accumulation of senescent cells, due to either inefficient clearance or increased number of cells undergoing this process, and, accordingly, SASP is considered a main driver of inflammaging. SASP, in turn, can ignite DNA damage response and synthesis of pro-inflammatory cytokines in surrounding cells in a self-amplifying loop, leading to the proposal that inflammaging can be a substantial driver of the increase in cancer incidence and progression observed in aged people (113).

The phenomenon of inflammaging (at the level of stem cell niche) can be therefore a risk factor for cancer development and, since inflammaging increases with age, this would account for association between cancer and old age. This can be exemplified by the case of myeloproliferative neoplasms (MPNs), which are acquired age-associated clonal disorders of the hematopoietic stem/progenitor cells (HSPCs). MPNs are characterized by a state of chronic inflammation due to the continuous release of inflammatory products from *in vivo* activated leukocytes. This state of chronic inflammation (or inflammaging) affects both the malignant HSPCs and the non-malignant/malignant microenvironment, likely being the main contributor in MPNs initiation/clonal evolution (114, 115). This inflammatory microenvironment is a key factor in MPNs pathogenesis, since strong evidences suggest that stromal cells are primed by the malignant hematopoietic clone, which, in turn, conditions the stroma to create a favorable microenvironment that nurtures and protects the malignant cells (116).

Among the classical component of inflammaging, IL-6 occupies a prominent place. It has been demonstrated that IL-6 drives the progression toward the acquisition of a malignant phenotype of cancer cells (15) and that the blockade of IL-6 signaling has strong effects *in vivo* on tumor progression, interfering broadly with tumor-supportive stromal functions, including angiogenesis, fibroblast infiltration, and myeloid suppressor cell recruitment in both the tumor and premetastatic niche (117).

As a whole, it is widely accepted that inflammation and cancer are strictly connected and that inflammation is involved in cancer onset and progression. Inflammaging seems to be an almost universal feature of human aging, so it can be hypothesized that if a subject could live long enough, the effect of inflammaging on his/her probability to get cancer would become very important. Similarly, it can be reasoned that a person who got cancer at 60 years of age is comparable (as far as inflammaging is concerned) to a much older person, thus it could be speculated that cancer is to a certain extent a consequence of an accelerated aging process. To further support this hypothesis, it is known that many syndromes of premature, accelerated aging like Werner syndrome and ataxia telangiectasia are also characterized by increased frequency of malignancies (118, 119). On the other side, centenarians (who can be considered to be biologically younger than their chronological age) rarely die by cancer (120). Of course many factors concur in malignant transformation other than inflammation; however, this fascinating hypothesis certainly deserves further investigations.

Neurodegenerative Diseases

Alzheimer's disease (AD) and PD are the most common neurodegenerative diseases in the world (121). These diseases are age-associated and most often have a long prodromic phase preceding the clinical manifestation with a subsequent stage of progression leading to signs of dementia with similar symptoms such as memory impairment, orientation problems, and difficulties in performing service functions among others. AD and PD are referred to as "protein misfolding" diseases because deposits of improperly folded modified proteins are detected in specific areas of the brain (122–124). In the case of AD, these deposits contain β-amyloid proteins and hyperphosphorylated tau protein (tau-P), which, respectively, form extracellular plaques and intracellular fibrillar tangles (125). In the case of PD, the deposits—called Lewy bodies—are formed by the accumulation of α-synuclein protein in dopaminergic neurons mainly of the *substantia nigra*, as well as in other regions of the brain (126). In both AD and PD, neurodegeneration processes are generally accompanied by neuroinflammation (127).

Alzheimer's Disease

The clear diagnosis of AD is made only postmortem, and no effective disease-modifying therapy exists at the moment (128). On living patients, AD is diagnosed by a combination of cognitive tests and neurobiological markers [brain imaging, decreased amyloid beta Abeta42 (Ab) level and/or increased total and hyperphosphorylated tau-P in cerebral spinal fluid] (129). These tissue changes precede the onset of clinical signs by several years, implying that AD neuropathological lesions may be found in a subset of cognitively normal elderly persons (130). This suggests that (i) although senile amyloid beta (Abeta) plaques play a role in the AD dementia, the scenario is more complex and other (major) drivers are also involved; (ii) there is a *continuum* between neurodegenerative AD dementia and the dementia-free brain aging. The limits of the current conceptualization on AD pathogenesis and of the amyloid cascade hypothesis are well summarized by two Nature Neuroscience papers released in 2015 (131, 132). In fact, many other potentially harmful phenomena take place in AD pathogenesis, some of them being shared with the aging process, such as oxidative stress, mitochondrial dysfunction, neuroinflammation,

decrease in proteasome activity (10) and deregulation of basic mechanisms of cell functioning (autophagy and DNA damage response). In many cases, these phenomena are not immediately connected to Abeta deposition and neurofibrillary changes. In particular, neuroinflammation in AD involves not only resident cells (microglia, astrocytes, and neurons) but also cells and soluble factors of the peripheral immune system that can enter into the brain (133). To this regard, inflammaging can stimulate the development of neuroinflammation and neurodegeneration (134, 135). This effect is due to soluble mediators that can enter the blood–brain barrier, essentially cytokines, whose network can be deranged in AD (136), therefore the assessment of peripheral inflammatory markers should be considered in the monitoring of the efficacy of therapeutic approaches. A meta-analysis demonstrated that increased serum levels of IL-6, TNF-α, IL-1β, TGFβ, IL-12, IL-18, and IFNγ characterize AD (7). Interestingly, IL-6 is capable of entering the blood–brain barrier and has a role in memory consolidation (137). The pro-inflammatory cytokines IL-1β and TNF-α exert variable (inhibiting or supporting) synapse-specific effects on long-term potentiation maintenance (138). It was also shown that IL-1β and TNF-α in combination with IFNγ can exacerbate the pathology in AD due to alterations of the β-amyloid precursor protein (βAPP) metabolism resulting in triggering the production of β-amyloid peptides (139, 140).

The balance of antioxidant and oxidant system activity is deranged in cells affected by AD. Elevated levels of oxidative stress markers are also present in mitochondria isolated from peripheral lymphocytes of AD patients (141). Mitochondrial DNA (mtDNA) inherited mutations have also been associated with AD onset (142). AD patients are characterized by significant increases in blood cells of markers of oxidative stress for both RNA (8-hydroxyguanosine) (8) and DNA (8-hydroxy-2′-deoxyguanosine), together with a considerable decrease in antioxidant defense (9, 143, 144). Moreover, high levels of oxidized proteins and of products of lipid peroxidation are also found. In particular, a significant increase in the degree of lipoprotein oxidation was observed in the peripheral blood of AD patients (145). Neutrophils are the main source of ROS production in the sites of inflammation. A possible participation of neutrophils in the development of AD has been demonstrated (146). Oxidative stress in neurons it is also able to produce a DNA damage response that in turn leads to apoptosis or cellular senescence (11). A potential contributor to age-related inflammation in the brain can then be cellular senescence, likely occurring in replication-competent glial cells. Recent studies from several laboratories suggest that senescent cells are detectable in the mammalian brain, where they could contribute to neurodegenerative processes with their pro-inflammatory SASP and/or disrupting cell–cell contacts needed for the structural and functional neuron–glial interaction that maintains neuronal ionic and metabolic homeostasis (147, 148). Senescent markers were recently reported to be present in astrocytes of autopsied human brain tissue; both p16INK4a and the SASP factor MMP3 increased significantly with age and were even higher in affected cortical brain tissues from AD patients compared with age-matched non-demented controls (149).

Gut and brain are deeply interconnected through the gut–brain axis (150). Inputs from the CNS can modify gut functions, while inputs from gut to CNS can modulate specific symptoms (151). Alterations of these bidirectional communications may contribute to neuroinflammation and the pathogenesis of CNS disorders (152). In particular, alterations of GM can activate pro-inflammatory cytokines and increase intestinal permeability, leading to the development of insulin resistance, which has also been associated with AD (12). In addition, bacteria of GM are known to excrete immunogenic mixtures of amyloids, lipopolysaccharides, and other microbial exudates into their surrounding environment (153, 154). Bacterial amyloids might activate signaling pathways known to play a role in neurodegeneration and AD pathogenesis, while GM might enhance inflammatory responses to cerebral accumulation of Ab (155). It is also interesting to mention that beside gut microbiota, the oral microbiota is involved in several pathologies including AD. Aging may favor the proliferation of anaerobes in the mouth eliciting a robust TNF-α response by the oral epithelium (156). In AD brains, a sevenfold higher presence of anaerobe oral bacteria compared with cognitively normal controls has been found (157). The causal link between bacteria and AD-like neurodegeneration has been further illustrated in a mouse model (158).

Parkinson's Disease

Parkinson's disease is caused by the selective loss of neurons of the substantia nigra due to improper accumulation of α-synuclein protein leading to motor alterations. Despite this apparently very specific cause, PD actually shares some feature with normal aging and could be considered a segmental accelerated aging that affects specific neurons in the brain and in many other anatomical sites. First of all, features of PD are found also in elderly without clinical sign of PD (159). A study on 2,500 old persons annually assessed for Parkinsonism showed that 744 of these subjects deceased without diagnosed PD (mean age at death: 88.5 years): (i) about one-third of cases had mild or more severe nigral neuronal loss; (ii) about 17% had Lewy bodies; and (iii) 10% of the brains showed both nigral neuronal loss and Lewy bodies (160). Thus, also in this condition there is an apparent continuum between physiological aging and neurodegenerative age-related motor disorders.

Recent data indicate that aging and PD share basic characteristics such as accumulation of senescent cells, inflammation, and propagation phenomena. It has been reported that senescent and inflammatory cells (astrocytes) are present in the brain of PD patients (23) and a "transmission hypothesis" has been proposed regarding the pathogenesis of "PD as a prion disease" (161) where intercellular transmission of pathological protein aggregates (α-synuclein) occurs, causing a prion-like spreading of neuronal damage and neuroinflammation (162, 163). Aggregated α-synuclein, released by neuronal degeneration, acts as an endogenous trigger inducing a strong inflammatory response in PD (164). Similar propagation phenomena have been described for beta-amyloid and Alzheimer's diseases (165).

Increasing evidence suggests that PD should be included on the growing list of diseases associated with vitamin D insufficiency and that we should routinely monitor vitamin D levels in

patients with PD (166). One of the most advanced and appealing hypotheses is that environmental stressors may contribute to age-related neurodegeneration by favoring cell senescence of glia, thus creating a chronically inflamed milieu in the brain (167). From this point of view it is important to note that a bidirectional axis between the brain and the GM does exist, and, importantly, GM is involved in the production of various neurotransmitters (serotonin, dopamine, noradrenaline, and GABA), and in the modulation of various behavioral and CNS functions (168, 169). Recent studies showed that PD is associated with gut dysbiosis (24, 170), the fecal concentration of short-chain fatty acids is significantly reduced in PD patients compared with controls, and this reduction could impact on CNS alterations and contribute to gastrointestinal dysmobility in PD (171). In a mouse model of PD, it has been demonstrated that GM is key player in motor deficits and microglia activation (172).

On the basis of the profound even if still unclear relationship between aging and PD, these data on PD microbiome should be interpreted on the background of the changes that occur in the GM during healthy aging. It has been recently showed that the GM undergoes profound changes with age (173), which likely contribute to inflammaging (174) and can have profound effects on the brain, owing to the increased abundance with age of bacteria involved in the tryptophan metabolism pathway (175), in agreement with the reduction of tryptophan (a precursor of serotonin) found in the serum of centenarians (32, 176). Accumulating evidence shows that the age-related dysbiosis is involved in the neurological decline and promotes inflammaging (177) that play a pivotal role in both the physiological and the pathological cognitive decline (25). The GM contributes to the regulation of the brain function modulating the metabolism of tryptophan, an essential amino acid derived from the diet that is able to cross the blood–brain barrier contributing to the synthesis of the serotonin in the central nervous system (25). The age-related changes are more evident in the amygdala, hippocampus, and frontal cortex. The function of these brain areas is strongly dependent from the serotonergic neurotransmission and thus involving the changes in the tryptophan GM-dependent metabolism. Alterations in the serotonin system could represent the common denominator of the alterations of the sleep, mood, and sexual conduction often observed in elderly as well as of other modifications such as diabetes and cardiovascular diseases (25). Tryptophan is also metabolized *via* the kynurenine pathway (KP), which can lead to the production of nicotinamide adenosine dinucleotide (NAD+) (168), as well as quinolic and kynurenic acid. These latter compounds are neuroactive metabolites that act on *N*-methyl-D-aspartate (NMDA) and alpha 7 nicotinic acetylcholine receptors in CNS and ENS. In the CNS, kynurenic acid has been long viewed as neuroprotective, while quinolinic acid is primarily considered an excitotoxic NMDA receptor agonist (178).

Alterations of the KP have been assessed in PD (as well as other neurodegenerative diseases). PD patients have higher L-kynurenine/tryptophan ratios in serum and CSF as compared with controls, suggesting upregulated activity of enzymes involved in catabolizing tryptophan to kynurenine [i.e., indoleamine-2, 3-di-oxygenase and tryptophan 2,3-dioxygenase]. Levels of 3-hydroxykynurenine have also been found to be increased in the putamen, prefrontal cortex, and substantia nigra pars compacta in PD patients (179).

Despite the fact that periodontal diseases resulted associated with PD, few data are present on the role of oral microbiota in PD. A recent paper showed that oral microbiota of PD patients differs from those of control subjects as assessed through beta diversity and differential abundance analyses. Differences were also detected between sexes, with a higher abundance of taxa that include opportunistic oral pathogens in males (180).

Other Pathologies: RA, Osteoarthritis (OA), Osteopenia, and Macular Degeneration

It is well known that chronic inflammatory (or autoimmune) diseases, such as RA, psoriasis, ankylosing spondylitis, OA, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel diseases, and pemphigus vulgaris among others, share an inflammatory component highly depending on immune system activation, self-epitopes, environment-associated variables, and genetic makeup. In this review, we focus on osteoarticular pathologies and macular degeneration since an impressive amount of data is recently emerged. These data converge on the chronic inflammatory process, which drives the evolution of the disease as a continuum. Among osteoarticular pathologies, elderly onset RA usually develops in persons older than 60–65 years of age. Main actors involved in the RA development are activated T/B cells, macrophages, and fibroblasts producing pro-inflammatory cytokines that play a key role in synovitis and tissue destruction. In particular, TNF-α and IL-1β are two of the main cytokines that enhance synovial proliferation and stimulate secretion of MMPs, other inflammatory cytokines, and adhesion molecules (181). Recently, the role of HGMB1, released from dead cells, has been focused as a mediator of local and systemic inflammation being able to bind to RAGE, TLR2, and TLR4, to activate NF-κB and to induce the expression of the downstream cytokines including IL-6 (27, 182). Importantly, both TNF-α and IL-1β are included in the cytokine profile characterizing inflammaging (183), and HGMB1 is hypothesized to be one of molecules fueling this process (40) suggesting that inflammaging can be an additional cofactor involved in the pathogenesis of RA.

Furthermore, scientists have also pointed out the tight molecular relationship between periodontitis and RA pathogenesis consisting in an increased numbers of citrullinated epitopes, likely produced by specific human bacteria (*Porphyromonas gingivalis*) able to express peptidylarginine deiminase, an enzyme that generates citrullinated epitopes that are recognized by anti-citrullinated protein antibodies. Both diseases involve chronic inflammation fueled by pro-inflammatory cytokines, connective tissue breakdown and bone erosion as reviewed very recently (26). Thus, other mechanisms, such as the release of damage-associated molecular patterns from neutrophils may accelerate local and systemic inflammation as well as occur during aging (40), making evident the network structure of the involved molecules/markers and propagation mechanisms.

Aging is also the major risk factor for OA, which begins with disruption of the superficial zone of cartilage without any involvement of immune system, leading to progressive cartilage erosion and bone remodeling, causing disability and decreasing the quality of life. HMGB2 expression is uniquely restricted to cells in the superficial zone of normal mature human articular cartilage, and importantly, joint aging in humans and mice leads to the loss of HMGB2 expression while HMGB1 expression results increased in human OA-affected cartilage compared with normal cartilage (184). The contribution of HMGB1 to localized or systemic inflammation is mediated by innate immunity receptors, as described previously, leading to the increase of inflammatory status due to the production of chemokines and in particular IL-8 (185). Furthermore, many molecular and cellular mechanisms involved in inflammaging, such as cellular senescence; mitochondrial dysfunction and oxidative stress, dysfunction in energy metabolism associated with reduced autophagy and alterations in cell signaling were recently highlighted as processes contributing also to the development of OA (21). These processes promote a pro-inflammatory, catabolic state accompanied by increased susceptibility to cell death that together lead to increased joint tissue destruction and defective repair of damaged matrix.

Osteopenia is a condition not only highly associated with the aging process but also to different acute inflammatory diseases, leading to episodic bone reabsorption. Long-term solicitation of this process (22) might induce low bone mass and lately osteoporosis. Indeed, bone loss is typical in chronic inflammatory diseases (186–192) and other conditions or syndromes such as sarcopenia, as recently described (30). Common mechanisms of bone reabsorption are also found during aging process, i.e., an increase of the levels of pro-osteoclastogenic inflammatory cytokines such as TNF-α and IL-6, a decrease of bone-anabolic factors such as gonadal hormones and adrenal androgens as previously reviewed (193). Increased C-reactive protein was linked to an augmented fracture rate due to osteoporosis (194, 195), and circulating levels of inflammatory markers predict change in bone mineral density and reabsorption in older adults (196).

Age-related macular degeneration (AMD) is a highly prevalent, multifactorial, polygenic, and complex retinal degenerative disease. It is now widely accepted that inflammation, inflammasome activation (20), and immune system play important roles in AMD pathogenesis (197), but recently inflammaging was proposed to give a crucial contribution in the onset of AMD (198–200). Furthermore, the interplay and cross talk between protein homeostasis, autophagy, the proteasome, and heat shock proteins (HSPs) in the pathogenesis of AMD has become increasingly investigated over the past few years and has been recently reviewed (201). The role of HSPs as gatekeepers of proteolytic pathways in the retinal pigment epithelium and the implications of the disruption of the HSP-mediated chaperone functions affecting autophagy regulation, accumulation of oxidative stress-induced damage, protein aggregation and lipofuscinogenesis have also been reviewed (202) as zwell as the inflammatory process and the insufficient tissue repair (203).

Genetic Syndromes Characterized by Accelerated Aging: A Focus on DS

One could reason that in genetic syndromes characterized by accelerated aging, the same molecular mechanisms involved in normal aging should be affected by similar, yet more precocious and intense, alterations. Actually, these syndromes, including mandibuloacral dysplasia (MADA and MADB) (204), Werner syndrome (4), and Hutchinson–Gilford progeria (2) are the subjects of intense research to understand whether the aging phenotype observed in the affected patients is superimposable to the normal one or rather it has peculiar features. In this section, we will focus on DS, which is the most common genetic cause of intellectual disability, caused by a partial or complete trisomy of chromosome 21. Life expectancy of DS persons has dramatically increased in the last two generations, and in 1988, it was calculated that about 44.4 and 13.6% of live born DS persons would survive to 60 and 68 years, respectively (205). A decade after, the average death age was 55.8 years (206). Nowadays the median life expectancy is about 60 years (207), and it is expected to further increase in the near future (208). This unprecedented increase of life expectancy, together with the early occurrence of age-related disorders let emerge a brand new phenomenon: the aging of DS persons. Actually, clinical and experimental findings lead support to the concept that DS has to be considered a premature aging syndrome, especially as far as the nervous system is concerned.

Dementia appears to be the most relevant health problem of adult DS persons, as it is the most important disorder related to mortality, together with mobility restrictions, visual impairment, and epilepsy. In addition, level of intellectual disability and institutionalization are associated with mortality (209). At the age of 50, typical neuropathological hallmarks of AD appear in DS persons, including deposition of senile plaques containing amyloid β-peptide (Aβ), neurofibrillary tangles composed of hyperphosphorylated tau-P, and cholinergic and serotoninergic reduction (210). However, signs of cognitive decline appear much earlier and are detectable already at 35–40 years of age (5, 211). This is due at least in part to the fact that APP gene is located in chromosome 21; however, other mechanisms are likely involved including endosomal–lysosomal pathway and autophagy (212). Similarly, to what occurs in the aging process, autophagy (and mitophagy in particular) is decreased in cells from DS persons, due to impaired lysosomal acidification and protease activity (212, 213).

The other major system affected by premature senescence in DS subjects is the immune system. Actually adult DS persons display a series of changes that largely recapitulate the normal aging process of the immune system. In particular, diminished NK activity (214), erosion of telomeres in T lymphocytes (215), decreased response to mitogenic stimuli of blood leukocytes (216), increased risk of autoimmune disorders (217), and decreased number of T and B lymphocytes (218). However, these commonalities with normal immunosenescence have also been interpreted as an intrinsic immunodeficiency typical of DS rather than a precocious senescence of the immune system (218). Another striking commonality with normal immunosenescence is the pro-inflammatory profile of cytokine production observed

in PBMC from DS, including the increased production of IFN-γ, TNF-α, and IL-2 (219) and the increased plasmatic levels of IL-6, IL-10, TNF-α, and metalloproteases (220). This strongly resembles the phenomenon of inflammaging in old persons (39).

Down syndrome displays other typical age-associated alterations such as increased oxidative burden due to mitochondrial dysfunction (221), and, recently, it has been demonstrated that this defect can be partially restored by a treatment with metformin, a drug able to reactivate mitochondrial biogenesis by acting on the transcriptional coactivator PGC-1a (222).

As a whole, these data suggest that DS is a segmental syndrome where at least two main systems devoted to body homeostasis, i.e., the nervous and the immune systems, are affected by a premature decline that largely recapitulates what occurs in normal aging. Further support to this idea came from studies on markers of biological age (see next paragraph). In particular, analyses conducted with two types of biomarkers reliably correlated with biological age, i.e., DNA methylation age and GlycoAgeTest (see below) showed that 1. Tissues from DS persons are characterized by levels of DNA methylation typical of persons that are on average 7 years older (223); 2. The age-sensitive N-glycan species identified as GlycoAgeTest displayed accelerated dynamics in DS persons vs non-trisomic, age-matched sibs (224).

MARKERS OF BIOLOGICAL AGE

Within this frame, there is growing interest around biomarkers of biological age. Biological age is intended as a synthetic index constituted by a single marker or the combination of few biological markers which, alone or integrated with functional markers, not only correlates with chronological age but is/are capable of identifying individuals "younger" or "older" than their chronological age in the same demographic cohorts.

With such biomarkers, it should be possible to obtain trajectories of aging, where the "accelerated" ones would predict unhealthy aging and diseases, while the "decelerated" ones would be associated with healthy aging and longevity. The possibility to draw trajectories of aging is a fascinating, far-reaching perspective, especially in consideration of the abovementioned long incubation preclinical period that characterizes most of the major age-related chronic diseases, and is considered the critical time window for effective treatments. Biomarkers of biological age could greatly contribute to identify the subjects characterized by higher risk to develop overt clinical diseases who would have a major benefit from tailored preventive treatments. However, these biomarkers are apparently informative about the status of deep molecular mechanisms (the seven pillars) underpinning the age-related decline which predisposes to ARDs but do not tell us which specific disease people characterized by accelerated biological age are predisposed to. Accordingly, a major biomedical aim is to identify the subjects at higher risk for each *specific* ARD at very early stage. At present, the combination of the new generation of effective biomarkers, capable of assessing the deep biological age, with the classical and innovative biochemical and functional disease-specific ones represents the best strategy to identify disease-specific aging trajectories. Within this perspective, particular attention has to be devoted to the genetics of each individual which is the complex result of the interaction between nuclear and mitochondrial genetics (stable with the exception of somatic mutations) and microbiomes's genetics (malleable and adaptative to the environment), focusing on GM for its capability to be modified by basic habits such as nutrition. In particular, we predict that it will be useful to combine the abovementioned integrated biomarkers' assessment with established and new genetic risk factors for ARDs, taking into account some criticalities related to population genetics and demographic birth cohorts (225).

To date, there are no clinically validated markers of biological age; however, a number of promising candidates have been proposed in the last years. We will discuss three of them: (i) DNA methylation markers, (ii) N-glycan markers, and (iii) GM biomarkers.

DNA Methylation Markers

DNA methylation variability gained a central position in the rush for the setup of markers of biological age since several years. In a seminal paper of 2005, Fraga et al. (226) showed for the first time that in human the DNA methylation patterns change profoundly with age. With the advent of microarray technology capable to quantify the DNA methylation levels in hundreds thousands of CpG sites across the genome, the knowledge regarding variability and dynamics of such molecules increased dramatically. In particular, DNA methylation proved to be a powerful source of robust biomarkers capable to correlate with different clinical conditions (227, 228). One of the most striking results from these epigenetic studies on human models is the occurrence of directional (229–231) and stochastic (232) DNA methylation changes that highly correlate with chronological age. These observations paved the way to the generation of a number of "methylation clocks" that result from the combination of different CpG sites whose methylation level correlates with chronological age. Many of such clocks have been developed for forensic applications (233–235), thus highlighting the elevated accuracy of the chronological age estimation that can be obtained from DNA methylation data.

Of all the different clocks, three have been tested thoroughly as possible markers of biological age: the one developed by Horvath (230), the one by Hannum et al. (231), and the one by Weidner et al. (236). To date, Horvath's DNA methylation clock provided the most interesting results as marker of biological age. This is probably due to the fact that is the only one that is applicable to all the tissues, whereas the other two clocks are validated only in blood. In many different studies, Horvath's clock has proven to grasp features of accelerated aging in many different age-related clinical conditions such as cancer (237, 238), neurodegeneration (239–241), progeroid genetic syndromes other than DS, such as Werner syndrome (242), and all-cause mortality (243, 244). Moreover, this clock was able to show a signature of decelerated aging in human models of longevity, such as Italian centenarians and their offspring (245, 246).

Despite such promising results, a lot of work has yet to be done to include such evaluation of the biological age in the clinical practice. In this perspective, it is necessary to devote a great

effort in the definition of epigenetic markers of biological age that rely on the analysis of a limited number of CpG sites to obtain an inexpensive clock suitable for large scale screenings. Indeed, both Horvath's and Hannum's clocks are based on the analysis of many CpG sites (353 and 75, respectively), with elevated costs that prevent their use in large scale for broad applications.

Glycomic Biomarkers

The relative quantification of the N-glycan species that constitute the sugar shell of circulating proteins is a wealthy source of reliable biomarkers. The characterization of circulating N-glycans from sera or plasma, hereafter referred as glycomics, has provided markers in several clinical fields such as hepatology (247–249), type 2 diabetes (250–254), RA (255–258), and cancer (259–262).

It is noteworthy to mention that in a 2011 study, Vanhooren et al. showed that the glycomic parameters are correlated with age also in mice (263). In particular, studying a short-living mice model, i.e., mice defective in klotho gene expression (kl/kl), a long-living one, i.e., slow-aging Snell Dwarf mice (dw/dw) and ice fed at calorically restricted diet they showed that the N-glycan variance catch the accelerated aging of the short-living mice and the decelerated aging of the long-living ones, demonstrating that the N-glycan profiling is a promising markers of biological age also for the mice model, thus representing a powerful tool to bridge preclinical and clinical studies on aging. In the same study, the author showed that the mechanism at the basis of the age-related N-glycan changes is likely due to the impairment of the liver glycosylation machinery.

A study by Borelli et al. (224) provided the characterization of the glycomic profile of DS persons (DS). In the study the, author obtained the glycomic quantification of DS by means of DSA-FACE protocol and of the high-throughput protocol of matrix-assisted laser desorption ionization-time-of-flight-mass spectrometry. With the combination of these two protocols, the authors were able to provide for the first time the specific glycomic signature of DS and showed that the age-sensitive N-glycan species show accelerated dynamics in DS vs non-trisomic siblings and mothers.

In a study on a Netherland model of familial longevity (264, 265), the authors reported that the glycomic profile showed features of decelerated biological age, correlated with metabolic health and cardiovascular events.

Finally, it has been suggested that the age-related glycomic changes could be a contributor to inflammaging by affecting IgG structure and function. In fact, IgGs devoid of terminal galactose residue in the di-antennary N-glycan at asparagine 297 (also called IgG-G0) can exert pro-inflammatory effects through a more efficient activation of complement's lectin pathway and phagocytosis, and their production is increased with age (266).

Gut Microbiota Biomarkers

The comparison of GM among young adults, elderly persons, and centenarians has highlighted that the mutualistic changes in the composition and diversity of the gut ecosystem do not follow a linear relation with age, remaining highly similar from young adults to 70-year-old persons while markedly changing in centenarians. Thus, GM seems to rest in a stable state from the 3rd to the 8th decade of life (174), while after 100 years of symbiotic association with the human host, it shows a profound, and possibly adaptive, remodeling. Centenarians stand out as a separate population, as their GM shows high diversity in terms of species composition (173). In centenarians, there is a shrinkage of dominant symbiotic bacterial taxa that is counterbalanced by an increase in longevity-adapted and possibly health-promoting subdominant species (e.g., *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*) (267). On the other hand, GM dysbiosis has been associated with several diseases suggesting that alteration of its composition may be involved in disease-related mechanisms (268).

A recent paper addressed the potential interaction between biological age and GM. The authors identified both global and specific changes in the GM that were closely associated with biological age but not chronological age (269), suggesting that GM could be used as a potential biomarker of age.

Overall, epigenetic (DNA methylation), glycomic, and GM markers seem to be valuable markers of biological age and promising tools to draw informative aging trajectories. Many other molecular parameters obtained in particular from –omic analyses are at present under evaluation for their possible use as markers of biological age. To this regard, it is worth mentioning studies on metabolomics (32), lipidomics (270), circulating nucleic acids, in particular miRNA (271) and cell-free mtDNA (272), and metagenomics (176) that showed complex age-related reshapes in both healthy elderly and ARDs.

CONCLUSION

The complex scenario emerging from the previous sections deserves and stimulates two different, even if complementary, types of conclusions. The former refers to the biomedical and molecular aspects, while the latter faces the philosophical, societal, and ethical implications and problems rose by the conceptualization here presented.

Biomedicine and Biology

A debate exists on whether aging is a disease in itself. Some authors suggest that physiological aging (or senescence) is not really distinguishable from pathology (273), while others argue that aging is different from age-related diseases and other pathologies (274, 275). It is interesting to stress that the answer to this question has important theoretical and practical consequences, taking into account that various strategies capable of setting back the aging clock are emerging (276–278). The most relevant consequence is that, if we agree that aging is equal to disease, all human beings have to be considered as patients to be treated, being an open question when this treatment should start. As we tried to summarize in this review, many mechanisms proposed to cause aging are the same as those known to underlie ARDs/GSs, lending support to the hypothesis that the aging phenotype and ARDs/GSs are not separate entities but rather the visible consequences of the same processes which likely proceed at different rates.

Within this conceptual framework, it can be somehow puzzling to pigeonhole the phenomenon of longevity, which is a peculiar

manifestation of aging. Longevity can indeed be considered the consequence of successful aging. So, why the same molecular mechanisms should lead to successful aging and longevity on one side and to unsuccessful aging and ARDs on the other? To further complicate the picture there is another important aspect, not discussed in this review, that should be, however, taken into account, i.e., the influence of gender on aging, longevity and ARDs. It is known that females have a survival advantage in advanced age, paradoxically characterized by a worse quality of life (279). In fact, females have an increased prevalence of many ARDs, in particular degenerative diseases and consequently an augment of disability. Therefore, men and women follow different trajectories to reach extreme longevity, have a diverse quantitative chance to attain longevity and the aging process is likely qualitatively different between genders (280). Several studies have also shown that sex hormones play a role in the host–microbiota interaction. Indeed, the term "microgenderome" defines the potential mediating and modulatory role of sex hormones on GM function and composition with implication for autoimmune and neuroimmune conditions (281).

The overall conceptual framework of the relationship between aging and ARDs/GSs, here presented, fits quite well into the concept of hormesis, which is considered an overarching conceptualization of aging and longevity (159, 282, 283). It is known that a stressful stimulus can determine both detrimental and positive effects depending on its intensity. If the intensity of the stress is low, the response of biological system (cell, organ, or whole organism) can produce benefits that overcome the damage caused by the stress (283–285). It is possible to apply this paradigm also to the aging process (**Figure 1**). If the intensity of the stresses (oxidative stress, inflammation, proteostatic stress, telomere attrition, etc.) does not exceed the threshold after which the detrimental effects of such stress are higher than the adaptive, protective effects of the organismal stress response, it is likely that a successful aging will follow. A corollary of this hypothesis is that low stress is better than no stress at all, as absence of stress likely does not trigger protective effects (286). Another corollary is that the more effective is the response to stress, the higher is the level of stress intensity that can be tolerated. If an individual succeeds in maintaining his/her responses as much as possible within the range of the "hormetic zone" (green line of **Figure 1**), his/her trajectory toward clinical symptoms and overt disease(s) will be delayed (**Figure 2A**, green line). On the contrary, strong detrimental effects will accelerate aging as well as the onset of chronic diseases (red line of **Figure 2A**). We recently argued that the adaptive hormetic paradigm could be applied to inflammaging (287) as well as to lifestyle such as Mediterranean diet, which counteract the deleterious effects of inflammaging (283).

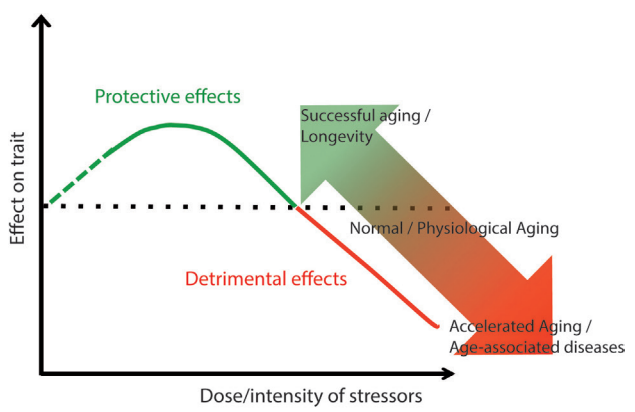

FIGURE 1 | Hormesis as a possible mechanism to account for the continuum between healthy aging and geriatric syndromes (GSs)/ARDs. Lifelong low-intensity stressors stimulate maintenance and repair mechanisms with positive effects for health. The increase of stressors' intensity can overcome the capability of the organs and systems to adapt and end up with detrimental effects (GSs/ARDs).

Which are the determinants that make the aging trajectories depicted in **Figure 2** more or less steep? First of all, the environmental conditions (intensity and types of stressors, as mentioned), but also and likely most important, the capacity of the body to respond and adapt to these stressors. This capacity is determined at least in part by the individual genetic background and by epigenetic changes mediating many phenomena of adaptation and remodeling. In any case, the processes underpinning the aging progression and the corresponding successful or unsuccessful adaptive mechanisms take time, and the eventual onset of clinically overt ARDs/GSs has a long period of incubation, preceded by years/decades of deep/hidden molecular and cellular alterations, which are difficult to pinpoint with present technology and knowledge. This situation is represented in the cartoon of **Figure 2B**, where the continuum among healthy status, GSs, and ARDs is represented as an iceberg. The tip of the iceberg is just the (clinically) visible part of a much longer process that goes from normality to pathology. Few persons like centenarians manage to remain "healthy," in the sense that they avoided or largely postponed the onset of ARDs/GSs even at old or very old age (green arrow), others proceed to GSs (orange arrow), while the majority develop ARDs (red arrow). Within this perspective, even "healthy" centenarians do not escape the physiological decline, and the accumulation of molecular scars that accompanies aging, but the rate of such processes is slow enough to let them stay below the threshold over which clinically overt pathologies ensue. We predict that biomarkers based on CpG DNA methylation as well as N-glycan profiling and GM composition are currently the most appropriate and powerful to distinguish biological vs chronological age and to measure the deep alterations that anticipate clinical symptoms. However, further studies are needed to assess the aging rate at the level of the various organs and systems of the body, in the same individual as required by personalized and precision medicine. Finally, beside the molecular mechanisms shared between aging and ARDs/GSs discussed earlier, a deeper level involving even more basic mechanisms (entropy failure) is likely present and will be the topic of future investigations.

Philosophical, Ethical, and Social Implications

The second conclusion is that medicine should combat aging to combat many ARDs at a time and not one by one. In this

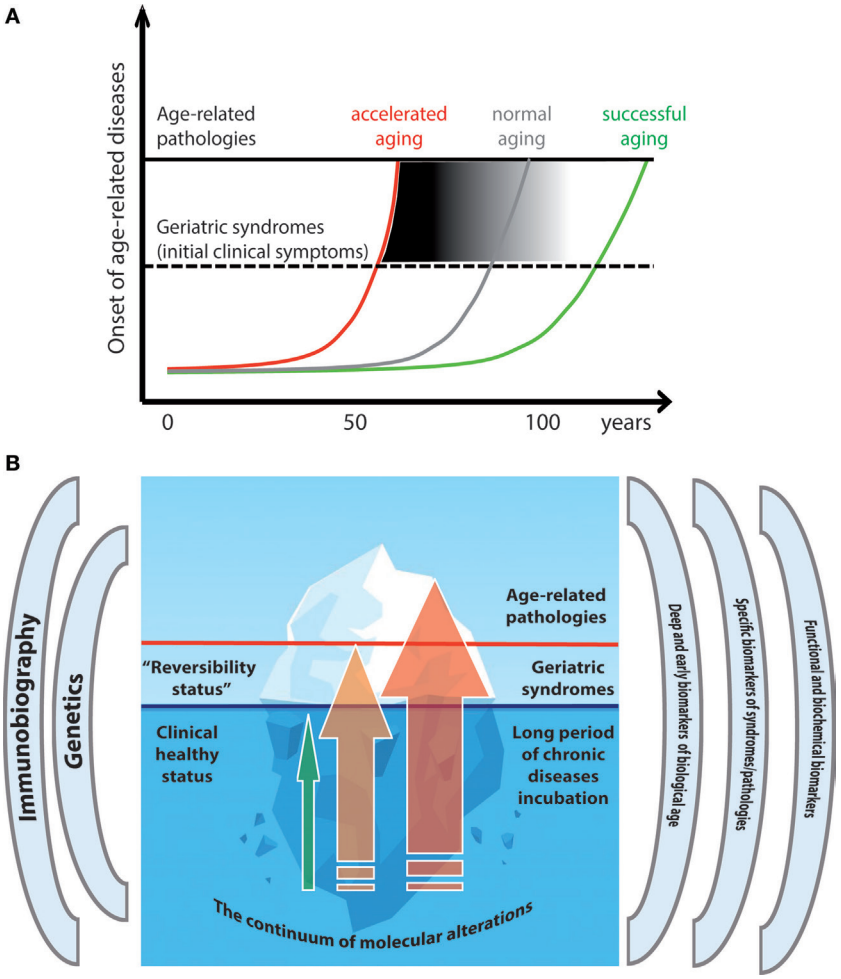

FIGURE 2 | Trajectories of healthy and unhealthy aging. **(A)** The different age-trajectories are depicted as lines with different slopes, each corresponding to a rate of response and adaptation to lifelong stressors, leading to accelerated, normal, or successful aging, and reaching the threshold for ARDs at different age. **(B)** The metaphor of the iceberg is used to illustrate the continuum between healthy aging and geriatric syndromes (GSs)/ARDs. The hidden part of the iceberg illustrates the long incubation period during which no clinical signs are apparent but markers of biological age can be accelerated. Green arrow: persons with slow-aging trajectory who do not develop (or pospone) GSs/ARSs; Orange arrow: persons with faster aging who develop GSs; Red arrow: persons with accelerated aging who develop ARDs.

perspective, one could envisage following two possible strategies to attain this result:

(A) Try to slow the aging rate through changes in life style, and possibly drugs or medical treatments that counteract the impairment of the abovementioned mechanisms (the seven pillars and maybe others). This strategy should help people to stay healthy and active as long as possible and pospone ARDs for decades, ideally until the apparently inevitable limit of human lifespan (288).
(B) More radically, try to rejuvenate human tissues, organs, and whole body. In this case, also the abovementioned limits of human lifespan could be likely overtaken.

We are relatively ready to the first strategy that appears more feasible and acceptable from an ethical and social point of view, as it would be very similar to what is already happening nowadays, i.e., an increase in life expectancy and in the number of people who attain 90 or 100 years of age and more in good health. Even a very long life for most people will engender various biomedical and societal problems, but this strategy has the advantage of being doable and allowing people to live longer and healthier, relieving burden from families and welfare states and, most of all, avoiding suffering, disability, and dependence.

We are instead not yet ready, in particular from a social and ethical point of view, for the second strategy, which opens uncanny scenarios of rejuvenating bodies and very long life for the bulk of the population, a topic addressed in utopian, dystopian, and science fiction novels. Taking into account, the fantastic, unprecedented rate of scientific discoveries in the field of aging and rejuvenation, it is timely and urgent to open a large debate, involving first of all the general public but also experts in different fields (economy, demography, philosophy, religion bioethics, among others). Indeed, such sensitive topics as doable age-prolongation and rejuvenation have

been either neglected or conceptualized according to the scanty scientific knowledge available until recently, i.e., incomparably less than that available today and likely in the next future.

AUTHOR CONTRIBUTIONS

CF conceived, designed, and coordinated the writing of the whole manuscript; PG, CM, MaC, AS, AG, DM, MiC, and SS revised literature and wrote the different parts of the manuscript; all the authors contributed to critically revise and approve the final version of this manuscript.

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APPENDIX

The History of Old Age As a Disease

The first theories of aging that appeared in ancient Greece and recovered during the Middle Age identified old age as a consequence of the gradual consumption of the innate heat with the inevitable loss of body moisture, according to Hippocrates' system of four humors (V BC). For Hippocrates (c. 460–c. 370 BC) every organism is born with a certain quantity of innate latent warmth (*calor innatus*), which progressively declines conducting to natural death (289).

Influenced by this theory, Aristotle's (384–322 BC) on youth and old age sees old age itself as a *morbus* (disease) or a *marasmus*. To represent such an ineluctable process of degeneration, Aristotle used the image of a lamp in which the life fuel has run out, a metaphor that enjoyed a wide currency in medical literature over the centuries (290, 291).

Combining Hippocratic medicine with Aristotelian theory, Galen's (AD 129–c. AD 199) *De Sanitate Tuenda*—where the term "gerocomy" first appeared—avoided to consider old age as a disease and rather described the health of elders as incomplete and correspondent to convalescents. More specifically, Galen saw senescence as a heterogeneous and postponable process since he observed that aging was an event impacting on population differently according to individual past history, lifestyle, and illnesses, and that by respecting a certain dietary regimen the arrival of aging might be retarded (292).

The old-age-as-a-disease idea was also present in ancient Rome. It can be detected in the comedy *Phormio* (161 BC) by the playwright Terence where the old Chremes affirms about his suffering that "the illness is old age itself," in Seneca (c. 4 BC–AD 65) who referred to old age as an incurable illness, and in Cicero's (106 BC–7 December 43 BC) *De Senectute* where the author argues that "we have to fight against aging, as we do against a disease" (291).

The idea that senescence was itself an illness and the image of the aged body as a consuming lamp was two of the main themes around which research into aging revolved from classical to medieval speculations, from Renaissance to eighteenth century.

Like Galen, the great Arab physician Avicenna (980–1037) refused to consider aging and death like a pathological entities, for he looked them as a result of a natural decrease of the *calor innatus* due to the consumption of the *humidum radicale*. Indeed, he was skeptical about the possibilities of medicine of retarding the aging process and then considered prolongevity not an appropriate medical goal (293). In the last centuries of the Middle Ages, of the two main medical schools of Salerno and Montpellier, the latter concentrated on the importance of the equilibrium between the four humors and the innate heat to enjoy a delayed and unimpaired senescence (294).

Interestingly, the unknown author of De retardatione accidentium senectutis (often ascribed to Roger Bacon, 1219–1292) in the thirteenth century realized that aging process can be identified with some characteristics (i.e., gray hairs), and consequently suggested that if such phenomena (*accidentia senectutis*) were to occur in adolescence, they would be called illnesses (291).

By around 1500, the old-age-illness became a prominent literary cliché that can be found both in Erasmus of Rotterdam's poem for the Basel physician Guilielmus Copus and in Martin Luther's comments of Ecclesiastes where he declares: "old age is *per se* a disease" (291).

During the eighteenth century, the most influential concept for the medicalization of old age was that of "*marasmus*," a concept related to wasting fever or exhaustion, which can be traced back to Galen (295). Referring to the deterioration of old people, the marasmus senilis was a generalized pathological state that was not the effect of a temporary illness and might occur without fever. Yet, this prevailing theoretical context did not impede to realize, as well illustrated by the German physician Burkhard Seiler (1779–1843) in his seminal work *Anatomia corporis humani senilis specimen* (1799), that most old people did not die because of the weaknesses of age or senility, but rather as a result of several illnesses with a cumulative mutual effect (291), a concept that will reappear in many major theories of aging in the twentieth century (289).

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Practical and Ethical Aspects of Advance Research Directives for Research on Healthy Aging: German and Israeli Professionals' Perspectives

Perla Werner[1] *and Silke Schicktanz*[2]*

[1] Department of Community Mental Health, University of Haifa, Haifa, Israel, [2] Institute of Medical Ethics and History of Medicine, University Medical Center Göttingen, Göttingen, Germany

Background: *Healthy aging* is the development and maintenance of optimal cognitive, social and physical well-being, and function in older adults. Preventing or minimizing disease is one of the main ways of achieving healthy aging. Dementia is one of the most prevalent and life-changing diseases of old age. Thus, dementia prevention research is defined as one of the main priorities worldwide. However, conducting research with persons who lack the capacity to give consent is a major ethical issue.

Objective: Our study attempts to explore if and how advance research directives (ARDs) may be used as a future tool to deal with the ethical and practical issues in dementia research.

Method: We conducted focus groups and in-depth interviews with German and Israeli professional stakeholders from the fields of gerontology, ethics, medical law, psychiatry, neurology and policy advice ($n = 16$), and analyzed the main topics discussed regarding cross-national similarities and controversies within the groups, as well as across the two national contexts.

Results: While both countries are in the midst of a developmental process and have recognized the importance and need for ARD as a tool for expanding healthy aging, Germany is in a more advanced stage than Israel because of the EU regulation process, which indicates the influence of international harmonization on these research-related ethical issues. Consensual themes within the qualitative material were identified: the need for a broader debate on ARD, the ethical importance of autonomy and risk–benefit assessment for ARD implementation, the role of the proxy and the need for the differentiation of types of dementia research. Controversies and dilemmas aroused around themes such as the current role of IRBs in each country, the need for limits, and how to guaranty safeguarding and control.

Discussion: Implementing a new tool is a step-by-step procedure requiring a thorough understanding of the current state of knowledge as well as of the challenges and hurdles ahead. As long as improving quality of life and promoting autonomy continue

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***Correspondence:**
Silke Schicktanz
sschick@gwdg.de

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to be core elements in the process of healthy aging, efforts to advance knowledge and solve dilemmas associated with the implementation of ARD is of the utmost importance.

Keywords: advance research directives, dementia, professionals' opinion focus group, healthy aging research, ethics, Germany, Israel

INTRODUCTION

As the population worldwide ages (1), the focus in gerontology and geriatrics is moving from the treatment and management of disease to the promotion of healthy aging. While multiple and sometimes controversial definitions have been used to describe healthy aging (2), all share several common attributes. First, healthy aging is conceptualized as a process occurring along the life course (3). Second, it includes a multi-dimensional approach encompassing the development and maintenance of optimal cognitive, social and physical well-being and function (4). Third, sustained independence and autonomy were defined as fundamental in the process of achieving and maintaining healthy aging (5, 6). Finally, increasing multidisciplinary research in the area of healthy aging has been defined as essential (3, 7). Advancing research and knowledge regarding healthy aging and dementia is of the utmost importance.

Dementia, defined as a syndrome of severe progressive cognitive deterioration that impairs daily functioning, is one of the most prevalent and life-changing diseases of old age (8). Increasing dementia prevention research has been defined by Alzheimer's Disease International and the World Health Organization as one of the main priorities worldwide (9). Such research cannot focus only on healthy participants, but needs to involve persons with dementia—whether in a comparative setting or in order to test for the long-term effects of particular treatments. Furthermore, this research might include long-term and large cohort studies, which means that participants' cognitive capacity might decline over time.

Recruiting and retaining people who lack the capacity to give consent has been defined as one of the main crises necessitating the advancement of research in the area of AD and dementia (10). Most importantly, this problem confronts researchers with serious ethical problems. While advance research directives (ARDs) might provide a potential tool to deal with these issues, knowledge in the area remains scant.

The aim of this article is to examine the literature on the topic of ARD, with a special focus on the participation of persons with decreased cognitive capacity, and to explore the attitudes and perceptions of professional stakeholders about the topic in Israel and Germany. The chapter opens with a review of available knowledge in the area of ARD, followed by a description of the findings of expert interviews (in a focus group or as individuals) conducted in Israel and Germany in 2017. We will conclude the chapter by discussing the findings of our focus groups and their relevance regarding international efforts to introduce ARD as a tool for expanding research with persons with deteriorated cognitive functioning.

ARD—A Brief Overview

The international ethics guidelines regarding research with persons lacking decisional competence is not very homogenous (11). In most countries, current research with persons with dementia relies—if at all allowed—on surrogate decision-making as "proxy consent." This is the decision made by a formal, legally appointed guardian, a power of attorney or sometimes an informal representative (e.g., a family member consenting to a specific study).

However, this surrogate decision-making has lately been criticized for two reasons: first, it only allows for research with "minimal risk," or for research with personal or patient group benefits—therefore, any research beyond minimal risk or for third parties cannot be conducted (12, 13). Second, surrogate decision-making is not fully in accordance with the ethical principle of patient self-determination—a principle which is increasingly gaining priority in international medical law and ethics.

Advance research directives might provide a potential way to overcome these criticisms. However, although the topic of ARD has been frequently discussed since the end of the previous century, clear legal regulations are still lacking in most European countries as well as in the United States (14–16). In the next subsections, we summarize what is known in the ARD literature till today. We limit our examination to ARD, but will refer to Advanced Health Care Directives when it was mentioned by the participants.

Defining the Concept

Advance research directives are legal documents allowing persons who have decisional capacity to express their preferences regarding participation in future research studies in the event they will lack this capacity to do so at the time of the research (12, 14). ARD differ from the concept of *advanced informed consent* because they document the individual's interest and desire regarding potential future research, in general, rather than specific studies (17).

Appointing a specific proxy (also described as power of attorney) can also be part of an ARD. The recognition and implementation of ARD are lately being encouraged as a formal strategy to complement surrogate decision-making (12, 18, 19), and as a mechanism to increase respect for autonomy and the exercise of self-determined decision-making (20).

The Ethical Basis of ARD

Similar to Advanced Health Care Directives (AHD), the core ethical principles mentioned in the literature as the basis for ARD are self-determination, autonomy and empowerment (12, 14, 16, 19, 21, 22). The main understanding is that a person should determine by his/herself, in advance, what should be decided on his/her behalf in case he/she loses the capacity to make decisions

(20). However, a common objection to this idea is that the will of the person might not remain the same in the current research situation, compared with when he/she decided and documented his/her will in the past. However, at this point he/she will no longer be able to express this change in attitude (23). Therefore, the idea of ARD serving "self-determination" in the current situation might be misleading. However, if we perceive *autonomy* as being relational, processual, and as self-expression through the support and interpretation of others, then ARD/AHD may be contextualized as justified instruments of autonomy (24, 25). Indeed, Jongsma and van de Vanthorst (20) discuss the dilemma between respect for autonomy and the "best interest" principle embedded in the ARD concept, and advocate for perceiving ARD as a morally defensible and reasonable basis for including persons with dementia, who lack the capacity to make decisions precisely because, in their view, respecting autonomy also means respecting preferences regarding their own future. Both claim further that research is different than care, as in the case of research, the anticipation of one's best interest is less evident than perhaps in the case of receiving the best standard of general health care. As Jongsma (26) maintains, thus, the ARD and proxy consent must not be perceived as excluding alternatives, but as a way of providing more evidence for future proxies about how to make decisions in concrete situations as well as guide the proxy about how to ensure the ARD is respected by the different professionals in charge.

Safeguards for ARD Implementation

The ethical principle of respecting autonomy in research is normally implemented in practice as "informed consent." In addition, an ARD should be based on informed consent in the sense that providing expanded, adequate information and education about the meaning of ARD as well as about different research scenarios and risk potentials is mandatory (19, 23). However, the overall aim of ARD implementation is twofold: protecting the subjects and ensuring self-determination on the one hand, while fostering research participation on the other. Therefore, it remains unclear whether ARD will actually increase or decrease research practice, and this might rely on how the research needs, risks and benefits are presented. Surrogates also need clear guidelines regarding their role, rights and duties when interpreting and acting on behalf of an ARD (12). Finally, close and steady contact and the monitoring of participants' well-being are mentioned as the main safeguards to be respected by researchers and professionals (20, 23).

Prevalence Rates and Correlates of ARD

While information about this topic is scant and relatively outdated, studies examining these issues consistently show low prevalence rates (18, 21, 27, 28), and three main correlates of ARD: previous research experience, health care directives and the level of risk or side effects involved in the research protocol. Finally, it should be noted that there is no knowledge at all regarding these issues either in Germany or in Israel.

In sum, ARD is convincingly suggested as a new ethical-legal tool to discuss and ensure more self-determined wishes regarding research participation. However, many practical and ethical issues remain unclear or unsolved regarding the "what?" ("What needs to be described in an ARD? For which type of research is ARD needed?"); the "when?" ("When is the best time to convey information to others and encourage the public or patients to compose an ARD?"); the "who?" ("Who or which group of persons should be approached for an ARD?"); and the "how?" ("How should ARD be implemented in practice and which kind of safeguards are needed?").

In the next section, we describe tentative responses to these questions as discussed in stakeholders' interviews conducted in Israel and Germany on the topic.

Comparing Professional Stakeholders' Perspectives in Israel and Germany

Israel and Germany provide an ideal basis for comparison, since they are both characterized by public health care systems in which the topic of dementia has gained particular attention over the last years. However, neither country has developed a concrete dementia action plan or a particular policy regarding research on dementia or on healthy aging at all. In both countries, legal requirements regarding research with persons with dementia are rather restrictive. The latest change in Germany occurred in 2016 when, according to a new EU clinical trial regulation group, it was decided that research should be allowed with cognitively impaired persons, even the research only benefits this class of patients, but not the patient him/herself (hereafter labeled as "patient group benefit"). The political compromise ended by allowing such research only on behalf of the existence of an ARD, without any public or more detailed expert debate defining the pros and cons for such an ARD (29, 30). In Israel, a National Strategic Plan to Address Alzheimer's and Other Types of Dementia was formulated in 2013 (31). While AHD are included in this plan as one of the main areas needing further development and awareness, the topic of ARD is not directly mentioned. Furthermore, advisory committees regarding dementia were established in both countries: In Germany, the so-called Alliance for People with Dementia has, since 2012, provided a platform to inform the Ministry of Health and the Federal Ministry for Family Affairs, Senior Citizens, Women and Youth with ideas and information related to dementia care. In Israel, the National Council for Dementia, established in 2013, focuses on improving training and research in the field of dementia.

MATERIALS AND METHODS

In order to explore the practical status of ARD and related ethical issues from a professional stakeholders' perspective, we conducted two expert focus groups and additionally, two individual expert interviews (because several professionals were not available for the time scheduled for the focus groups) in Israel and Germany between March and September 2017. We use the term "expert" in a broad sense, as this includes scientific experts from a particular field (neurology, clinical geriatrics or social gerontology, bioethics, legal studies), as well as representatives of patient organizations or persons with practical expertise (e.g., on decision-making processes in ethics or policy committees). The

experts we invited are also seen as professional stakeholders in the sense that they present legitimate interests and concerns of their field or their academic organization into the broader public or health policy debate (direct *via* policy advice or indirect by publications or presentations, newspaper comments, etc.). Focus groups were chosen as the method of inquiry because they create a shared space for group discussion in addition to allowing participants to expand the scope of the topic (32).

Participants

A purposive sampling technique was used. A total of 16 participants from different fields (ethics, medicine, medical law, gerontology, dementia research, patient representation and health politics/insurance) participated. Seven experts took part in Germany: four experts had a background in medical ethics/medical law; one in clinical dementia care and research; one from gerontology; and one representative of a patient organization, who was also part of a ministerial board for dementia (gender ratio: four women, three men). In Israel, overall 9 professional stakeholders took part: three from medical ethics/medical law; two from clinical dementia care or research practice; three from gerontology; and one from ministerial administration (gender ratio: seven women and two men). The experts' work experience ranged from 4 to 40 years.

Participants were promised anonymity for publication to allow a free and open-minded discussion.

Procedure

Participants were recruited through the researchers' professional networks while ensuring the experts' well-known status of expertise by their documented research/working profile. We also considered the various disciplinary backgrounds for reaching heterogeneity. Focus group discussions were held until saturation of new information was reached (32). Before each focus group, all participants were asked to complete a short questionnaire, including demographic and professional information. Focus group meetings lasted on average almost 120 min. Discussions were audio taped and transcribed. The facilitator (SS) in both cases was skilled and experienced in conducting focus groups. In Israel, the meetings were conducted in English; in Germany, they were conducted in German. The main parts of the German transcript were translated into English for further comparative content analysis. For the purpose of publication, all original quotes are anonymized and only the professional background is mentioned. Our comparative qualitative content analysis was supported by using the scientific software ATLAS.Ti® and was guided by the aim to first find similar topics and perspectives. The second step involved searching for cross-national specificities or professional peculiarities.

Interview Guide

According to the recommended focus group methodology (32), a semi-structured interview guide containing open questions was developed by the research team (see Supplementary Material). The aim of the interview guide was to cover the following key themes: (a) professional experience and background knowledge of ARD; (b) assessing content and practical implementation of ARD; (c) overall perspectives on advanced planning in health issues; (d) assessment of the current dementia research setting and legal status in the respective country. The guide was developed jointly in English and afterward translated into German for the focus group in Germany. For the two additional individual expert interviews, we used the similar semi-structured interview guide.

The moderator also made sure to show enough flexibility to allow for open discussions among the participants.

RESULTS

The main topics emerging from the discussions are summarized in **Tables 1** and **2**. **Table 1** provides an overview of topics and opinions that were shared by the majority or consensually discussed in the focus groups and showed similarities across German and Israeli stakeholders. These consensual topics can be categorized into the following main areas: Concept and need of ARD, ethical issues such as autonomy and risk–benefit assessment, the role of proxy, and desirable ARD content.

Overall, professionals in both groups recognized the need to find a mechanism to allow increased research activity involving persons with dementia who have diminished decisional capacity. In both countries, the need stemmed from the increasing individual and social costs faced by health systems because of the world's ongoing demographic changes. Furthermore, it was also argued that the current restrictions on research with persons with dementia deprive this group of patients of evidence-based treatments. Disseminating and expanding knowledge about ARD among clinicians and the public was discussed in both groups and most agreed there was a need for more information and for conducting open discussions on the topic. Stakeholders in both countries extensively discussed implementation issues. The main common topic in this area referred to the connection between AHD and ARD and how research needs more safeguards and monitoring than treatment.

Participants conceptualized ARD as part of the process of respecting a person's decision-making preferences and autonomy regarding research, but only in relation to his/her general wishes, and not, for example, in regard to veto rights regarding withdrawing/withholding treatment at the end of life. ARD were seen by most as a tool—if correctly done and based on proper information—to respect patients' autonomy and personal wishes. However, proper risk–benefit assessment was seen as an important safety measure, in cases of persons with dementia who were seen as a vulnerable group. The majority agreed that research without personal benefit, but rather the benefit of the group, should only be conducted on minimal risk/minimal burden level. However, the notion of whether or not ARD might also allow for more than minimal risk/burden proved to be very controversial (see below). During the discussion about ARD, other common themes of research ethics were also mentioned. These themes mainly included IRBs and researcher's responsibility. These basic principles were perceived as indicative of allowing research and monitoring during the study to determine whether any signs of burdening or objection might occur with the patient, independent of whether an ARD is available or not. The majority of

TABLE 1 | Overview of main topics consensually discussed with the majorities' opinion in the German and Israeli focus groups.

Main areas discussed	Consensual opinions
Concept and need of ARD	• The need of advance research directives (ARD) must be further discussed and explored within the public and the professional community. • Research in dementia and healthy aging studies would benefit from the increased participation of persons with dementia. • Patients' interest in taking part in research is high. • The demarcation between ARD and AHD needs to be clarified: ARD might be a practical subpart of AHD, but as they cover research where the patient does not always benefit personally, there is a risk of therapeutic misconception or misuse. • It is ineffective to approach the general public to sign ARD; instead, persons in early stages of dementia/Mild-cognitive impairment or if there is genetic disposition for a kind of dementia should be approached.
Ethical issues: Autonomy	• ARD is a good tool for empowering patients and allowing them to express their own wishes regarding research participation, but demented persons remain a vulnerable population. • Competency and capacities to compose an ARD are needed: any layperson might need a lot of information about the potential research and limitations of ARD. • Approaching potential candidates for ARD needs to be done with sensibility and caution. • ARD is not similar to consent; If an ARD states the wish to take part in research, it does still not imply a professional duty to include the patient in research • ARD resamples AHD if the wish not to take in research is stated as this is a veto right for any research participation
Ethical issues: risk–benefit assessment	• IRBs still have the main responsibility to assess the risks and benefits of a particular research study; ARD cannot replace the continuous monitoring and safeguarding of the patient's best interests and actual opinions/desires. • Misuse in research needs to be identified and avoided (responsibility of IRBs and researchers). • Conflict of interest (research/career vs. care for and protection of patients) remain problematic, even when an ARD exists. • Training of professionals and the IRB are crucial to implementing ARD properly, including monitoring the use and interpretation of ARD during a study. • Undue burden must in all cases be avoided
Role of proxy	• The role of proxy remains very important as a safeguard; in regard to concrete decisions, the proxy needs to balance the patient's welfare and his/her future wishes.
ARD Content, type of research	• Differentiation between various types of studies is needed and the public must be educated about these differences (e.g., what differences exist among observational studies, invasive vs. intervention studies; longitudinal epidemiological studies, cohort studies, etc.)

TABLE 2 | Overview of main controversial topics discussed in German and Israeli focus groups.

Controversial issues/dissent	Israel	Germany
Current IRB/research practices	• Disagreement as to whether non-invasive, observational studies are allowed under the current Israeli law (the heterogeneous practice might be due to the local IRB's interpretation of what a risk or benefit actually entails)	• Disagreement about whether non-invasive, observational studies are allowed under the old German law (the heterogeneous practice might be due to the local IRB's interpretation of what a minimal risk or benefit actually entails) • Disagreement about whether a once-given informed consent is still valid in longitudinal studies, during which the research participants gradually become demented, according to the current law.
Need of ARD	• Controversy about whether a power of attorney is sufficient or even a better tool than advance research directives (ARD). • Controversy about whether an ARD should also allow research that has neither a personal nor a/patient group benefit, but would be only benefit the public good.	• Controversy about whether neither ARD nor a proxy should be an ethico-legal condition to allow research with persons with dementia if the research lacks any personal benefit • Minority opinion that there is an ethical "slippery slope" in broadening the patient group benefit criteria to include any third party benefit in the future
Ethical Issues	• Lack of clarity regarding concrete rights and responsibilities of *de facto* legal or informal guardians, family proxies or a power of attorney: who is best for a person with dementia? • Uncertainty and doubts about how to monitor the well-being of research participants with dementia: neither an IRB nor a power of attorney have the skills to fulfill this type of monitoring	• Concerns about lay persons' competency to decide about research issues
Future ARD practice	• Uncertainty over whether forms or pre-formulated texts are needed and what the patients preferences might be • Disagreement about whether the low public motivation to compose an ARD/AHD can be explained by people's tendency to deny death, aging and dementia or by the Israeli cultural attitude to put high trust in family for informal care.	• Uncertainty about to whom and how information should be provided to patients/potential research candidates. • Concern that there are problems in the interpretation of ARD, similar to AHD: the documents do not comply with clinical complexity and people change their minds during the course of a disease.

participants in both countries also agreed that clear definitions of what is minimal risk/minimal burden and how exactly personal benefit vs. patient group benefit is determined is, in many cases, not easy: standards for defining these important issues do not exist. This also resulted in sharing the observation that there are heterogeneous decision policies in local IRBs in both countries regarding how restrictive or permissive research is assessed (see below and in **Table 2**).

Despite the different levels of knowledge and use found between both countries, it should be noted that the discussion about safeguard mechanisms for the use of ARD was extensive and far-reaching in both countries. Developing a monitoring system to follow the individual progress and status of each participant during the research project was one of the main concerns in both countries. Here, IRBs, researchers and legally appointed guardians were mentioned as the ones who "ensure" the well-being of research participants with dementia. Similarly, being aware of the different requirements for ARD depending on the research type (clinical, non-clinical) and the level of risk (no side effects, serious side effects; invasive, non-invasive) was a central topic in both countries. There was an overall consensus that it would be a good idea to inform patients and laypersons about the different types of research, their general risks and benefits, and to support the composing of such ARDs with forms/guidelines.

Despite these similarities, considerable differences and/or disagreements emerged in the focus groups regarding knowledge and attitudes. **Table 2** provides an overview of the main topics that were rather controversially discussed by the stakeholders in both countries. These included: whether there is a real need for ARD; how ARD should be practically implemented; ethical issues such as the role of the proxy; what autonomous decisions means, and whether monitoring and safeguarding will work; and how the current practice in IRBs about dementia research or similar cases takes place.

Interestingly, the level of knowledge and familiarity of the professionals in both countries with the concept of ARD varied. While in Israel many participants mentioned that they had been exposed to the term (not the concept) for the first time during the focus group, professionals in the German study were well acquainted with it. The reason for the latter is the current legal change (see above) in Germany. The lack of familiarity with the term in Israel might also explain the stronger focus and longer discussions devoted to the current role of proxy and guardians in Israel compared with Germany. However, most of the German professional stakeholders also showed considerable unfamiliarity with the exact legal regulation and in regard to what ARD will mean in detail for future research practice.

When talking about implementation issues, both groups emphasized different areas. While professionals in Israel very intensively discussed the role of IRBs in the implementation of ARD and in monitoring the concrete research participation, professionals in Germany discussed the role of an IRB mainly in connection to risk–benefit assessment. Overall, there were different interpretations about the precise meaning of "minimal risk" or the exact definition of "potential benefit" for the research participant. The participants' experiences varied in respect to how these two important criteria were currently interpreted and assessed in local IRBs. Dissent or even obscurity within both groups was expressed regarding whether, under the current law, studies with pure patient group benefit, but no or minimal risk (e.g., social scientific observational studies in care facilities or diagnostic studies based on blood examples) would be approved by different IRBs. Some participants reported they had never heard of any problems, while others reported that researchers had to go abroad for this kind of research because it is handled very strictly in their respective country. One German professional, as well as a patient organization representative, expressed skepticism toward ARD because the ARD practice already shows limitations regarding their interpretation.

Finally, **Figure 1** presents an overview showing how far the process of discussing and implementing ARD in Germany and Israel has evolved. The figure indicates that while Germany is a bit more advanced regarding how to discuss the main concepts and involvement of the public, both countries are still in a rather preliminary stage of ARD implementation.

DISCUSSION

The present article compared professionals' knowledge and attitudes regarding ARD in Israel and Germany. We identified a large spectrum of themes which were similarly discussed in both national focus groups, along with several differences. While the concept of ARD is relevant to other populations—such as psychiatric patients, ICU ventilated patients or patients with traumatic brain injuries, in both groups the focus was mainly on dementia, as a unique field for ARD implementation. Indeed, other populations were rarely mentioned by the participants in the current study because of two main reasons. First, the primary expertise of the participants in both countries was dementia. Second, while all these conditions are associated with difficulties to initiate ARD, unlike dementia, the other above-mentioned conditions do not have a specific, foreseeable trajectory and in some cases, chances of reversibility exist. Importantly, AHD is currently being intensively discussed for psychiatry (33), especially to enhance patients' self-determination through potential phases of decision incapacity. However, as this review (33) reveals, the acceptance and uptake rate for AHD in psychiatry is still very low, and the question remains whether the willingness for research participation is not even lower.

Another important finding of our study is that there is a need to discuss the relevance and helpfulness of ARD in relation to different types of research. Research in dementia has tremendously evolved from single-patient studies from the Alois Alzheimer's period to large-scale, international epidemiological or intervention studies with often more than thousands of patients. As the so-called "subsidiarity principle" (34) of the current EU directive (15) indicates, if the research in question can be answered by including competent patients, this should be the preferred research method. Individuals who lack competence should only be included in the study design if the research cannot be conducted without their inclusion. This subsidiarity principle for research with persons with dementia was already proposed in early work published on the topic of ARD in the 1990s (35). However, although much of the research about healthy aging will

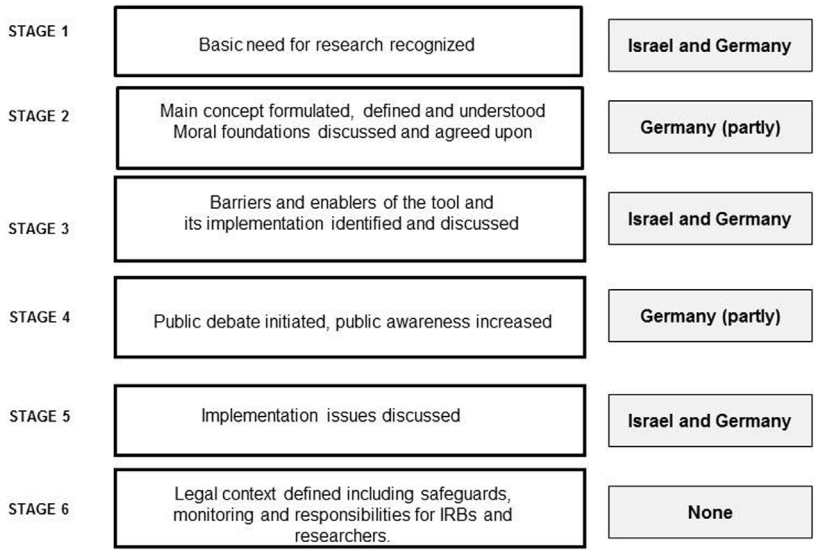

FIGURE 1 | Suggested process of defining, regulating, and implementing ARDs and the observed situation in Germany and Israel.

very likely include competent persons at first, longitudinal studies might have to deal with the loss of such competence—a point that can be addressed by the implementation of ARD ahead of time.

Another crucial topic addressed in both countries related to risk–benefit assessment and, more specifically, to the underlying definitions of risk and benefit. Regulations in Germany and Israel allow research with persons lacking competence only under the minimal risk/minimal burden paradigm (36). However, the question of what "minimal risk/minimal burden" actually entails in practice, is not always easy to answer (36). The latest version of the Declaration of Helsinki by the World Medical Association (37) addresses the topic of research with persons being unable to give informed consent in Article §24. Although the concept of ARD is not explicitly considered there, it is stated that informed consent should be obtained from the "legally authorized representative in accordance with applicable law". In the U.S.A., according to the National Bioethics Advisory Commission, third-interest research with persons who are cognitively impaired is allowed under restrictions for proxy consent and minimal risk or if a legally authorized representative consents and there are no signs of objection by the incompetent person (38). The American College of Physicians (39) has added that if research participation entails more than minimal risk, a national IRB should review the research application.

In contrast, the German expert discourse on ethics and law is less permissive. For example, Germany has still not signed the "Oviedo Bioethics Convention" (40) developed by the Council of Europe in 1997. This is because the convention allows third-interest research with cognitively impaired persons. However, the Central Ethics Board of German Chamber of Physicians (41) suggested allowing such research, but only if there is a minimal burden, consent by a legal representative, and no opposing behavior on the part of the patient. In Israel, these topics are even less developed.

In sum, while ARD is an emerging concept internationally, a number of unsolved practical issues and ethical questions still await further clarifications. However, ARD remain an important tool for future research given their overall advantages. To advance knowledge in this area, it seems important that professionals from law, ethics and social sciences, as well as researchers in the field of healthy aging engage in a joint interdisciplinary and international discourse to exchange experiences regarding both the limits and benefits of such a tool, and to ensure best practice regarding information, monitoring and safeguarding mechanisms.

Knowledge About ARD in the Public and Scientific Community

Our inspection of the literature and the knowledge emerging from the focus group study with professionals in the two countries showed that, while the importance of conducting research in the area of dementia and involving persons with dementia is increasing worldwide, the role and understanding of ARD to attain this goal is still in its developmental phases.

Conceptually, the definition of ARD is still blurred and the uniqueness of this tool compared with surrogate decision-making and other venues for anticipated decision-making, are not always clear. This theoretical fuzziness might explain the fact that public perceptions and knowledge about this tool is also lacking in research attention; the few studies that did examine these subject found very low prevalence rates. However, the low prevalence rates reported by these studies underline the importance of expanding knowledge in this area. Potential ways to attain this goal include: engaging the general public in a discourse on the topic *via* print and social media, and engaging with specific groups of affected persons, such as persons diagnosed with Mild Cognitive Impairment or early dementia *via* memory clinics or

patient organizations. Indeed, a new project was recently initiated by the authors with the aim of elaborating in more detail on how to improve the public and scientific community's knowledge and interests in ARD. In this new project (2018–2020), we will explore the extent to which patients and family members' perspectives can actively contribute to a better conceptualization of ARD and related concepts, such as Advance Care Planning and communication about dementia, especially as prodromal and early diagnosis are undertaken more often.

In sum, while the legal status of ARD is still to be determined in each country regarding national laws and recommendations, it will gain relevance as more countries strive for legal and ethical harmonization in medical research, following the three main international documents dealing with the topic: the Council of Europe Convention on Biomedicine and Human Rights, its additional protocol on Biomedical Research, and the EU Directive 2001/20/EC on Clinical Trials on Medicine Products (15). Furthermore, if international cohort studies gain increasingly more relevance for healthy aging research, the interest in harmonization regarding research ethical standards might also increase, and ARD might serve as a promoter of this research.

ARD as a Promoter of Healthy Aging Research

Similar to AHD and as discussed above, autonomy and self-determination are underlying principles of ARD (20). Thus, ARD might promote healthy aging in the area of dementia through two different although complementary avenues:

First, it might increase the amount of research conducted in the area by increasing the participation of persons with dementia who have consented *a priori* to be part of research projects. Second, it might facilitate researchers to conduct research in the field of dementia prevention if they know there is an available pool of persons who have completed ARD and might serve as potential participants. However, the first step in the process of executing ARD in order to improve the quality of life and death of persons with dementia should be providing knowledge and extending the awareness of professionals regarding the meaning and importance of this tool. Also the leading European patient organization, Alzheimer Europe (42) supports the use of advance directives for research. However, they mentioned that many practical and ethical issues regarding implementation, information and safe guarding are not yet sufficiently solved (pp. 59ff).

Another critical issue that remains is to clarify the meaning of "benefit" and for whom. For some, the distinction between patient group benefit and third-party benefit is too vague and even problematic. However, the current legal shift in Germany allowing research for the same "class of patient" resembles the existing U.S. guidelines (39). This additional dimension of benefit assessment needs additional normative justification and clarification. The first justification refers to the collective dimension. This is because historically any benefits related to the risk–benefit assessment in research ethics was addressed only to the individual patient-participant (43). The newer focus is now on other patients, rather than the patient-participant; this is based on the assumption that any clinical research should also have social value. To gain such social value, Buchanan and Miller (43) have suggested that any research design should explicitly address public health considerations. This would entail considerations that research should focus on treatments, cost effectiveness and fair access to such new treatments for a larger patient population. Research for healthy aging is likely to be in line with these social value conditions, but it is necessary to show this in a case-by-case manner. The second point refers to the conceptual and ethical issues: how and why to distinguish between "patients of the same class" and "other patients" when assessing collective benefit. Regarding patients' altruistic motivations for research participation, for many it might be irrelevant whether only dementia patients would benefit from the research or only patients with another condition. The assumption that patients prefer to help patients within the same class of disease has—to our knowledge—not yet been empirically substantiated. ARD would be a chance to overcome this difficulty by giving citizens their own opportunity to set priorities.

Limitations of Our Study

Comparing two countries such as Israel and Germany allows only limited representative knowledge regarding the professions on an international level. By covering various fields of expertise, we increased heterogeneity. The experts in our study were not randomly selected (which is always a difficult issue for expert interviews), but were identified by their professional backgrounds documented by their work profiles or academic CVs. Only two German experts and none of the Israeli experts had explicitly published on ARD, so for the most part, we had no particular ideas about what they would say during the focus group discussions. However, similar studies in additional countries, and including a wider variety of participants, will help provide a broader, more sustained picture.

Summary and Conclusion

Implementing a new research or organizational tool is a step-by-step procedure requiring a thorough understanding of the current state of knowledge, as well as the challenges and hurdles ahead. Thus, this article aimed to describe the state of knowledge in the area of ARD and to discuss the main ethical and practical dilemmas in their implementation, while comparing Israeli and German professional stakeholders' perspectives on the topic.

Overall, from our qualitative exploration of focus group discussions, several similarities and dissimilarities between the countries emerged. While differences in the cultural and legal environments of both countries might explain these finding, they may also reflect the fact that these societies are in different stages of the ARD implementation process. First, as represented in **Figure 1**, a complete analysis of the focus groups showed that in both countries the evolution of ARD seems to follow a process similar to the development and implementation of new medical technologies. While both countries are in the midst of a developmental process and have recognized the importance and the need for ARD as a tool for expanding healthy aging research, Germany is in a more advanced stage than Israel.

This is because of the EU regulation process, which indicates the influence of international harmonization on these research ethical issues.

As long as improving quality of life and promoting autonomy continue to be core elements in the process of healthy aging, efforts to advance knowledge and solve dilemmas associated with ARD implementation is of the utmost importance. This article provided a small but important step in this direction.

ETHICS STATEMENT

The University of Haifa Ethics Committee approved this project. In Germany, no IRB is needed for collecting data by interviewing experts or professionals' perspectives. However, all participants, in Germany as well as in Israel received an information sheet and a consent letter explaining the aim of the study and ensuring their consent for a pseudonymonized data analysis and publication of data in a fully anonymized way.

AUTHOR CONTRIBUTIONS

PW and SiS conceptualized the questionnaire for the focus groups and jointly analyzed the material and wrote the article. SiS facilitated both focus groups as the moderator and conducted the additional expert interviews.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fmed.2018.00081/full#supplementary-material.

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REVIEW
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Genomic Instabilities, Cellular Senescence, and Aging: *In Vitro*, *In Vivo* and Aging-Like Human Syndromes

*Gabriel Lidzbarsky†, Danielle Gutman†, Huda Adwan Shekhidem, Lital Sharvit and Gil Atzmon**

Department of Human Biology, University of Haifa, Haifa, Israel

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Edited by:
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***Correspondence:**
Gil Atzmon
gatzmon@univ.haifa.ac.il

†These authors have contributed equally to this work.

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As average life span and elderly people prevalence in the western world population is gradually increasing, the incidence of age-related diseases such as cancer, heart diseases, diabetes, and dementia is increasing, bearing social and economic consequences worldwide. Understanding the molecular basis of aging-related processes can help extend the organism's health span, i.e., the life period in which the organism is free of chronic diseases or decrease in basic body functions. During the last few decades, immense progress was made in the understanding of major components of aging and healthy aging biology, including genomic instability, telomere attrition, epigenetic changes, proteostasis, nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and intracellular communications. This progress has been made by three spear-headed strategies: *in vitro* (cell and tissue culture from various sources), *in vivo* (includes diverse model and non-model organisms), both can be manipulated and translated to human biology, and the study of aging-like human syndromes and human populations. Herein, we will focus on current repository of genomic "senescence" stage of aging, which includes health decline, structural changes of the genome, faulty DNA damage response and DNA damage, telomere shortening, and epigenetic alterations. Although aging is a complex process, many of the "hallmarks" of aging are directly related to DNA structure and function. This review will illustrate the variety of these studies, done in *in vitro*, *in vivo* and human levels, and highlight the unique potential and contribution of each research level and eventually the link between them.

Keywords: aging, cellular senescence, DNA damage, telomeres, epigenetics

GENERAL INTRODUCTION

During an organism's lifetime, cells are constantly exposed to exogenous and endogenous stressful agents. Cells can cope with these stressors by various response mechanisms, or in case of irreversible damage, programmed cell death (apoptosis), or permanent cell-cycle arrest (cellular senescence). Cellular senescence is characterized by a halt in cellular replication, accompanied by a specific molecular phenotype (1–3). This phenotype can be the result of a few factors, such as accumulation of DNA damage, telomere attrition, and various epigenetic alterations (4).

In this review, we will highlight the major efforts to unveil the role of senescence in healthy aging by three main strategies: *in vitro*, *in vivo*, and human. Each strategy has advantages and limitations, yet when stratified and combined can elucidate molecular and physiological mechanisms and phenotypes, in general, and in healthy aging in particular.

CELLULAR SENESCENCE AND PHYSIOLOGICAL AGING

The aging process is a complex trait that combines different biologic levels. Aging at the organism level includes failure to maintain internal environment and regular function, alongside increased susceptibility to diseases. Aging at the tissue level may involve, for example, chronic inflammation, which in turn contributes to cardiovascular and neurodegenerative disorders (5). The mechanisms of aging are affected by cellular and non-cellular pathways. The buildup of chronic stress, for example, is significant for the aging phenotype, but it is an organism-level phenotype (6). Structural deterioration of the body will influence an organism's ability to forage, resulting in bad nutritional state that in turn will speed the aging process. Cellular senescence is one of the cellular pathways contributing to organismal aging. This process is triggered by several factors such as accumulation of DNA damage, telomere attrition, and various epigenetic alterations and involves the activation of permanent cell-cycle arrest. Yet, unlike quiescence and other kinds of no-proliferation conditions, it is followed by a typical gene expression, metabolic activity, and a senescence-associated secretory phenotype (SASP). Cellular senescence is a multistage path. Once activated, the arrested cells shift from unstable to steady cell-cycle arrest, in a procedure that involves p21, $p16^{Ink4a}$, and p53 (**Figure 1**). Next, alterations in chromatin methylation are generated. Senescent cells can accumulate in tissues and organs and can ultimately result in tissue lesions that will cause organ dysfunction (7, 8), and thus the cellular processes can lead to organism-level decay in function and health.

FROM CELL CULTURE TO HUMAN SUBJECTS: STRATEGIES IN AGING RESEARCH

In Vitro

Cell cultures are used in biological research since 1912. Carrel (10) isolated and cultured chicken cells to study aging processes (10). He concluded that the single cell is immortal, and aging and death are multicellular organism-related phenotypes. It was not until 1961 that Hayflick and Moorehead proved that Carrel was wrong and normal cells have limited proliferation capability in culture (10–12), also known as the Hayflick limit. Hayflick and Moorehead also discovered that normal cells looked "old" after they exhausted their replication potential. They speculated that single-celled replicative senescence contributed to the organism's aging (11), which promoted the use of cell cultures to study aging processes in the full organism (12). Since the study by Hayflick and Moorehead, *in vitro* studies became the basis for every study in human biology. *In vitro* studies enable comparisons between many types of cells including mesenchymal stem cells, peripheral blood mononuclear cells, lymphoblast cells, muscle satellite cells (SCs), skin fibroblasts, endothelial cells, and embryonic stem cells, cultures from different organisms and different donor's ages, enabling use for studying the genetics and biology of aging. Another advantage of *in vitro* studies is the capability to easily perform manipulations and treatments directly on the cells and to study the responses isolated from the original environment. The biggest limitation of *in vitro* studies is the translation to a whole organism (13). In culture, cells "behave" differently due to the loss of the cross talk between cells and the extracellular matrix from other regions in the body (such as immune system or hormonal signals). Though it helps with eliminating background pathway signaling noise when investigating certain mechanisms or pathways, it is a setback when trying to translate the effect of a manipulation or treatment to the whole organism. In attempt to compensate for the main *in vitro* limitation (i.e., translation drawback), researchers turn to *in vivo* (animal model) studies.

In Vivo

In vivo studies can further test the effect of a manipulation or treatment, either targeted or scattered, on the whole organism. Most of these biological models offer many advantages over humans, for instance, their basic biology and genomes are well documented and are easier to manipulate genetically. Furthermore, they have much shorter life spans than humans, enabling longitudinal studies, while ethical issues, long natural life span, environmental influences, genetic heterogeneity, and various other limiting factors complicate the use of human subjects in aging research. Regardless of the advantages listed earlier and the eminent contribution to our understanding of the aging process, the use of animal models in aging studies has its own limitations. Aging is not a simple process, and there is no genuine agreement about what it is and how to define it (14, 15), despite the agreement on being a multifactorial and complex phenomenon. Additionally, there is conflicting evidence about aging as a process that is similar across all organisms or particular to each species (15, 16). Therefore, it is important to draw attention to the fact that animal models are usually chosen for convenience rather than for specific features applicable to human aging. Hence, choosing the suitable animal model to answer the specific question we aim to understand is of high importance in these types of studies. Among the most prevalent aging model organisms are *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus*. As a single-celled organism, *S. cerevisiae* is easily grown, manipulated, and observed; together with a well-characterized genome that bares much resemblance to bigger and more complex organisms, this model organism among others is a convenient platform for the study of the aging phenotype. Another important model system for studying a range of biological processes, including aging, is the nematode *C. elegans*. *C. elegans* has a short adult life span of ~2 weeks and a well-documented anatomy which is visible using a microscope. This enables easy observations of aging-related changes in the whole organism, in specific tissues and organs, and even on molecular and cellular levels (17–21). The classic genetic model organism, *D. melanogaster*, is also used

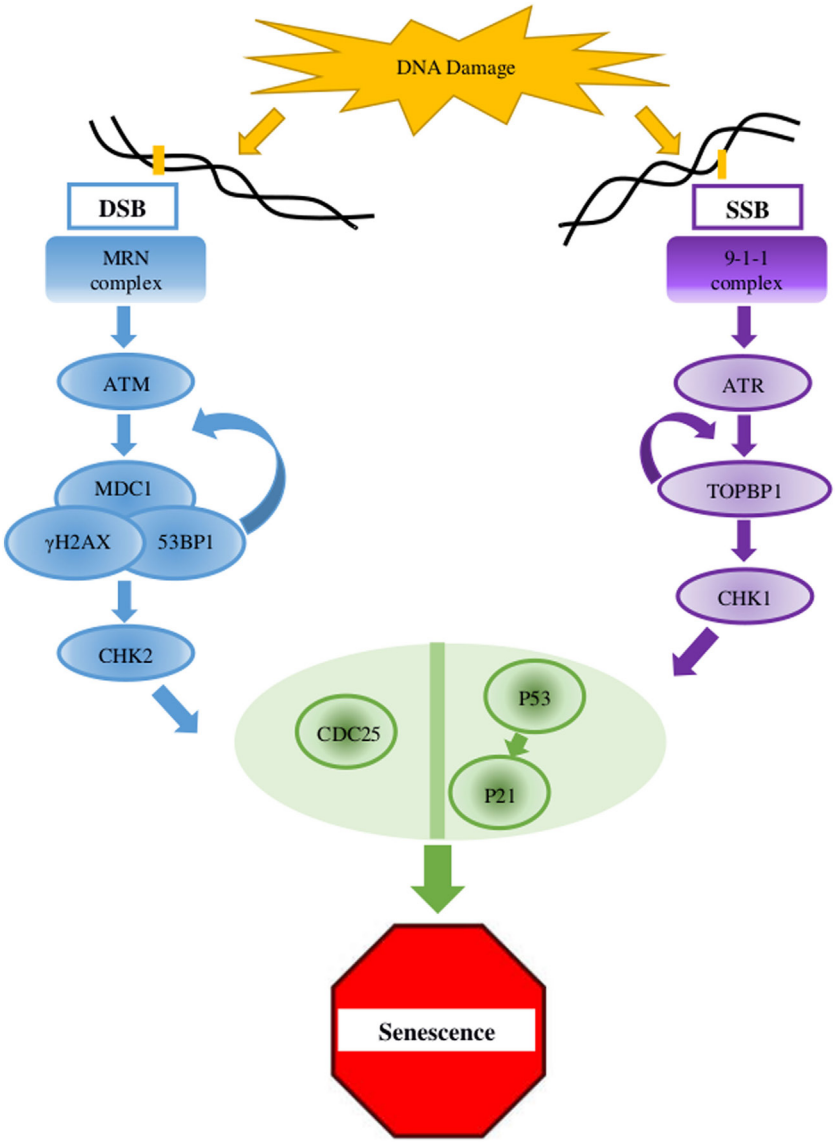

FIGURE 1 | Key elements in the DNA damage response (DDR) pathway. In case of double-strand breaks (DSB), the DNA damage sensor MRN complex recruits the protein kinase ATM which activates γH2AX at the damaged site. γH2AX connects to MDC1, and this complex amplifies the activity of the MRN complex which, in a positive feedback, amplifies the ATM activity and the dispersal of γH2AX along the chromosome. MDC1 and 53BP1 further mediates the activation of CHK2 which carries the signal to distant locations on the genome. For single-strand breaks (SSB), the protein kinase ATR is activated and amplified by the 9-1-1 complex and TOPBP1, which also mediates the activation of CHK1. The signaling pathway cascades toward the key factors p53 and CDC25. When the lesion is repaired, the DDR complexes are dismantled (2, 4, 9).

in the study of aging. Studies conducted in these flies have identified single gene mutations that influence their life span. One of the strengths of Drosophila as a model organism is the capability to illustrate how genes that have an established role in regulating organismal life span particularly influence cellular and tissue function, how they work together, and how their tissue-specific functions might be linked (22–25). That said, Drosophila is far from being a good model for human aging as they share only 60% of the human genome. A better similarity is achieved with *M. musculus*, the mouse. It is the most commonly used model in biological research for various reasons. Mice are small, have a short generation time, and an accelerated life span which means they are not expensive and require only little space and time, compared to larger animal models. Another important reason is the fact that the mouse genome is well documented and can be easily manipulated. In addition, they are biologically similar to humans, exhibiting many of the same diseases and conditions. Nevertheless, mice do not develop several important age-related diseases naturally (e.g., atherosclerosis and diabetes), a fact that limits their potential as an aging model. All the organisms described earlier are short-lived, which is one of their desired traits as model organisms. However, that may not be appropriate for the

study of human aging. Thus, in recent years there have been more studies conducted on non-model long-living organisms such as the naked mole rats and bats, which may be more appropriate models in understanding healthy human aging. The naked mole rat (*Heterocephalus glaber*) is a very important non-model organism in cancer and aging studies. This subterranean, mouse-sized, eusocial rodent is known as the longest-living rodent, living 4–17 years in the wild and with captive individuals demonstrating exceptional longevity that exceeds 30 years (26)—almost an order of magnitude longer than mice. Moreover, until a few years ago no cancer cases were reported in NMRs, and researchers failed to induce tumorigenesis, placing this rodent as a novel model for cancer studies. Bats are the second most speciose mammalian order after rodents. Little brown bats (Myotis) are the smallest bats (3–30 g) with the highest longevity records (*Myotis myotis* live for 37.1 years and *M. brandti* live for 41 years). Nevertheless, longevity is generally high in all bat lineages, which makes them an interesting model in biogerontology. One of the most interesting non-model organisms adopted for aging research is the Bowhead whale (*Balaena mysticetus*), which is estimated to be the longest-living mammal, reaching the age of ~200 years and also one of the biggest species, with length and weight of 20 m and 100 tons (6, 27). Bowhead whales live in arctic environment and are well adapted to these harsh surroundings. They are considered to be resistance to cancer and age-related diseases, and thus, though research is very technically complicated, the study of Bowhead whale in the context of longevity could improve our understanding of molecular mechanisms of healthy aging (27).

Human Aging-Like Syndromes

The limitations of *in vitro* and *in vivo* studies, and the great power of inferring from human studies on the human population, lead researchers to focus on aging-like human models. There are obvious moral and ethical limitations when working with human subjects, for this reason, most information on human aging was obtained from various progeroid syndromes, especially Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome (28). These genetic conditions offer a glimpse into the molecular and physiological mechanisms of the aging cell and body, yet they do not capture the entire complexity of the aging and senescence phenotypes. Another approach for this purpose is using genome- and epigenome-wide association studies (GWAS and EWAS, respectively), which utilize the great improvement in whole genome sequencing technologies. Such studies have highlighted aging-related genes such as *APOE* (apolipoprotein E) (29–31) and have alleviated the dependency on *in vitro* and *in vivo* models by using direct human samples.

AGE-RELATED DNA DAMAGE AND DNA DAMAGE RESPONSE (DDR) ACTIVITY

Age-related accumulation of DNA damage has been studied thoroughly, showing correlation between age and damage levels or mutation frequency (32, 33). In the presence of DNA lesions or abnormalities, the DDR, a complex multigenic pathway, is activated and can eventually lead to cell cycle arrest (**Figure 1**) (2, 4, 9). In older organisms, accumulation of DNA damage and loss of regenerative potential consequently increase the number of senescent cells, leading to aging cells, tissues, organs (4), and inevitable death (2, 34, 35). The general term DNA damage encompasses different types of lesions in the DNA, including large chromosomal lesions such as double-strand breaks (DSBs) and small, local lesions such as single-strand breaks (SSBs) and mismatched bases. To prevent the deleterious effect of these lesions, cells have evolved four DNA damage repair mechanisms. For large DSBs, such as the case in DSBs, cells utilize homologous recombination (HR) or non-homologous end joining (NHEJ). SSBs are resolved *via* the base- or nucleotide-excision repair pathways (BER and NER, respectively) (33, 36), and mismatched bases are corrected by the mismatch repair (MMR) mechanism (37).

BER Reactive Oxygen Species (ROS)-Related DNA Damage Repair Efficiency, *In Vitro*

Wang et al. (38) tested lens samples isolated from age-related cataract (ARC) patients and age-matched patients with unrelated eye diseases (38). ARC was found to be affected by ROS and oxidative DNA damage, which is repaired by the BER pathway. The study showed that in ARC patients the expression levels of 8-oxoguanine DNA glycosylase (OGG1), a core member of the BER pathway, were significantly low. In addition, hypermethylation was demonstrated in the first exon of *OGG1*, hinting at the role of faulty DDR in the formation of ARC. Age-related BER activity was also studied with human foreskin fibroblasts derived from 20 to 64-year-old healthy donors, with similar results showing BER efficiency decrease with age. However, among several BER-related factors that were assessed, only Polβ (DNA polymerase beta) and XRCC1 (X-Ray Repair Cross Complementing 1) showed correlation between expression levels and age. In addition, a negative correlation was observed between age and the expression of Sirtuin 6 (SIRT6), which is connected to DNA maintenance and DSB repair (39), demonstrating a correlation between SIRT6 expression levels and BER quality. While overexpression of SIRT6 increased BER activity, SIRT6 knockout decreased BER activity, in the human foreskin fibroblasts (39). Related results were found in young and old rat MSCs. Here, increased cellular ROS production was observed with age. A hinting cause for the increased ROS level was the low superoxide dismutase (SOD) 1 (a central gene in the ROS response pathway) expression suggesting potential DNA damage (40). ROS is a known cause for DNA damage, from single base oxidation to single and DSBs, indicating that high ROS levels have an erroneous effect on genomic integrity (41).

DSB Repair Efficiency, *In Vitro* and *In Vivo*

A similar approach was implemented on eyelid fibroblast cells originating from different ages of healthy donors, showing that the efficiency and quality of DNA repair through NHEJ and HR pathways decreased with age (42).

The role of faulty DNA repair machinery in age-related genomic instability was also found in *S. cerevisiae* and Drosophila. Mutations in the *sgs1* and *srs2* genes [encoding for RecQ helicase,

homologous to the human *WRN* (43)] shortened *S. cerevisiae* life span through two distinct pathways: *sgs1*- and *srs2*-mutated cells stopped dividing randomly in an age-independent manner that required the RAD9 (cell cycle checkpoint control protein) DNA damage checkpoint, but late-generation *sgs1*- and *srs2*-mutated cells exhibited premature aging. The double sgs1/srs2-mutated yeast cells showed a high rate of terminal G2/M arrest. This arrest was suppressed by knockouts of *RAD51* (DNA repair protein RAD51 homolog 1), *RAD52* (DNA repair protein), and *RAD57* (DNA repair protein), hinting for malfunctioning HR. In a similar study, knockout of *DNA2*, encoding RecQ helicase-like protein, caused premature aging phenotypes including longer cell cycle time, transcriptional silencing, genomic alterations, and eventually shorter life span (44). Shaposhnikov et al. (45) used *D. melanogaster* to evaluate the effect of overexpression of DNA repair genes in several locations in the body and several time points during the life period on the Drosophila life span. Beneficial effects on life span were observed with overexpression of Hus1 (checkpoint clamp component), mnk (MAPK interacting protein kinases), mei-9 (meiotic 9, *D. melanogaster*), mus210 (Xeroderma pigmentosum, complementation group C, *D. melanogaster*), spn-B (spindle B, *D. melanogaster*), and WRNexo (WRN exonuclease, *D. melanogaster*), which control the processes of DNA damage recognition and repair (45). Myc, a key regulator protein of cell growth and proliferation, was shown to act as a pro-aging factor, probably by its ability to increase genomic instability. Overexpression of Myc in Drosophila increased the frequency of large genome rearrangements associated with faulty repair of DNA DSBs and decreased adult life span. *Myc* knockdowns demonstrated reduced mutation rate and extended life span (46). In aged mice, increased levels of DNA breaks or unrepaired DNA damage as illustrated by the formation of γH2AX (phosphorylated variant histone H2A) foci were observed (47–49). A positive effect on longevity was observed with overexpression of the human enzyme hMTH1 (MutT Human Homolog 1), which eliminates oxidized purine18 and deacetylase Sirt6 (50). Overexpression of SIRT6 promotes DSB repair by the activation of PARP1 [Poly (ADP-ribose) polymerase 1] and facilitating the recruitment of Rad51 (51) and NBS1 (Nijmegen Breakage Syndrome 1) (52) to DNA lesions.

Evidence From Omics Experiments, *In Vitro*

The accumulation of genomic abnormalities is influenced by the quality of the repair pathways, which may also decline with age. Laurie et al. (53) studied age-related DNA damage in peripheral blood cells using single nucleotide polymorphism (SNP) microarray data from over 50,000 individuals. The frequency of detectable genomic abnormalities was low (<0.5%) at birth and rose to 2–3% in 50-year-old donors (53). Peripheral blood cells were also studied using whole-exome sequencing data from DNA of 17,182 individuals lacking hematologic phenotypes. Somatic mutations were rare in young donors (~40 years old) but became more frequent with age. Furthermore, while studying subjects at 70–79 years, compared with 90–108 years, mutation frequency rose from 9.5 to 18.4%, respectively (54). In some cases, the accumulation of damage was noticeable in relatively advanced ages and not as a linear progression. Goronzy et al. (55) found that memory T cells from healthy donors showed steady increase in levels of DNA damage in different ages, up to 65 years (55). All these findings lay the basis for longitudinal *in vivo* studies in model organisms to decipher the mechanistic view of this phenomenon (i.e., accumulation of DNA damage with age) in a manageable life span.

DNA Repair in Long-Lived Animals

Analysis of two bat genomes showed that DNA repair and DNA damage signaling genes ATMh (human ataxia telangiectasia mutated), TP53 (tumor protein 53), RAD50 (DNA repair protein), and KU70 (XRCC6 protein product) are under selection in bats, suggesting that genome maintenance systems are under selective pressure in longer lived species (56). The study of Bowhead whales in the context of longevity is relatively new, but some insights have already been generated. Keane et al. (27) found duplications in genes linked to DNA damage repair and aging, such as *PCNA* (proliferating cell nuclear antigen). According to RNA-seq, both the PCNA copies were expressed. Several DNA damage and aging-associated genes, such as *ERCC1* and *ERCC3* (excision repair cross-complementing rodent repair), had unique mutations (compared to short-living animals) that were found to be under positive selection (27, 57). Mice with deleted *ERCC1* suffered from liver dysfunction and died prematurely before weaning, a phenotype that was rescued by overexpression of ERCC1 (58). It is interesting to notice that similar unique mutations in DNA repair genes (including *ERCC1* and *ERCC3*) were also found in naked mole rats and several species of bats (56, 59), hinting again at the role of DDR in longevity.

Comparative Studies of Short-Lived and Long-Lived Animals

Long-lived organisms are suggested to possess more efficient genome maintenance mechanisms than short-lived ones. For instance, in a comparative study conducted on both short- and long-lived wild bats, the MMR system and the levels of DNA damage as well as the antioxidant enzymatic activities were compared (60). By analyzing the DNA MMR proteins MSH2 (DNA MMR protein) and MLH1 (MutL homolog 1) in the liver, lung, and brain of young, adult, and old bats, the study showed that the short-lived bats presented with a decrease in protein levels and an increase in microsatellite instability antioxidant activity with age while the long-lived bats exhibited higher levels of antioxidant enzyme activities. These results suggest that the antioxidant response of those animals is important to attain a long life span. Several genes associated with the repair of DNA damage have been reported as overexpressed in long-lived subterranean rodents than in short-lived surface-dwelling rodents. In addition, when comparing blind mole rats (the genus *spalax*) to rats, the long-lived *spalax* showed more transcript abundance in genes that encode for DNA damage repair proteins (61). In another comparative study performed on mice, naked mole rats, and humans, studying the expression levels of DNA repair genes in livers found that humans and naked mole rats exhibit higher levels of expression of DNA repair enzymes that are important for DNA damage sensing and the MMR, NHEJ, and the BER

pathways (62). This evidence supports the hypothesis that long-lived organisms have better genome maintenance techniques than short-lived animals.

Antioxidants have been more attentively studied in naked mole rats than in bats. When comparing the activity of antioxidant enzymes such as SODs, catalase, and cGPx (human cellular glutathione peroxidase) in the livers of young, middle-aged, and old naked mole rats with mice, their activity was higher in at least one age class in mole rats (63). More importantly, Csiszar et al. found that relative expression of numerous antioxidant enzymes in naked mole rat blood vessels remained constant with age which may distinguish this species from other short-lived species, such as mice (64). Comparative *in vitro* studies were performed as well. One recent example of such a comparative study is the study performed by Ma et al. (59) which compared primary skin fibroblasts of 16 different mammalian species and highlighted differences in fibroblast profiles among long- and short-lived species (59). In contrast to these findings, the work of Page et al. (65) did not find correlation between DDR activity and life span. Page and Stuart (65) compared DNA repair rates and life span values by studying BER activity in brain and liver tissues from 15 species including mice, hamster, bat, sheep, dogs, pigs, and two bird species, quail and finch. The BER activity was found to be (negatively) correlated only with body mass (65).

Contradicting Evidence, *In Vitro* and *In Vivo*

Despite the body of evidence mentioned here and in other reviews, some studies report contrary results. In a study performed by Schellenberg et al. (66), using long-term cultures of hMSC, Karyotype analyses at early passage and late passage did not reveal age-related chromosomal abnormalities and SNP array analysis did not reveal passage-related changes (66). A similar trend was observed when the efficiency of DNA MMR pathway was studied using $CD4^+$ T cells from 25 to 80-year-old healthy donors. In this study, there was no connection between MMR frequency and donor's age. Only when mutations were chemically induced, there was a negative correlation between MMR efficiency and age, but only among the younger age groups, 25–40 years old; no such connection was found for the older donors (67). Similar contradictions were also established in *in vivo* studies. Though there is a documented phenotype of DNA instability in aging yeast cells, it is still under debate whether accumulation of mutations is a cause of aging for yeast. Ijpma and Greider (68) found that chromosome loss was not related to loss of viability (68, 69). Daughter cells produced in early stages of their mother cell life live as long as their progenitors, yet cells produced later had reduced life span. However, the last cell created by a specific mother cell is still capable of bearing offspring. The observed increase in division time, which corresponded with an age-specific decline in reproduction in old mother cells, was only partially passed on to the daughter cells, and they resumed normal division time after a few budding cycles (70, 71). Kaya et al. (72) studied *de novo* mutations during multiple replications in daughter cells of mother cells at different ages. Mutations were found to increase with age, but their frequency was very low, and no effect on viability was detected (72). All these observations suggest genome integrity conservation through generations and question the role of genomic changes in aging in yeast. A possible explanation for aging-related genomic instability in yeast could be found in extra-chromosomal rDNA circles (ERCs), which were shown to be correlated with premature aging and short life span in yeast. *sgs1* mutant accumulated more ERCs than wild-type cells, causing shorter life span (73), while knockouts of FOB1 (DNA replication fork blocking protein) decreased the formation of ERCs and extending life span (74).

Progeroid Diseases as Models for Aging

As mentioned earlier, age-related genomic instabilities in humans are studied through progeroid diseases. The first three genes causally linked to human aging *(according to HAGRID)* are progeroid phenotype causing genes: *LMNA* (Lamin A/C), *WRN* (Werner Syndrome RecQ-Like Helicase), and *ERCC8* (DNA excision repair protein) (75). *LMNA* is a gene coding for a nuclear envelope scaffolding protein, mutations in which lead to genomic instability which in turn cause HGPS. This syndrome serves as a model for human aging since progerin (the mutated LMNA protein) can be found in normally aging cells and is believed to cause cellular toxicity and senescence (76). Mutates *WRN* (RecQ-like helicase) causes Werner syndrome and is involved in the DNA DSB repair pathway, similar to the *S. cerevisiae SGS1* (43, 77). *ERCC8*, mutated in Cockayne syndrome patients, is a protein involved in the NER pathway, mutations in which cause high sensitivity to UV due to loss of ability to repair UV-induced DNA damage (78). These genes exemplify the effect of the DNA damage repair quality on aging, as brought forth by the previously mentioned *in vitro* and *in vivo* studies. Besides these three genes, another, more recently described gene is the *SPRTN* (SprT-Like N-Terminal Domain) gene whose translated protein product acts in the translation repair pathway, allowing DNA replication despite single nucleotide lesions. Mutations in this genes cause Werner-like progeria, probably due to their disabling effect on this replication pathway (79). Additional support for the importance of genomic integrity in the aging process is 53BP1 (p53 binding protein 1) (76). This protein is crucial for DNA DSB repair mediation and proteins' recruitment. First described as a p53 binding protein, 53BP1 recognizes DSB histone code and recruits the repair proteins to the site in different mechanisms depending on different stages of the cell cycle (80). The DNA DSB repair is crucial as it is well established that DSBs lead to premature aging and senescence (81, 82).

TELOMERE ALTERATIONS AND CELLULAR SENESCENCE

Besides direct DNA damage, cellular senescence can be induced by diverse mechanisms, the principal among them is telomere attrition. Telomeres are short tandem repeats that serve as "caps" that protect the ends of the chromosomes from being recognized as DSBs and prevent the cascade of DDR in the cell and actively participate in genome maintenance. With every cellular division, the telomeres shorten by several repeats.

Evidence From *In Vitro* Studies

In most organisms, telomere elongation is controlled by the enzyme telomerase under tight regulation to ensure sufficient number of replications, yet when this number is reached, telomere elongation is seized (2, 83). Once telomeres reach the critical length, the cells undergo senescence and stop proliferating (84). This process is believed to be the trigger for the aging process, according to the telomere theory (11, 85, 86). It is further supported by Bodnar et al. who proved that telomere elongation caused by ectopic expression of telomerase avoids the senescence phenotype (87). His work relied on one of the earliest studies linking telomere shortening to aging which was performed by Harley et al. on human fibroblast cells (88). In their paper, they describe the shortening of telomeres in aging fibroblasts alongside chromosomal abnormalities, specifically the fusion of two chromosomes at the telomeric region and chromosomal rearrangement, while hinting at a biological significance to the shortening process. Since this early study, numerous studies have emerged strengthening this association and aiming to elucidate the exact underlying mechanism of telomere shortening. Murillo-Ortiz et al. (89) studied telomere alterations using T, B, and NK cells from 20 to 25-year-old and 60 to 65-year-old donors. Treatment with concanavalin A (a mitogen of T cells) caused increase in telomere length and number of replications in the samples from the young donors, but did not improve the samples from the older donors, which exhibited loss of telomere parts, decrease in telomere length, and decreased proliferation potential (89). Age-related changes in telomere length were also established in bone marrow hMSC in a long-term *in vitro* study (90). *COMET* assay revealed higher levels of damage in cells from older donors (91). Similar results were obtained in the study of $CD34^-$ and $CD34^+$ cells isolated from healthy donors of different ages. However, some of the cells exhibited telomere shortening that was not correlated with age. It seems that $CD34^+$ cells from older donor suffer from increased non-telomeric DNA damage, but the variation among the cultures hints for multiple factors contributing to DNA damage (92).

The Question of Telomere-Related Senescence in *S. cerevisiae*

For *S. cerevisiae*, various studies were performed on the effect of missing/broken telomere and mutated telomerase on the physiology of the organism. Genetic manipulations of *S. cerevisiae* cells caused decreased growth, irregular shape, and eventually, cellular senescence (69). Several genes, such as *EST1* (telomere elongation protein), *EST2* (telomere reverse transcriptase), *EST3* (telomere replication protein), *TLC1* (template RNA component), *RAD9*, *RAP1* (DNA binding protein), *CDC13* (cell division control protein 13), *TEL1* (serine/threonine protein kinase), *MEC1* (serine/threonine protein kinase), and *MRC1* (macrophage mannose receptor 1 precursor) were studied in connection to telomere-related senescence; however, despite the extensive experimental work put into using mutated cells, the role of eroded telomeres in "natural" cellular senescence in yeast remained questionable (93). For example, *EST1-4* (ever short telomere) mutants began to lose viability after 60 doublings, but late knockout cultures continued to maintain proliferation potential (94). Cells with mutated telomerase exhibited irregular morphology and short telomeres, but these changes did not cause deadly damage and determinate senescence (95). One hypothesis connects aging to telomere erosion through the transcription of subtelomeric genes. Genes located in subtelomeric regions are affected by transcriptional silencing which was found to change in an age-related manner. Kim et al. (96) found that silencing of genes in subtelomeric regions declined during the cell's senescence, hinting at a connection between the transcription of subtelomeric regions and cellular senescence in yeast (96). The work of Austriaco and Guarente (97) reinforced this model, as they found that mutated telomerase extended life span (relatively to the wild type), probably by hanging the silencing procedure in the subtelomeric locations (97).

Telomere Alterations in *C. elegans*

The evidence for the role of telomere attrition in the senescence of *C. elegans* are contradicting and are influenced by the worm's unique physiology, as the adult worm go through a short reproductive stage, followed by a "post-mitotic life" with a definite number of steady post-mitotic cells (98, 99). Overexpression of HRP1 (Heterogeneous nuclear Ribonucleo Protein 1) was found to increase telomere length and, subsequently, the life span of transgenic worms. The resulting prolonged life span was reliant on *DAF16* (Forkhead box protein O gene, *C. elegans*) (100), which codes for a FOXO (Forkhead Box protein O) transcription factor and is required also for the effect of the insulin/IGF-1 pathway on life span in *C. elegans* (98). This connects to the first life span-related gene that was discovered in *C. elegans—AGE-1*. *AGE-1* encodes a phosphatidylinositol-3-kinase that functions in the insulin/IGF-1 signaling pathway. Mutations in this gene cause delay in age-related deterioration of body movement and muscle deterioration a twofold extension of the life span (17, 101, 102). Opposing results were obtained by Raices et al. (103) that found no correlation between telomere length and the life span of *daf-2* and *daf-16* mutants. Furthermore, a study of different wild-type populations with diverse telomere lengths found again that the length of the telomeres was not correlated with life span (103). Similar phenomena were observed in mutants of *TRT*-1, a catalytic subunit of telomerase. The mutants reproduced regularly for several generations but eventually became sterile (104). The telomeres shortened by ~125 nucleotides per generation and suffered from sequence abnormalities, but the mutation and other telomere-shortening manipulations did not affect post-mitotic aging (104, 105). Mutations in *MRT-2*, a gene in the same pathway as *TRT-1*, caused similar phenotypes including telomere shortening, accumulation of DNA damage, and sterility. Similarly, the mutation had no effect on life span (106).

Relevance of Drosophila and Mice in the Study of Telomere-Related Senescence

While most organisms have a tandem repeat-based telomere and a telomerase for its maintenance, Drosophila telomeres are composed of randomly ordered retrotransposable elements that are maintained by retrotransposition (107–110). Although the length of the drosophila telomere is close to the human telomere (~10–12 kb), its structure is much more complex since each building block contains its own promoter regions, coding

sequences, and regulatory elements (110, 111). These might be the reasons why there are no evidence for connection between telomere shortening and aging in Drosophila. Walter et al. (112) found that like *C. elegans*, the length of the telomeres in Drosophila did not affect life span, but it was correlated with fertility and fecundity (112). Study of age-related transcriptional changes did not find any telomere-related modifications (113). As in *C. elegans*, the FOXO-mediated insulin/IGF-1 pathway can affect the Drosophila life span (114), but a possible connection to telomere length was not studied.

Similar to Drosophila, the relevance of mice telomeres studies is also debated and unclear. Several studies show that mice with shortened or lengthened telomeres exhibit decreased or increased life span, respectively (115–119). The premature aging of telomerase-deficient mice was reverted when telomerase was genetically reactivated in aged mice (120), and systematic viral transduction of telomerase in adult wild-type mice delayed normal physiological aging (121). Mice with telomerase deficiencies exhibited signs of accelerated aging, but only after several generations and that overexpressing telomerase did not alter aging (122). The delayed phenotype implies that for senescence activation, telomeres need to be shortened extensively, in a manner that might not be realistic during the regular mouse life span. Mice are interesting models for the research of human telomere diseases. Telomerase dysfunction in humans causes a disease called dyskeratosis congenita (DKC), which shares some features with telomerase-deficient mice (123). However, the use of mice as a model for telomere-related human aging and aging-related human diseases is very questionable since the telomeres of most laboratory mice are 5–10 times longer than in humans (~40–50 kb), yet their life span is 30 times shorter (111, 124). Like *S. cerevisiae*, although genetic manipulations of telomere and telomerase may influence the organism's life span, this effect might be overlooked while observing naive mice.

Telomere-Related Senescence in Long-Lived Animals

In a study conducted on four wild populations of long-lived bats, telomeres were shown to maintain their length in blood fibroblasts in the *M. myotis* species, and similar to humans, they also showed no signs of telomerase expression (125). In naked mole rats, genes involved in the function and regulation of telomerase, Tep1 (telomerase-associated protein 1) and Terf1 (telomeric repeat binding factor 1), were found to have undergone positive selection which may contribute to their slow rate of aging, though contradicting results were also published (126). For instance, a different study established that similar to mice (but unlike humans), naked mole rat somatic cells express telomerase, although at lower levels, and are not amenable to telomere-dependent replicative senescence. Gomes et al. (124) studied the telomeres of the bowhead whale lung fibroblast cells and found that the average telomere lengths was ~9 kb, in resemblance to human telomere length (124). The bowhead whale telomerase had repressed activity as well, again, similar to human telomerase (124, 127). Lai et al. (128) tested cultured bowhead whale lung fibroblasts at different population doublings and found age-related telomere shortening (128).

Human Diseases—Telomeropathies

In humans, early telomere attrition or exhaustion leads to telomeropathies (telomere syndromes) and age-related diseases (129). Telomeropathies are divided into two subgroups: primary and secondary telomeropathies. Primary telomeropathies are disorders of impaired telomere maintenance, or in other words, telomere disorders, while secondary telomeropathies are disorders in which the main mutated gene has a role in DNA repair, thus affecting telomere maintenance without actual damage to the telomere maintenance biology (130, 131). As previously mentioned, human genetic diseases are the main mode of "*in vivo*" research in humans. Almost all secondary telomeropathies, such as Werner syndrome and Hutchinson–Gilford progeria, are associated with premature aging and increased disease risk. Yet, most of the primary telomeropathies, such as the various forms of DKC, do not present with a progeroid phenotype but do have a wide phenotypic range which includes bone marrow failure, hair loss, emphysema, liver cirrhosis, osteoporosis, and pulmonary fibrosis. All these symptoms are also associated with aging, linking once again, the deterioration of bodily functions to shortening telomeres (130). A study conducted on 274 pairs of aged twins concluded that shortened telomeres can forecast death in the elderly (132). There are supporting (133, 134) and contradicting (135–137) evidence for this, yet the authors used intrapair comparisons on same-sex twins in order to eliminate biases of gender, genetic background, and age differences, providing another strong supportive evidence.

Telomere Position Effect—Over Long Distances

An additional effect of telomere shortening is the increase in expression of TPE-OLD (Telomere Position Effect—Over Long Distances) genes. Robin et al. demonstrated, using high-resolution Hi-C (an unbiased 3D chromatin capture technique), that long telomeres form chromatin loops reaching up to 10 Mb away from them. This loop is highly condensed causing epigenetic silencing of the genes in that region (called TPE-OLD genes). When the telomeres shorten, this loop is no longer able to form and in turn, the epigenetic regulation is changed to activation of the TPE-OLD genes. This happens before the telomeres reach the critical length that causes activation of DDR, thus leading to another earlier possible effect of telomere shortening on aging (138, 139). Interestingly, a following study by Kim et al. showed that one of the TPE-OLD sensitive genes is *hTERT*, the core reverse transcriptase component of telomerase (140). This is also supported by the abovementioned studies of subtelomeric regions performed in yeast.

SENESCENCE-RELATED EPIGENETIC ALTERATIONS

Epigenetics as a field, and specifically epigenetics of aging, has gained much interest in recent years. According to Pal and Tyler (141), genetics only explain 20–30% of the aging phenomenon and researchers now aim to elucidate the remaining 70–80% mainly through epigenetics. Epigenetics can be broadly defined

as changes in gene regulation without changes to the DNA coding sequence. It encompasses a range of possible changes; DNA methylation (142), histone modifications (143), various non-coding RNAs (144), and recently emerging evidence show that change in chromatin structure offers epigenetic regulation as well (145).

DNA Methylation

Age-related epigenetic modifications were shown in long-term cultures of hMSC. DNA methylation profiles of early and later passage were compared and revealed highly consistent senescence-associated (SA) modifications at specific CpG sites (66). Similar results were obtained in a long-term *in vitro* study of bone marrow hMSC. DNA methylation analysis revealed methylation changes between early and advanced passages. At early passages, 61.6% of all CpG islands were methylated while later, methylation decreased to 44.7% (90). A related phenotype was also observed in skeletal muscle stem cells (SCs) from young and old mice. Epigenetic profiles revealed age-related accumulation of epigenetic changes (145). Additionally, DNA methylation profiles were compared between different passages in order to identify SA changes. 1,702 CpG sites were SA hypermethylated, and 2,116 CpG sites were SA hypomethylated. SA hypermethylation was enriched in inter- and intragenic regions, and in the 3′UTR, while SA hypomethylation was highly enriched in intergenic regions (146).

The gene *dDNMT2* (DNA methyltransferase) was found to be necessary for maintenance of the average life span of the flies, as mutants suffered from shorten life span. Overexpression of *dDNMT2*, however, extended *Drosophila* life span (147).

DNA methylation is also used as an "aging clock" to predict a person's age. Horvath has provided a breakthrough "epigenetic clock" in his study from 2013. He used 8,000 samples from 82 publicly available datasets of Illumina DNA methylation arrays, including 51 tissues and cell types. This clock was able to detect the age of the sample using only 353 CpGs (148). This remarkable clock was later further improved, using fresh human blood samples, and now contains just three CpG sites (149).

Age- and Radiation-Related DNA Methylation, *In Vitro*

Koch et al. (146) studied age-related methylation profile in bone marrow hMSCs under several conditions and after different number of passages. Their results reveal that ionizing radiation (IR), although connected to DNA damage, did not affect age-related methylation profile. Chemical immortalization of the cells increased telomere length, but the cells still exhibited a senescence-related methylation profile. The only treatment that completely inhibited the age-related profile was "reprogramming" the cells back to their pluripotent stage (induced pluripotent stem cells) (146). It seems that although senescence has an epigenetic regulation, IR and immortalization are not connected to this process.

Histone Deacetylation—Sirtuin 2 (SIR2) and RPD3, *In Vivo*

Epigenetic alterations were also found to play a major role in *S. Cerevisiae*, *C. elegans*, and *Drosophila* life span. The histone deacetylase SIR2 was found to extend yeast life span when overexpressed, as was found in worms and flies (150, 151). A double mutant of the *C. elegans SIR2* homolog significantly induced life span, and analysis revealed that the *sir-2.1* functions upstream of *daf-16* in the insulin-like signaling pathway (152). Also, it was found that during aging, histone H4K16 acetylation increases while H3K56 acetylation decreases (153). This is thought to be a result of the decline of SIR2 that occurs naturally during aging, which leads to H4K16 deacetylation (154). Moreover, all histone protein levels were found to descend with age which has a direct effect on the life span of the cells (155). RPD3, another histone deacetylase targeting H4K16, was also found to affect longevity in several organisms. *RPD3* deletion increased *S. cerevisiae* life span by increasing silencing at three loci, the silent mating type *(HMR*), subtelomeric, and rDNA loci (96). Similarly, a fractional decrease in the levels of Rpd3 resulted in a 30–50% increase in life span of Drosophila (156, 157). Yet, Drosophila life span was not affected through gene silencing. It seems that in flies, the two deacetylases, SIR2 and RPD3, function opposingly at the euchromatin influencing gene expression and affecting longevity (156).

Age-Related Histone Deacetylation—Sirtuin Family, *In Vivo*

In mice, numerous sirtuin paralogs were found to improve different characteristics of aging (158, 159). Transgenic overexpression of SIRT1, an ortholog of the histone deacetylase SIR2 in yeast, improved healthy aging but did not increase longevity (160). The mechanisms involved in the beneficial effects of SIRT1 are complex and interconnected, including improved genomic stability (161, 162). Other convincing evidence for the sirtuin role in prolongevity is the SIRT6 that modulates genomic stability through histone H3K9 deacetylation (163–165). Mutant mice that lack SIRT6 exhibit accelerated aging (166), while overexpression in male transgenic mice leads to longer life span compared to wild-type animals, an effect that is associated with reduced serum IGF-1 (Insulin Growth Factor 1) and other indicators of IGF-1 signaling (50). It has been reported that SIRT3 improves the regenerative ability of aged hematopoietic stem cells (167). Therefore, in mice, SIRT1, SIRT3, and SIRT6 contribute to healthy aging. SIRT6 has been associated with aging and disease protection through repression of aging and cancer-related transcription factors, promotion of chromatin changes essential for DNA repair, maintenance of telomere structure, and thus preventing genomic instability and senescence, in humans as well (168).

Histone Methylation, *In Vivo*

Greer et al. (169) discovered a crucial role for histone methylation in aging. They examined chromatin in different states and its effect on life span by investigating different enzymatic complexes and performing a targeted RNAi screening in fertile *C. elegans*. They discovered what is now known as the COMPASS complex, a key regulator of worm life span that acts in germline cells. This complex trimethylates histone H3 at a lysine residue (H3K4me3), and deficiencies in its members including the H3K4 methyltransferase SET2 extend life span (169). On the other hand, loss of function of the H3K4 demethylase RBR2 leads to a decreased life span, which agrees with the key idea that an increase in H3K trimethylation

activates chromatin, thus promoting aging. When studying histone marks associated with repressed chromatin, Maures et al. discovered that absence of the demethylase for the repressive H3K27me3 mark—UTX1, increased worm life span separately of the germline. This mark significantly declines with normal aging in soma cell, which means that repressive H3K27me3 levels allow somatic maintenance during aging (170). Related phenotypes for H3K4me3 were also discovered in Drosophila. Overexpression of *LID*, a RBR*2* homolog, extends life span, while its knockdown shortens life span of male flies by 18% (171). Siebold et al. (172) found that heterozygous mutations in two core subunits of PRC2 (Polycomb Repressive Complex 2), the histone H3 lysine 27 (H3K27)-specific methyltransferase E(Z), and the H3 binding protein ESC, enhanced life span and decreased H3K27me3 levels in adults. Mutations in *trithorax* (*trx*), an antagonist of Polycomb silencing, reversed the H3K27me3 level of the *E(z)* mutants and suppressed their enhanced longevity and resistance to oxidative stress and starvation, hinting that the reduced levels of H3K27me3 are connected to longevity and stress resistance in the PRC2 mutants (172). In drosophila, H3K27me3 seems to influence life span in an opposite manner compared to *C. elegans*. Mutations in H3K27 methyltransferase (PRC2) subunits E(Z) and ESC reduce global levels of H3K27me3 and extend life span of male drosophila by activating target genes *Abd-B* (abdominal B) and *Odc1* (Ornithine Decarboxylase 1) (172).

Large-Scale Chromatin Remodeling, *In Vitro*

Epigenetic alterations include also genomic organization and large-scale chromatin remodeling which are facilitated by smaller scale epigenetic changes such as DNA methylation and histone post-translational modifications (PTMs). Human MSCs were also used in a recent study performed by Dillinger et al. (173) showing genomic organizational changes associated with senescence. In this study, they show using Hi-C data that there is little change in nucleolus-associated chromosomal domains between proliferating and senescent cells, yet there are large satellite repeat clusters that dissociate from centromeric and pericentromeric regions in the nucleolus during senescence (173). These findings relate back to the established aging-associated genomic instability and chromatin remodeling as discussed earlier.

Chromosomal Rearrangements, *In Vivo*

An examination of chromatin structure during aging in Drosophila revealed significant age-associated chromosomal rearrangements (174). In young flies, H3K9me3 and HP1 were enriched in the pericentric regions, in chromosome 4, and in heterochromatin islands spread throughout the genome. However, this enrichment decreased in an age-associated manner, equalizing H3K9me3 and HP1 levels in the pericentric regions, chromosome 4, heterochromatin, and euchromatin. Furthermore, single-cell immunohistochemistry showed changes in nuclear distribution of H3K9me3 and HP1 marks with age.

miR's Activity, *In Vivo*

miR's also play a role in aging. Liu et al. (175) showed that miR-34 regulates age-related effects and long-term brain stability in *Drosophila*. Expression of the drosophila *mir-34* exhibits adult-onset, brain-enriched, and age-related phenotypes. While *mir-34* loss induced genetic profile of brain aging, late-onset brain degeneration, and a significant decline in life span, *mir-34* upregulation extended life span and reduced neurodegeneration evoked by human pathogenic polyglutamine disease protein (175). miRNAs also affect gene expression during the aging process in mice (176) and modulate senescence in human cell lines (177). Studies have found that miRNAs work in groups by modulating gene expression and silencing that can lead to age-dependent disease states or alternatively to longevity (178). Inherited epigenetic effects in miRNA loci cause changes in gene expression that modulate longevity (179), and miRNAs that target the insulin/IGF-1 pathway can foresee up to 47% of life span variations (180). Some loci show positive effects on life span, promoting longevity, while others show the opposite effect, causing a shorter life span (181). Ugalde et al. have reported that alteration in the expression of two miRNAs leads to a progeroid phenotype in a mouse model for a progeria syndrome by effecting key components of the DNA-damage response pathways (182).

Epigenetic Alterations in Long-Lived Animal Model

Only a few studies were conducted on the epigenome of the naked mole rats, especially in the context of aging. Sequencing the naked mole rat genome (183) showed that its genome had relatively low CpG density and higher fraction of CpG dinucleotides within CpG islands compared to the human genome. CpG dinucleotides within CpG islands contribute less to genetic variation because of their lower methylation rate. In a different study of the reprogramming of naked mole rat cells, analyzing the global histone landscape revealed that naked mole rats had higher levels of repressive H3K27 methylation marks and lower levels of activating H3K27 acetylation marks than mice which suggests that naked mole rats display a more stable epigenome that resists de-differentiation contributing to its longevity as well as to its resistant to cancer.

DNA Methylation—Twin Studies

Since the genomic methylation profile of each person is unique, comparative studies are needed. Monozygotic (MZ) twins have identical methylation and epigenetic patterns immediately after birth and in early childhood, making them a perfect platform for the study of methylation and epigenetic changes in general. Such a study performed in 2005 by Fraga et al. has provided many insights on the genomic methylation and gene expression changes in MZ twins of different ages. Fraga et al. were the first to look into epigenetics of MZ twins, and in their paper, they described the changes in methylation with age between the twins as "epigenetic drift." Epigenetic drift, as they define it, is changes in the methylation profile over time due to accumulating "small defects" in transmitting epigenetic information over successive cell divisions. In other words, changes in the epigenome of an organism over time are due to random changes in methylation (184). The effect of epigenetic drift on the genome can be small or large, depending on where those changes occur. Keeping in mind that hypermethylation of promoter regions is associated

with transcriptional repression, epigenetic drift can, and indeed does, cause changes in gene expression. The pattern of elevated methylation with age was also shown for general human populations (not twins) by Horvath (148) and Hannum et al. (185).

Histone PTMs

Human studies of histone PTMs related to aging are emerging and following are a few recent advances. There is accumulating evidence to the role of histones in memory and cognitive functions (186, 187) in the human brain. Hohl et al. showed that the histone methyltransferase SUV39H1 plays a role together with HDAC4 (histone deacetylase 4) in repression of pro-hypertrophic genes in the human heart (188) linking histone PTMs to cardiac stress and aging. Ucar et al. most recently published results indicating association of chromatin condensation with age in 27 histone-related genes. Among those genes were a few coding for histones (HIST1H3D, HIST1H3E, and HIST4H4) and histone modifiers such as EZH1 and SETD7 (189). These results strengthen the previously established patterns of reduction in core histone expression and changes in histone modifications (190).

TABLE 1 | Evidence for correlation between DNA damage accumulation, telomeres attrition and epigenetic alterations, and aging in *In Vitro*, *In Vivo*, and aging-like human syndromes studies.

	Age-related accumulation of DNA damage	Telomere attrition	Epigenetic modifications
Cell cultures (human and mice)	+	+	+
Saccharomyces cerevisiae	Debatable (contradicting results)	–	+
Caenorhabditis elegans	–	Debatable (contradicting results)	+
Drosophila melanogaster	+	–	+
Mus musculus	+	–	+
Human	+	+	+
Heterocephalus glaber (NMR)	+	Contradicting results	N/A
Bats (spp. *Myotis*)	+	+	N/A
Balaena mysticetus	+	+	N/A

CONCLUSION

Healthy aging and cellular senescence are complex processes of great interest to researchers. The multigenic nature of both of them complicates studies and necessitates creative and novel approaches in the path for understanding those phenomena. The three spear-headed strategies implemented for this purpose have brought forth much information and knowledge, yet there is still much to learn in these fields. The doubting and contradicting results in *in vivo* studies are influenced both by physiological and genetic differences between the model organisms and humans and the differences in the possible research methodologies between *in vitro* and *in vivo* studies. In many cases, the age-related phenotypes searched for and studied *in vitro* are not visible *in vivo* or not relevant for the model organism (**Table 1**.).

Molecular processes such as DNA damage repair, telomere shortening, and epigenetic alterations discussed earlier are the driving forces of the aging process in human, but their significance is varied in other organisms. Many evidence for age-related accumulation of DNA damage were found in *in vitro* studies, both in human and mice cell cultures. The connection between DNA damage and aging is emphasized by the secretion of senescence-associated proteins during cellular senescence, a phenotype which is activated by DNA damage and is common for both human and mice. Human progeroid diseases also show the connection between early aging and faulty DNA repair. In yeast, flies and mice, however, although some evidence for age-related damage and faulty DNA repair mechanisms were found, contradicting and debating results highlight the complexity of the use of these model organisms in this aging research. The study of telomeres in relation to aging demonstrates the questions derived from both physiological differences between organisms and differences in research approaches. The connection between telomere attrition and aging is very present in human aging (both in *in vitro* studies and as telomeropathies such as DKC, Werner syndrome, and Hutchinson–Gilford progeria) but not relevant in model organisms. In *C. elegans*, the evidence are contradicting. In drosophila, maybe because of the unique telomere structure, there are no evidence connecting telomere attrition to aging. In yeast and mice, genetic manipulations enabled the study of telomere-aging relations, but such relations were not seen in wild-type subjects. The study of telomere-related aging in mice especially feature the difficulties of comparing human and model organisms, since the telomeres of most laboratory mice are 5–10 times longer than in humans, but their life span is much shorter.

Interestingly, the only common effector on aging found among cell cultures, different model organisms, and humans is epigenetic modifications. Epigenetic modifications are indeed a part of every genetic response in the cell, but the existence of common age-related modifications and key-players is intriguing. Epigenetic alterations are "core" elements in cellular responses. They play an upstream role to specific cellular processes, and this might be the reason for the relatively joint phenotypes. Furthermore, epigenetic modifications that are related to age-associated chromosomal rearrangements in yeast and flies might be a link to age-related DNA damage, where direct evidence were not found.

Though much progress has been achieved, full understanding of these mechanisms has still a long way to go. New tools such as GWAS and EWAS studies hold the potential to further elucidate the aging phenotype by investigating large datasets obtained from human subjects, but, it is still important and useful to study the above discussed strategies and organisms. However, the selection of those organisms will have to be more conscious and target-based.

AUTHOR CONTRIBUTIONS

GL, DG, and HS wrote and edited the article; LS and GA edited the article.

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REVIEW
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The Role of Genetics in Advancing Precision Medicine for Alzheimer's Disease—A Narrative Review

Yun Freudenberg-Hua[1,2], Wentian Li[3] and Peter Davies[1]*

[1] Litwin-Zucker Center for the study of Alzheimer's Disease, The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, United States, [2] Division of Geriatric Psychiatry, Zucker Hillside Hospital, Northwell Health, Glen Oaks, NY, United States, [3] Robert S Boas Center for Genomics and Human Genetics, The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, United States

Alzheimer's disease (AD) is the most common type of dementia, which has a substantial genetic component. AD affects predominantly older people. Accordingly, the prevalence of dementia has been rising as the population ages. To date, there are no effective interventions that can cure or halt the progression of AD. The only available treatments are the management of certain symptoms and consequences of dementia. The current state-of-the-art medical care for AD comprises three simple principles: prevent the preventable, achieve early diagnosis, and manage the manageable symptoms. This review provides a summary of the current state of knowledge of risk factors for AD, biological diagnostic testing, and prospects for treatment. Special emphasis is given to recent advances in genetics of AD and the way genomic data may support prevention, early intervention, and development of effective pharmacological treatments. Mutations in the *APP*, *PSEN1,* and *PSEN2* genes cause early onset Alzheimer's disease (EOAD) that follows a Mendelian inheritance pattern. For late onset Alzheimer's disease (LOAD), *APOE4* was identified as a major risk allele more than two decades ago. Population-based genome-wide association studies of late onset AD have now additionally identified common variants at roughly 30 genetic loci. Furthermore, rare variants (allele frequency <1%) that influence the risk for LOAD have been identified in several genes. These genetic advances have broadened our insights into the biological underpinnings of AD. Moreover, the known genetic risk variants could be used to identify presymptomatic individuals at risk for AD and support diagnostic assessment of symptomatic subjects. Genetic knowledge may also facilitate precision medicine. The goal of precision medicine is to use biological knowledge and other health information to predict individual disease risk, understand disease etiology, identify disease subcategories, improve diagnosis, and provide personalized treatment strategies. We discuss the potential role of genetics in advancing precision medicine for AD along with its ethical challenges. We outline strategies to implement genomics into translational clinical research that will not only improve accuracy of dementia diagnosis, thus enabling more personalized treatment strategies, but may also speed up the discovery of novel drugs and interventions.

Keywords: Alzheimer's disease, genetics, genomics, risk factors, risk variants, precision medicine, genome sequencing

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Wee Shiong Lim,
Tan Tock Seng Hospital, Singapore

****Correspondence:***
Yun Freudenberg-Hua
yfreuden@northwell.edu

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia (1) accounting for 60–80% of dementia diagnosis and affects nearly 50 million people worldwide (2). The worldwide number of affected individuals is expected to reach 66 million by 2030, and 131 million by 2050 (3) as the number of older adults increases. One in 10 people over age 65 and every third person over age 85 in the US has a diagnosis of AD (4). The global financial toll of dementia was estimated to be 818 billion US dollars in 2015, an increase of 35% since 2010 and this cost is expected to further rise together with the prevalence of AD (2). The majority of the costs are related to family and social care of patients, rather than medical care. About 5% of all AD patients show cognitive symptoms before age 65 and are classified as early onset Alzheimer's disease (EOAD) (5). Patients showing clinical symptoms after age 65 are classified as having late onset Alzheimer's disease (LOAD). Here, we provide a summary of the clinical, neuropathological, fluid, and imaging biomarkers of AD along with a more comprehensive review of genetic findings in both Mendelian and sporadic forms of AD. We discuss how genetic analysis as applied in Mendelian randomization (MR) may be helpful in validating causality of modifiable risk factors that could advance preventive measures. Moreover, genetic data may be useful to facilitate precision medicine. The goal of precision medicine is to integrate clinical, genetic, and life style data to enable clinicians to efficiently and accurately predict the most appropriate course of action for a patient (6). We emphasize the ways genetics may facilitate precision medicine in AD: (1) identifying at risk individuals through risk prediction, (2) improving diagnostic precision, and (3) expediting the discovery of targetable disease mechanisms for drug development. Due to the large number of published articles in biomedical research of AD, we refer to more recent comprehensive reviews written by domain experts and supplement these with other findings.

LITERATURE SELECTION

Our goal of writing this narrative review (7) is to discuss how genetics may not only advance basic research on disease mechanisms but also play a role in facilitating precision medicine in AD. We provide summaries about clinical and neuropathological features, research on imaging and fluid biomarkers, as well as modifiable risk factors of AD by referring to high-quality recent systematic reviews and meta-analyses. Unpublished or original data, submitted manuscripts, or personal communications are excluded. More recent scientifically rigorous and high-impact studies on these topics that were found in the PubMed database, but not previously reviewed and those having a historical impact were also included. Over the past 20 years, our understanding about genetic research has expanded together with the rapidly advancing technology. The quality requirement for genetic studies has also evolved from candidate gene approaches, which were often criticized for producing inconsistent and non-replicable results (8), to more thoroughly conducted and well-powered genome-wide studies (9). We included publications of the Mendelian AD genes as well as publications that were referred and curated by the National Human Genome Research Institute-European Bioinformatics Institute (NHGRI-EBI) Catalog of published genome-wide association studies (GWAS Catalog) (10). In addition, we included high-quality association studies reporting rare variants that meet the "analytically rigorous" criteria for GWAS (9) or are otherwise statistically thorough.

CLINICAL FEATURES OF AD

In 1906, the German psychiatrist Alois Alzheimer first described the clinical features of an early-onset case of AD with its pathognomonic hallmarks—extracellular amyloid (neuritic) plaques and intracellular neurofibrillary tangles (NFT) (11). Patients typically show an insidious onset and continuous cognitive decline, which typically starts with an amnestic presentation with impaired ability to remember new information. The cognitive decline may further affect language, reasoning, executive function, visuospatial abilities, and the illness is often accompanied by personality and behavioral changes that affect the social function of the patient. In an advanced disease stage, patients are completely dependent on their caregivers for daily functioning such as getting dressed, toileting, mobility, and eating. The NINCDS-ADRDA criteria for diagnosing possible and probable AD are being widely used (12) and have a sensitivity and specificity of ~70% for distinguishing between AD patients and people without dementia. However, they were less accurate distinguishing between different types of dementias (13, 14). The median survival time of patients from the symptom onset is reported to be 9 years (15).

NEUROPATHOLOGY OF AD

Over many years, definitive diagnosis of AD could only be made by the "gold standard" of postmortem neuropathological examination, using a combination of CERAD score for neuritic plaques containing amyloid beta (Aβ) (16) together with Braak staging of NFT consisting of abnormally hyperphosphorylated tau (17). This had been defined in the National Institute on Aging (NIA)-Reagan Criteria (18). However, only half of the brains of patients with the clinical diagnosis of probable AD showed "pure" AD pathology (19). In 2011, the NIA and the Alzheimer's Association (AA) revised the diagnostic criteria aimed at integrating the advances of imaging and cerebrospinal fluid (CSF) biomarkers to model the three stages of AD that include preclinical stage, mild cognitive impairment, and dementia (12, 20–22). The updated criteria are now used in AD research and ongoing efforts exist

Abbreviations: AA, Alzheimer's Association; Aβ, amyloid beta; AD, Alzheimer's disease; BMI, body mass index; CSF, cerebrospinal fluid; DIAN, Dominantly Inherited Alzheimer Network; EOAD, early onset Alzheimer's disease; EHR, electronic health record; FDG, 18-fluoro-deoxyglucose; GWAS, genome-wide association studies; LOAD, late onset Alzheimer's disease; MR, Mendelian randomization; NFT, neurofibrillary tangles; NHGRI-EBI, National Human Genome Research Institute-European Bioinformatics Institute; NIH, National Institute of Health; NIA, National Institute on Aging; PD, Parkinson's diseases; PET, positron emission tomography; PI, physical interaction; PIB, Pittsburgh compound B; PRS, polygenic risks score; SNP, single-nucleotide polymorphism; WES, whole exome sequencing; WGS, whole genome sequencing.

to refine these criteria (23). It is important to emphasize that Aβ deposits have not been proven to be causal for late onset AD. In addition to Aβ and NFT, other neuropathological features such as TDP-43 immunoreactive inclusions and Lewy bodies may coexist, along with findings like cerebral amyloid angiopathy, cerebrovascular disease, and hippocampal sclerosis (19, 24–27). It is important to note that AD pathologies were also found in nearly all brain autopsies of cognitively normal individuals above age 80, even among those considered as high-cognitive performers (28, 29). Although some cognitively normal elderly had severe AD pathologies, as a group, they showed less severe AD pathologies than dementia patients. Signs of vascular injuries ranged from 32% among high cognitive performers to 64% in late dementia subjects.

IMAGING AND BIOMARKERS

To provide early and accurate diagnosis of AD, extensive efforts have been made into developing sophisticated methods to assess pathology in the living human brain. However, to date, no test or combination of tests that could accurately diagnose AD is available for broad clinical use outside of AD research centers (4). CSF levels of Aβ42, tau, and hyperphosphorylated tau (ptau) as markers for amyloid, neuronal injury, and tangles, respectively, have been the main fluid biomarkers used in AD research (30, 31). In CSF of AD patients, a decreased level of Aβ42 has been consistently found (32), whereas the concentrations of tau and ptau are increased (31). Levels of CSF tau and ptau, but not Aβ42, were found to correlate with brain atrophy in AD (33). Interestingly, a reduction of CSF Aβ42 had been shown to correlate with brain atrophy in non-demented elderly indicating a potential preclinical stage (33).

Unaddressed problems preventing broad clinical utility of biomarkers include incomplete clinical validity, inconsistent predictive value, and assay variability (34). The consensus from experts in the field of biomarkers concludes that CSF AD biomarkers may be used alongside clinical measures to identify or exclude AD as an underlying cause particularly in uncertain and atypical clinical presentations (35).

In parallel to CSF biomarkers, major advances were made to measure Aβ and tau deposits *in vivo* with help of brain imaging. Using a combination of 18-fluoro-deoxyglucose-positron emission tomography (PET), which measures cerebral glucose metabolism, and Pittsburgh compound B (PIB) PET measuring the Aβ deposition along with CSF biomarkers, it was demonstrated that subjects with known Mendelian AD mutations have CSF Aβ changes, brain amyloidosis, tauopathy, brain atrophy, and decreased glucose metabolism in that same temporal order starting 20 years before the clinical onset of AD (36). More recently, voxel-based hierarchical clustering was applied to cross-sectional flortaucipir PET imaging for ptau and PIB–PET for Aβ in 88 elderly cognitively normal individuals (37). The study identified four tau clusters and four Aβ clusters based on spatial features. It shows that tau clusters map to the temporal lobe and orbitofrontal cortex and expand to parietal and frontal lobes roughly corresponding to Braak tau stages (38), whereas Aβ deposits are dispersed in widespread heteromodal cortex. The finding that tau and Aβ deposits displayed distinguishable locations with some overlap, particularly in the association cortex, suggested that AD is a tau-centered disease with amyloid effects.

RISK FACTORS FOR AD

Currently known risk factors for AD include age, sex, cardiovascular risk factors, metabolic risk factors, sleep apnea, family history, and certain genetic variants (2, 4). Thus, both modifiable and non-modifiable risk factors have been associated with LOAD risk. The non-modifiable factors include sex, aging, and the genetic risk.

GENETICS OF AUTOSOMAL DOMINANT AD

A recent systematic review of studies from the US, Europe, India, and China shows that the worldwide proportion of EOAD is around 5% of all AD cases (39). Of note, only 30–60% of EOAD patients have a positive family history for dementia, and about 10–14% have a family history that is consistent with autosomal dominant inheritance (40–42). Thus, in addition to the Mendelian disease presentation of EOAD, a substantial proportion of EOAD cases fall into the category of sporadic and genetically complex disease. For the Mendelian cases, three genes that carry mutations causal for autosomal dominant AD were identified in the 1990s, namely *APP* (43), *PSEN1* (44), and *PSEN2* (45, 46). The *APP* gene encodes amyloid precursor protein which is proteolytically processed into Aβ peptides by β- and γ-secretase. Most pathogenic mutations in *APP* have been reported to either increase Aβ production or influence the ratio of Aβ peptides of different length (e.g., the Aβ42/Aβ40 ratio) resulting in increased self-aggregation (47). Notably, at the same site of a disease causing *APP* mutation that increases APP cleavage, a protective variant leading to a different amino acid change was found that decreases APP cleavage (48). *PSEN1* and *PSEN2* genes encode part of the γ-secretase complex and *PSEN1* accounts for most of the known mutations for autosomal dominant AD. The majority of pathogenic *PSEN1* mutations impair γ-secretase-dependent cleavage of APP and decrease the production of both Aβ42 and Aβ40 (49). These genetic findings in autosomal dominantly inherited EOAD (48, 50) provide strong support for the amyloid hypothesis implicating that Aβ plays an initiating role in AD. A recent review presented a large body of evidence from over 25 years of research supporting the generalizability of amyloid hypothesis (51). However, there are also findings that contradict amyloid being the main driving cause for the more common sporadic manifestations of AD (52). For example, elevated amyloid deposition is frequently found in cognitively normal subjects (28, 53–55) and CSF level of Aβ and Aβ imaging with PIB–PET do not correlate with cognitive decline (56). Furthermore, Aβ production is reduced by most *PSEN1* mutations (49). The anatomic and temporal discordance between Aβ pathology, tau aggregation, and neurodegeneration has led to the postulation of Aβ being an initiator of a complex cascade that ends in tau-medicated neurodegeneration (57).

GENETICS OF LOAD

For the majority of AD patients, no known causal genetic mutations have been identified. LOAD as well as many cases of EOAD are genetically complex and have multifactorial causes, which is similar to other chronic common diseases. A large population-based twin study estimated that genetic factors contribute 58–79% of etiologic role for LOAD (58). More than 20 years ago, *APOE4* (also called *APOE* ε4) allele of the *APOE* gene has been identified as a major genetic risk factor for LOAD (59, 60). The *APOE* gene has two missense variants at amino acid residues 112 and 158 leading to three common haplotypes, which are typically referred to as APOE alleles ε2 (Cys and Cys), ε3 (Cys and Arg), and ε4 (Arg and Arg). Among Caucasians, homozygous ε4 carriers show the highest life time risk for AD (68–91%) (61–64) with an odds ratio (OR) of 11–12.9 compared with homozygous ε3 carriers. Individuals carrying one copy of ε4 have a threefold risk increase for AD compared with people having no ε4 allele, and the ε2 allele is protective against AD (**Figure 1**). In African-Americans and Hispanic populations the OR of *APOE4* is found to be less pronounced compared to Caucasians. It is important to note that unlike the mutations in autosomal dominant forms of AD, *APOE4* is not a sufficient determinant of AD even in old aged individuals. We have previously reported a homozygous *APOE4* carrier who reached the age of 95 years without overt signs of dementia (65).

APOE encodes a lipid carrier Apolipoprotein E (ApoE) that is found both in the periphery and the central nervous system (66). The risk effects of *APOE4* in AD were linked to ApoE's pleiotropic functions that lead to reduced cholesterol transport, less efficient Aβ clearance and more aggregation, triggering neurotoxicity through Tau phosphorylation, increased brain neuronal activity and atrophy, reduced synaptic plasticity, and greater neuroinflammation. The large body of literature investigating the functional mechanism of ApoE in AD has been recently summarized (67–69). Most recently, ApoE has been shown to affect tau pathogenesis, neuroinflammation, and tau-mediated neurodegeneration independently of amyloid-β pathology in transgenic mice (70).

In addition to the well-established effects of *APOE*, GWAS have identified more than 30 genomic loci that are associated with AD risk. Unlike the *APOE* variants, the majorities of GWAS identified risk variants do not alter the protein sequence and are not necessarily the actual causal variants. Instead, an associated variant may be in linkage disequilibrium with an unidentified causal variant that may alter protein sequence, splicing patterns,

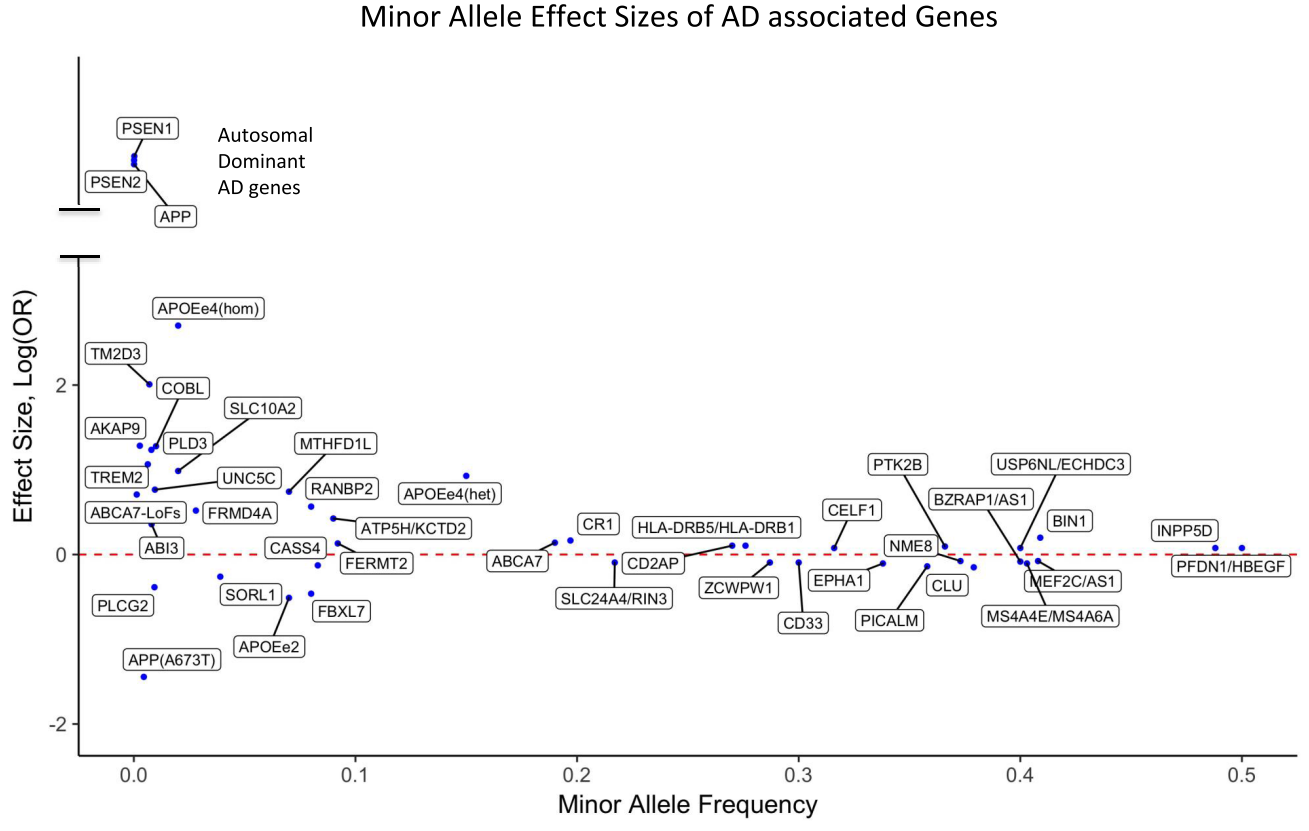

FIGURE 1 | Effect sizes of AD associated variants for the respective minor alleles. The red dotted line indicates OR = 1 [log(OR) = 0]. Minor alleles with log(OR) above the line are risk alleles and below the line are protective. Abbreviations: APOEe4(hom), homozygosity for the *APOE4* allele; APOEe4(het), heterozygosity for the APOE4 allele; ABCA7-LoFs, aggregated effects of loss-of-function variants in ABCA7; OR, odds ratio; AD, Alzheimer's disease.

or gene expression. In GWAS for LOAD, genes that are located near the associated variants are considered potential risk genes, but further evidences are necessary to support their actual etiological role. As of September 1, 2017, the NHGRI-EBI GWAS Catalog (10) listed 74 published GWAS studies on LOAD. We manually curated this list by merging multiple reports for the same locus into one row (**Table 1**). It is clear that some gene loci have been replicated by two or more GWAS or meta-analysis. These genes are *BIN1, CD2AP, CLU, CR1, EPHA1, MS4A4E/MS4A6A, PICALM, and TREM2*. The confidence for these genes to be actual AD genes is higher compared with those genes supported by a distant variant in one single study. For example, one association signal on Chromosome 2 was supported by an intergenic variant rs17034806 that is located 200 kb from the gene *RANBP2* (71). In **Table 1**, if a locus is implicated in more than one association study or is supported by meta-analysis, we show the strongest association signal.

Although GWAS have been a powerful method to uncover risk loci in AD, they are less suitable to discover infrequent or rare variants. A recent estimate indicates that only 30.6% of the genetic variance can be explained by known AD single-nucleotide polymorphisms (SNPs), but a sizeable fraction of the unidentified risk variants may be located close to the known risk SNPs, potentially as rare variants (72). Consistent with an important role of rare variants, our investigation using whole genome sequencing (WGS) showed an increased burden of rare loss-of-function variants in immune genes in AD compared with cognitively healthy centenarians (73). Large-scale sequencing, such as whole exome sequencing (WES) and WGS, has already identified new genes that harbor rare variants typically missed by GWAS. Rare variants that increase the risk for AD have been identified in *TREM2* (74, 75), *PLD3* (76, 77), *UNC5C* (78), *AKAP9* (79), *ADAM10* (80), and *ABI3* (81). Moreover, the burden of rare coding variants in risk genes identified by GWAS such as *ABCA7* (82–84) as well as in Mendelian genes for AD had been found to be increased among LOAD patients compared with unaffected general population (85, 86). The potential impact of rare variants in AD is further underscored by rare and low-frequency protective variants such as *APOE2* allele (61, 67), *APP* A673T (48), and *PLGC2* P522R (81). The effect sizes of both GWAS loci and genes harboring reported rare AD-associated variants are presented in **Figure 1**.

Undoubtedly, the search for rare risk variants with high-effect sizes for LOAD faces many obstacles. First, many studies are underpowered to separate true signals from false-positive associations as tens of thousands of cases and controls are usually required to achieve genome-wide significance level of $P < 5E{-}8$. Second, allele frequencies of rare variants are more likely to vary between population cohorts of different ethnic backgrounds due to founder effects, making replication studies difficult to conduct. For example, risk allele frequencies in *PLD3* in controls of one cohort may be higher than that of cases in another cohort, while combined result may be nominally significant (77) or not significant at all (87–89). Third, the necessarily small number of carriers of rare variants makes the respective association studies particularly prone to be impacted by factors such as age, *APOE4* carrier status, and different genotyping and sequencing platforms.

TABLE 1 | AD associated loci from the NHGRI-EBI GWAS Catalog.

CHR	Region	Gene locus	Risk allele frequency	*P*-value	Risk allele OR
1	1q32.2	CR1	0.197	6.0E−24	1.18
2	2q13	RANBP2	0.08	4.0E−08	1.76
2	2q14.3	BIN1	0.409	7.0E−44	1.22
2	2q37.1	INPP5D	0.488	3.0E−08	1.08
5	5p15.1	FBXL7	0.92	5.0E−08	1.59
5	5q14.3	MEF2C	0.592	3.0E−08	1.08
5	5q31.3	PFDN1, HBEGF	0.5	7.0E−09	1.08
6	6p21.32	HLA-DRB5, HLA-DRB1	0.276	3.0E−12	1.11
6	6p21.1	TREM2	0.0063	2.0E−12	2.9
6	6p12.3	CD2AP	0.27	9.0E−09	1.11
6	6q25.1	MTHFD1L	0.07	2.0E−10	2.1
7	7p14.1	NME8	0.627	5.0E−09	1.08
7	7p12.1	COBL	0.991	4.0E−08	3.59
7	7q22.1	ZCWPW1	0.713	6.0E−10	1.1
7	7q35	EPHA1	0.662	1.0E−13	1.11
8	8p21.2	PTK2B	0.366	7.0E−14	1.1
8	8p21.1	CLU	0.621	3.0E−25	1.16
10	10p14	USP6NL, ECHDC3	0.4	3.0E−08	1.08
10	10p13	FRMD4A	0.028	1.0E−10	1.68
11	11p11.2	CELF1	0.316	1.0E−08	1.08
11	11q12.2	MS4A4E/MS4A6A	0.597	6.0E−16	1.11
11	11q14.2	PICALM	0.642	9.0E−26	1.15
11	11q24.1	SORL1	0.961	1.0E−14	1.30
13	13q33.1	SLC10A2	0.985	5.0E−08	2.68
14	14q22.1	FERMT2	0.092	8.0E−09	1.14
14	14q32.12	SLC24A4, RIN3	0.783	6.0E−09	1.1
17	17q22	BZRAP1	0.6	4.0E−08	1.09
17	17q25.1	ATP5H, KCTD2	0.09	4.7E−09	1.53
19	19p13.3	ABCA7	0.19	1.0E−15	1.15
19	19q13.32	APOE	0.15	2.0E−157	2.53
19	19q13.41	CD33	0.7	2.0E−09	1.1
20	20q13.31	CASS4	0.917	3.0E−08	1.14

The database was queried on September 1, 2017 for association studies on AD. If an association locus is reported by multiple GWAS, we merged the results by reporting the most significant P-value for that locus.

CHR, chromosome; OR, odds ratio; AD, Alzheimer's disease; GWAS, genome-wide association studies; NHGRI-EBI, National Human Genome Research Institute-European Bioinformatics Institute.

PATHWAYS IMPLICATED BY RISK GENES FOR AD

The established AD associated genes exert pleiotropic functions across many molecular pathways. Several of these pathways stand out by providing insights for the disease mechanisms that may play a role in the etiology of AD (90–92). Major pathways include inflammatory response (*ABCA7, CD33, CLU, CR1, MS4A, INPP5D, TREM2, PLCG2, PTK2B,* and *ABI3*), lipid metabolism (*APOE, CLU, ABCA7*, and *PLCG2*), as well as endocytosis/vesicle-mediated transport (*BIN1, PICALM, CD2AP, EPHA1*, and *SORL1*). Other functional categories include regulation of cell cycle (*RANBP2*), oxidative stress response (*MEF2C*), and axon guidance (*UNC5C*).

A role of innate immunity and inflammation in AD etiology is independently supported by a large body of functional evidence (93–95). Among the risk genes from the immune pathways, *TREM2* stands out with its high effect-size of AD risk (74, 75). *TREM2* stands for triggering receptor expressed on myeloid

cells 2, a single-transmembrane protein expressed by monocytic myeloid cells. Both ApoE and Clusterin (encoded by *CLU*) are extracellular chaperons that prevent protein aggregation. In addition, both bind to the microglial receptor TREM2 and thus may promote uptake of Aβ by microglia (96). Studies on animal and human brains indicated that the TREM2 risk variant p.R47H impairs TREM2 detection of lipid ligands leading to microglia dysfunction (97, 98). In addition to *TREM2*, the two newly identified AD risk genes *ABI3* and *PLCG2* are highly expressed in microglia as well (81).

The abundance of genomics data in the public domain can be utilized not only to confirm the known connections among AD genes but also to reveal potentially new genes involved in the disease. **Figure 2** shows an example of a network representation of AD genes by the GeneMANIA software tool (99). AD genes, as well as other genes deemed to be appropriate by the program, can be linked by criteria such as coexpression, physical interaction (PI) studies, or being part of the same pathway. **Figure 2** shows an example of visualization of PI and pathways of a subset of AD genes reviewed in this article. The known high impact AD genes (*APP*, *APOE*, *PSEN2*, and *PSEN1*) are also highly connected genes. New genes introduced by this program may be further investigated as potential candidate genes for AD. As the computational methods to integrate larger biological data sets continue to improve and be refined, known risk genes may predict gene sets (100) and pathways that can be targeted by drugs.

POLYGENIC RISK SCORES

Because many AD risk SNPs are common variants, every individual necessarily inherits multiple such risk alleles. A polygenic risks score (PRS) (101) can be calculated based on the

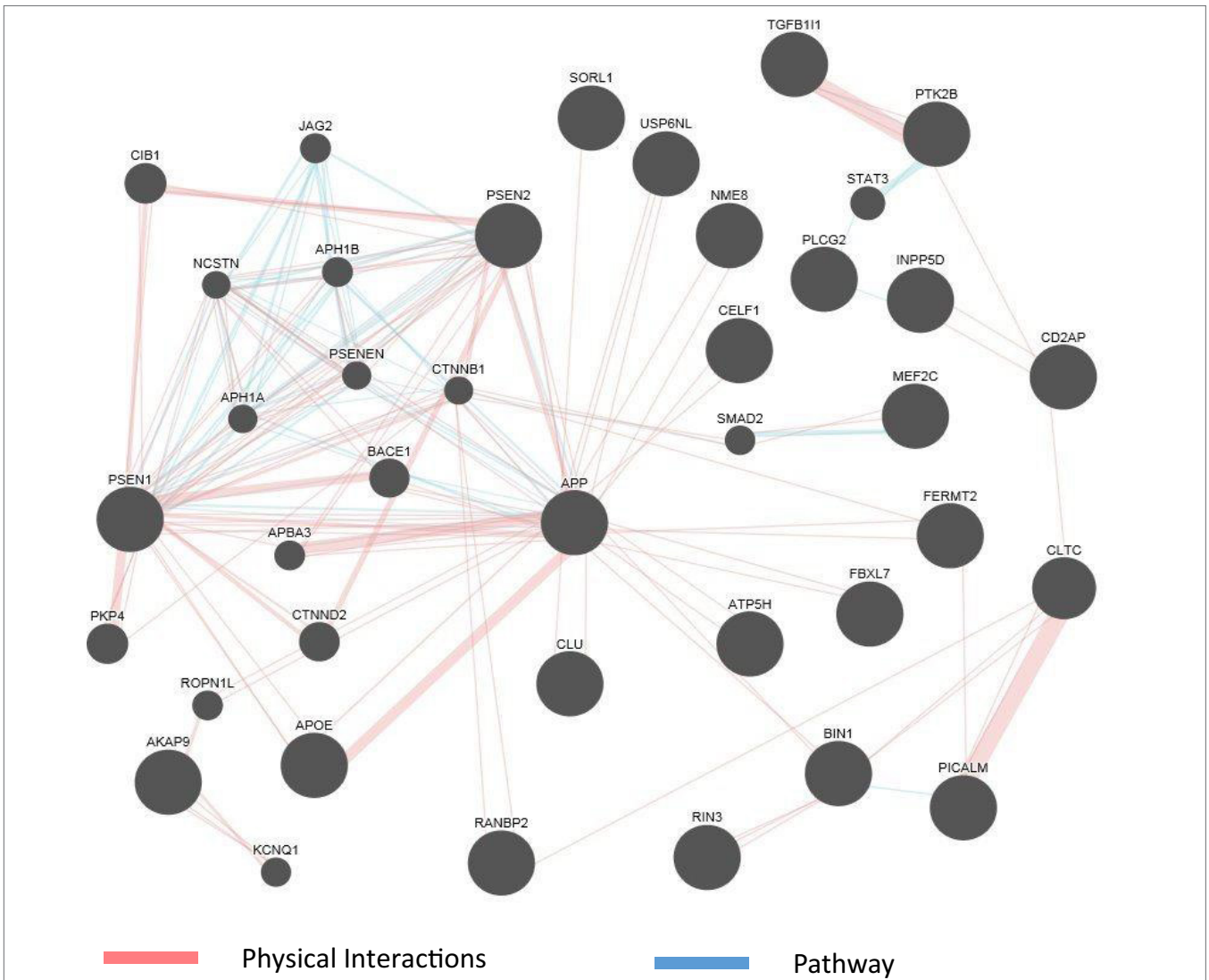

FIGURE 2 | GeneMania network for physical interaction (PI) and pathway. An example of GeneMANIA network when only the PI and pathway links are used. Alzheimer's disease genes from input list are presented as large black circles, and other genes deemed to be associated with the cluster are small black circles. Genes not linked to the main cluster are discarded.

number of common genetic risk factors present in an individual's genome, which may be used as predictor for AD risk (102, 103). Using the area under the curve receiver operator characteristic method, PRS may capture nearly all of the genetic liability from common risk variants for AD. However, the efficacy of a genetic predictor is dependent on prevalence and heritability of a disease (104). In AD, the prevalence is highly dependent on age. For the younger age group (65–74) PRS profile captured 90% of the phenotypic variance that can be attributed to common SNPs, which was estimated to be about 24%. Even though it is still controversial whether PRS is a good enough predictor for clinical use (105, 106), it may be useful to identify high-risk subjects where disease prevention studies can focus.

MODIFIABLE RISK FACTORS FOR AD

Observational studies have suggested that diabetes, mid-life obesity, mid-life hypertension, high cholesterol, and smoking are modifiable risk factors for AD (107). In terms of modifiable protective factors, education has been robustly shown to reduce AD risk (108). However, for many modifiable factors, no consistent pattern was found across studies (109). A recent comprehensive meta-analysis of 93 modifiable risk factors was conducted from 323 retrospective case/control and prospective cohort studies, which were selected after a systematic review of 16,906 publications (110). This study analyzed associations between AD risk and medical, dietary and occupational exposures as well as serum biochemistry, preexisting diseases, lifestyle, and psychological factors. The identified potentially protective factors include medical exposures of estrogen, statin, antihypertensive medications, and non-steroidal anti-inflammatory drugs, along with dietary exposures to folate, vitamin E/C, and coffee. Other potentially beneficial factors include a history of arthritis, heart disease, and cancer, cognitive activity, current smoking (in Western population), light-to-moderate drinking, and stress. Factors associated with increased risk were hyperhomocysteinemia, depression, frailty, carotid atherosclerosis, hypertension, low diastolic blood pressure, and low education. Evidence for metabolic factors appeared to be inconsistent. Notably, type 2 diabetes mellitus was associated with increased risk in an Asian population, but metabolic syndrome was associated with decreased risk. Moreover, both high body mass index (BMI) in mid-life and low BMI in late-life were associated with increased risk. Most recently, the Lancet Commissions estimated the population attributable fraction of the following modifiable risk factors: hearing loss (9.1%), "less education" (7.5%), followed by smoking, depression, physical inactivity, social isolation, hypertension, diabetes, and mid-life obesity in a declining order (2). The authors estimated that about 35% of total dementia risk may be attributable to a combination of these risk factors. Any preventive interventions addressing these factors can be applied independently of the presence of other factors like genetic risk. However, identifying individuals who would benefit most from a certain intervention due to their genetic risks remains an open question.

It has been widely hypothesized that factors such as physical activities that protect cardiovascular health would also protect the brain from AD and other dementias. A prospective interventional trial (111) along with observational studies (112–117) supports the notion that physical activity may reduce dementia risk. However, a recent meta-analysis of several randomized controlled trials (118) does not support the beneficial effects of long-term exercise on dementia or cognitive decline. A recent large trial with random assignment of intensive lifestyle intervention over 10 years showed that sustained relative weight loss and increases in physical activity did not alter the subsequent prevalence of cognitive impairment in diabetic and obese patients (119). It is currently uncertain whether life style intervention would prevent AD.

Another method to address the causal relationship of a potential modifiable risk factor (exposure) with an outcome such AD is MR. MR infers causation between the exposure and the outcome if the genetic variants associated with the exposure are also associated with the outcome. In other words, if a clinical risk factor P1 is causal for a disease P2, then genetic risk variants G associated with P1 would also be associated with P2 ($G \rightarrow P1 \rightarrow P2$) (120, 121). In principle, MR is expected to avoid bias from reverse causation and generally reduce confounding from other modifiable environmental exposures as it is a common problem in observational studies. Thus, it may provide relatively unbiased estimates of the effect of the modifiable risk factor being studied (122). A limitation of the MR approach is that at least one genetic variant that can reliably predict the exposure is required.

Larsson et al. (123) applied MR on genetic data from over 17,000 AD cases and over 37,000 controls to analyze the effect of 24 potentially modifiable risk factors. Assuming linear association and absence of any alternative causal pathways, genetically predicted higher educational attainment was found to significantly lower odds for AD. This finding is consistent with observational studies. Surprisingly, suggestive evidence was also found for genetically predicted higher quantity of cigarette smoking and lower odds of AD, which is inconsistent with results from cohort studies (124). In addition, genetically predicted higher 25-hydroxyvitamin D concentrations were associated with decreased AD odds, whereas higher coffee consumption with increased odds. Genetically predicted alcohol consumption, serum folate, serum vitamin B12, homocysteine, cardiometabolic factors, and C reactive proteins were not predicted to influence AD risk. One limitation of this study is that the authors used summary of association results rather than actual genotypes. Another MR study using different intermediate factors on the same set of GWAS data found that genetically predicted higher systolic blood pressure may be protective for AD (125), which is compatible with the reported protective effect of higher diastolic blood pressure (110). This result is nonetheless counterintuitive, given the known detrimental health effects of hypertension. This study also found a protective effect of genetically predicted higher smoking quantity. In addition, findings on cholesterol were not consistent with a causal effect on AD risk, after controlling for the confounding effect of *APOE*. Clearly, more research on larger datasets that include recorded clinical and lifestyle factors are needed to confirm or reject causal implications of some modifiable risk factors of AD.

In addition to the MR approach, there are other attempts to find interplay between genetic and environmental factors.

An example is to study gene–environment interactions (126) and one study have shown that estrogen use may be associated with less cognitive decline among *APOE4* negative women (127).

CURRENT STATE OF DEVELOPMENT OF TREATMENT FOR AD AND FUTURE OUTLOOK

Currently, no disease modifying treatment is available for AD. The only treatments available are treating symptoms, but not the causes of the disease and its progression (128). This statement holds despite the stunning fact that between 2002 and 2014, more than 400 drug trials for AD have been performed but subsequently failed (129). More recently, several large drug trials aiming at reducing the amyloid burden had failed to show efficacy. Attempts to reduce Aβ production (130) as well as immunotherapeutic approaches to clear amyloid plaques from the brain did not show efficacy in slowing down or halting the course of AD (131, 132). Biogen's immunotherapeutic drug Aducanumab reported positive Phase 1 results on removing brain Aβ plaques and clinical benefits (133). The result of a larger phase 3 trial is still pending.

Explanations of the failure of so many drug trials targeting Aβ argue for possible flaws in the amyloid hypothesis, or the possibility that the disease being too advanced at the time of intervention (131, 134). Drug trials in presymptomatic mutation carriers of autosomal dominant AD may shed light on whether targeting amyloid will yield any therapeutic effect (135). Ongoing drug trials include targeting anti-amyloid, anti-tau, anti-inflammatory, neuroprotection, stem cell therapy, and metabolism (136).

Advances of information technology have enabled health care providers to collect, store, and analyze large quantities of individual health data ranging from clinical information such as diagnostic test results and medication history to lifestyle factors such as smoking. At the same time, scientific community is equipped with methods to generate, process, and analyze large datasets from genomics, imaging, transcriptomics, and many other data-intensive researches. The current concept of precision medicine (137) considers clinical, behavioral, and molecular data to predict personalized disease risk, implement preventive measures, make more accurate diagnosis, and recommend treatments that maximize therapeutic effects and minimize adverse effects. To facilitate precision medicine the National Institute of Health (NIH) launched the *All of Us* research program, which plans to enroll one million participants (https://allofus.nih.gov/about/about-all-us-research-program).

Under the assumption that the treatment success of a potentially effective pharmacological intervention depends on its initiation in the presymptomatic stage, the identification of at risk subjects will be crucial to maximize treatment effect. Currently, a prevention trial as part of the Dominantly Inherited Alzheimer Network (DIAN) is under way (138). However, results from DIAN may not be representative for the majority of at risk subjects, as most AD patients do not carry Mendelian mutations. Independently, imaging amyloid and tau was shown to identify such at risk subjects (139). In reality, however, large-scale application of imaging biomarkers as a broad population screening method is difficult to implement, due to its invasiveness, high cost, and limited availability of equipment. Other fluid biomarkers have been useful in research studies (21), but their broad use in clinical settings was limited due to lack of established reproducible assays and the reluctance of patients to agree to lumbar puncture procedure (140). Most recently, reports on high-performance plasma amyloid-β biomarkers showed promising accuracy in predicting brain amyloid-β burden (141). Unlike these biomarkers, known genetic risks of a subject remain stable over time and are not influenced by any confounding factors. Currently, genetic risk factors can be assessed at a very low cost starting at around $50 per sample for array-based genotyping data. These arrays cover common variants that may include disease risk variants, which can be further used to impute additional disease risk variants. Moreover, the cost for more comprehensive WES and WGS is down trending toward several hundred dollars. Thus, it is feasible that genetic risk profiles may be used alone or combined with other biomarkers to select at risk subjects in preclinical stage for closer follow-ups and enrollment into preventive studies.

Genetic testing may also increase diagnostic precision in patients with dementia. A recent study showed that known pathogenic mutations for AD and frontotemporal dementia were found with similar proportion in familial LOAD and sporadic LOAD patients. Mutations for Parkinson's diseases (PD) and amyotrophic lateral sclerosis were also found in clinically diagnosed AD subjects (86). Therefore, genetic testing may prevent other neurodegenerative diseases, which may even have some treatment options, from being misdiagnosed as AD. Combined with fluid and imaging biomarkers, genetics may further increase diagnostic accuracy to ensure clinical trials are done in truly AD patients. Furthermore, instead of treating AD as a homogeneous disease, genetics and other diagnostic methods hold the potential to identify functional disease subtypes that could be specifically targeted.

Another advantage of genetic screening, especially in subjects with family history of dementia, would be the improved risk assessment. An accurate risk assessment could lead to specific consultation for preventive measures addressing modifiable risk factors, such as early use of hearing aids and managing metabolic symptoms. Linking genomic data and electronic health record (EHR) may further help researchers to identify how genetic factors interact with other health conditions such as the impact of medication use on disease risk. For example, an EHR-based analysis found that salbutamol, a β2-adrenoreceptor agonist often prescribed for asthma, is associated with a 34% lower risk of PD and propranolol, a drug frequently prescribed for hypertension, with increased risk (142). Similar approaches of EHR mining may discover medications that alter AD risk. Genetic risk factors had strongly supported a role of immune pathways in AD. Analysis of large EHR data could find out whether drugs that target the immune system had an impact on risk for AD.

Large-scale genetic testing may come from consumer genetic services as they become more broadly available. More than three million people already had their DNA tested at 23&Me and received their carrier status of *APOE4* among other risk variants affecting health. Currently, there are hundreds of companies offering similar services and the list is growing (143). The number

of people equipped with personal genetic data will likely continue to increase in the general population. Such consumer genetic data may be integrated into EHR to assist diagnostic assessments and choice of treatment. For example, clinicians may consider avoiding propranolol and other β-blockers for patients with genetic predisposition for PD.

In addition to risk variants, genetic studies will identify more protective variants against AD. As the sample size becomes larger, researchers may identify potentially protective factors in subjects who carry strong risk factors such as homozygosity of *APOE4*, but do not develop AD at an advanced age (65). Identification of protective variants in such a population may lead to possible new drugs that act through a similar mechanism. A recent example for protective genetic variants fueling new effective therapeutics was the development of PCSK9 inhibitor for hypercholesterolemia (144, 145). The newly identified gene *PLCG2* that harbors rare protective variants is highly expressed in microglia and may be a target to be exploited for drug discovery in AD (81). Certainly, a hope is that ongoing sequencing efforts (146) would identify more protective variants that can be targeted by drugs.

A workflow for clinical translational research implementing clinical assessments, genetics, and biomarkers into clinical research (as discussed above) is graphically described in **Figure 3**. Of course, large-scale population level genetic testing also brings ethical challenges. Clinicians and researchers need to take into account the respective guidelines for genetic testing (147). Current studies indicate that the majority of individuals tested for autosomal dominant forms of AD under a standardized counseling protocol demonstrated effective coping skills. Negative psychological reactions were absent after several months and the testing was perceived to be beneficial. The potential benefits, harms, and dilemmas of genetic testing and impacts on family members were detailed in a case report (148). If results of risk factors are returned to the participants, counseling needs to be provided and psychosocial support should be made available. It is important that patients and customers of consumer genetics services understand that typical risk factors are not deterministic for AD. The ethical, legal, and social implications of genetic testing such as testing-induced harm and discrimination are an active area of research at NIH (149).

In summary, the current approach for AD consists of optimizing modifiable risk factors to reduce and delay symptom onset as well as symptomatic treatment after disease onset. The dawn of the big data era may make it feasible to advance precision

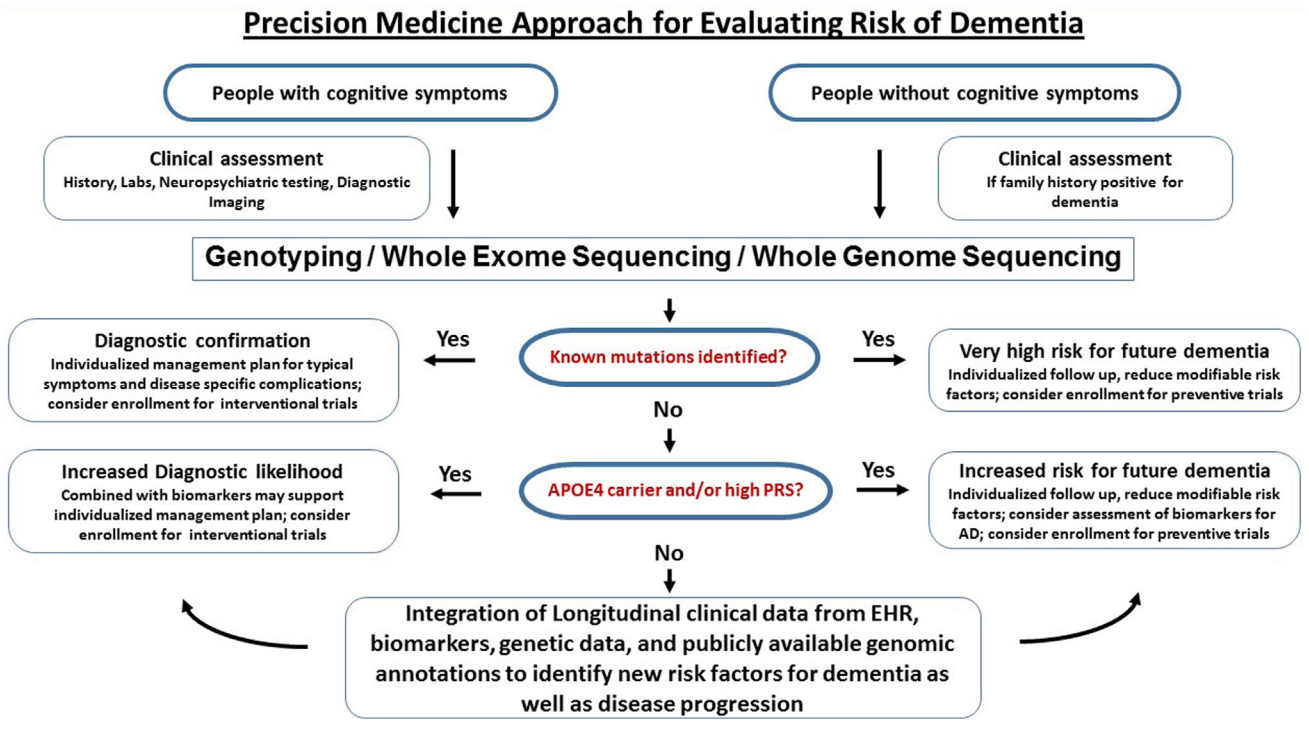

FIGURE 3 | Precision medicine approach for dementia. This is a graphical outline of how genetic and genomic information could be combined and integrated with electronic health records (EHRs) to improve the accuracy of dementia diagnosis and facilitate drug discovery. Middle-aged and older people (e.g., age > 50) are enrolled in an ongoing protocol that includes medical and family history, diagnostic assessment, and access to EHR. For those who have signs of cognitive impairment, genetic testing using either mutation-panels, genotyping arrays, whole exome sequencing or whole genome sequencing depending on the clinical question is performed alongside biomarkers. If a dementia diagnosis is confirmed through genetics and biomarkers, the patients are referred to specialized behavioral and pharmacological intervention and have the option to participate in drug trials. For the majority of subjects who do not have definitive biological findings, a likelihood risk score may be estimated based on the genetic and biomarker profiles. These risk scores may provide support for clinical diagnosis and identify subjects at risk for dementia. The presymptomatic at risk subject may be enrolled in longitudinal studies on prevention and those who never develop dementia despite having high risk may be studied to identify protective factors.

medicine by systematically integrating massive biological data generated by next-generation genomic sequencing, biomarker testing, and EHRs. This development is likely to shed more light to the complex biology of AD and accelerate development of better prevention, diagnosis, and treatments.

AUTHOR CONTRIBUTIONS

YF-H contributed to conception of the work, data collection, literature research, data interpretation, drafting of the article, critical revision, and final approval of the version to be published. WL contributed to data interpretation, drafting of the article, and critical revision. PD contributed to conception of the work, critical revision, and final approval of the version to be published.

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Genetic Insights Into Frailty: Association of 9p21-23 Locus With Frailty

*Sanish Sathyan[1], Nir Barzilai[2,3], Gil Atzmon[2,3,4], Sofiya Milman[2], Emmeline Ayers[1] and Joe Verghese[1,2]**

[1] Department of Neurology, Albert Einstein College of Medicine, Bronx, NY, United States, [2] Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, United States, [3] Department of Genetics, Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY, United States, [4] Department of Biology, Faculty of Natural Science, University of Haifa, Haifa, Israel

Frailty is a complex aging phenotype associated with increased vulnerability to disability and death. Understanding the biological antecedents of frailty may provide clues to healthy aging. The genome-wide association study hotspot, 9p21-23 region, is a risk locus for a number of age-related complex disorders associated with frailty. Hence, we conducted an association study to examine whether variations in 9p21-23 locus plays a role in the pathogenesis of frailty in 637 community-dwelling Ashkenazi Jewish adults aged 65 and older enrolled in the LonGenity study. The strongest association with frailty (adjusted for age and gender) was found with the SNP rs518054 (odds ratio: 1.635, 95% CI = 1.241–2.154; *p*-value: 4.81×10^{-04}) intergenic and located between LOC105375977 and C9orf146. The prevalence of four SNPs (rs1324192, rs7019262, rs518054, and rs571221) risk alleles haplotype in this region was significantly higher (compared with other haplotypes) in frail older adults compared with non-frail older adults (29.7 vs. 20.8%, $p = 0.0005$, respectively). Functional analyses using *in silico* approaches placed rs518054 in the CTCF binding site as well as DNase hypersensitive region. Furthermore, rs518054 was found to be in an enhancer site of *NFIB* gene located downstream. *NFIB* is a transcription factor that promotes cell differentiation during development, has antiapoptotic effect, maintains stem cell populations in adult tissues, and also acts as epigenetic regulators. Our study found novel association of SNPs in the regulatory region in the 9p21-23 region with the frailty phenotype; signifying the importance of this locus in aging.

Keywords: frailty, fried index, 9p21-23 locus, aging, genetics

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***Correspondence:**
Joe Verghese
joe.verghese@einstein.yu.edu

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INTRODUCTION

Frailty is a complex phenotype seen in aging, which is associated with low physiologic reserves and with increased vulnerability to adverse outcomes such as disability, hospitalization, and death (1, 2). The prevalence of frailty has been reported to range from 7 to 32% in older populations and is higher in women (3). Given the emergent aging pandemic worldwide (4), a major public health challenge is to find ways to enhance functional independence in older adults and to increase years free from disabilities. Hence, understanding the biological antecedents of frailty may provide insights into healthy aging strategies.

Frailty is a multidimensional construct involving several domains—physical, cognitive, psychological, and social domains (5–7). Even though expression and biomarker studies have pointed toward the involvement of various biological pathways in frailty (8, 9), genetic studies have not yielded consistent results. Candidate gene studies of *IL6, TNF* and *IGF1* have shown either no association with frailty or provided contradictory results (10). This might be mainly explained by the multifactorial nature of frailty with involvement of genetic, lifestyle, and epigenetic factors (11, 12). This multidimensionality and multifactorial or complex origin of frailty is further supported by the etiological overlap between frailty and various age-related complex or multifactorial disorders (13). Prevalent frailty was a strong risk factor for cardiovascular diseases (CVD) as well as associated mortality (14). In the Cardiovascular Health Study, a cross-sectional analyses showed 38% of frail individuals had prevalent heart disease compared with 17% in non-frail individuals (15). Frailty and diabetes are strongly linked (16) with a higher incidence of type 2 diabetes seen in individuals with frailty (17). Frailty is associated with postmortem Alzheimer pathology in older adults with and without an antemortem history of dementia (18, 19). All of these point toward overlapping biological mechanism for frailty and other complex disorders. It is also possible that complex disorders may alter the frailty risk conferred by specific biological pathways.

Complex or multifactorial diseases are caused by a combination of genetic, lifestyle, and other environment factors. Genome wide association studies (GWASs) have identified a large number of genetic variants associated with complex disorders (20, 21). In particular, 9p21-23 has been shown to be a risk-associated locus with many complex disorders. For example, *9p21* has been reported to be associated with CVD (22, 23), abdominal aortic aneurysm (24), arterial stiffness (25), peripheral artery disease (26), intracranial aneurysm (27), various types of cancers (28, 29), amyotrophic lateral sclerosis (30), primary open-angle glaucoma (29), vascular dementia, and Alzheimer's disease (31). The *9p23* region was associated with restless legs syndrome (32) and obsessive-compulsive disorder (33). Distinct haplotype blocks at the 9p21-23 region were associated with CVD and type 1 diabetes (34). This locus harbors several genes including *ANRIL*, a long non-coding RNA gene implicated in the pathogenesis CVD and strokes, three candidate tumor suppressor genes; *CDKN2A* (cyclin-dependent kinase inhibitor 2A) encoding p16 protein, *CDKN2B* encoding p15 protein, and *p14/ARF* encoding p14ARF protein (35). *C9ORF72* gene was found to be associated with amyotrophic lateral sclerosis-frontotemporal dementia (36). Furthermore, protein tyrosine phosphatase receptor type delta (*PTPRD*) at 9p23 region was associated with restless legs syndrome (32) as well as cancers (37). While there is substantial overlap in the diseases-associated with frailty and the 9p21-23 locus, to the best of our knowledge, the association of this locus with frailty has not been specifically examined.

Discovering new biological pathways that prevent or delay frailty would increase current therapeutic options for clinicians and increase health span for individuals. Interestingly, rs2811712 located in *ANRIL* gene in the 9p21 locus is associated with physical function in older people with the minor allele being associated with reduced physical impairment (38). Furthermore, rs71321217 in *PTPRD* in the 9p23 locus is associated with gait rhythm (39). Based on these observations, we hypothesized that genetic variants in the chromosome 9p21-23 locus will increase the risk of developing frailty in older adults. To elucidate the role of the 9p21-23 locus in the pathogenesis of frailty, we conducted a preliminary cross-sectional study in 637 community-residing Ashkenazi Jewish (AJ) older adults participating in the LonGenity Study (40, 41). This population is homogenous genetically and socioeconomically (42) and allows for greater power for genetic analysis with fewer number of participants. Establishing the genetic underpinnings of frailty may provide new insights into preventive strategies to delay the occurrence of frailty and other related comorbidities as well as to promote healthy aging.

MATERIALS AND METHODS

LonGenity Cohort

The LonGenity study, established in 2007, recruited a cohort of AJ adults age 65 and older, who were defined as either Offspring of Parents with Exceptional Longevity (OPEL) (having at least one parent who lived to age 95 or older) or Offspring of Parents with Usual Survival (OPUS) (neither parent survived to age 95). The goal of the LonGenity study is to identify genotypes associated with longevity and their association with successful aging. Study participants were recruited through contacts at synagogues, community organizations and advertisements in Jewish newspapers in the New York City area. Potential participants were contacted by telephone to assess interest and eligibility. They were invited to our research center for further evaluation. Exclusion criterion included diagnosis of dementia [previous physician diagnosed dementia or telephone Memory Impairment Screen scores in the dementia range (43)] as well as presence of severe visual or hearing impairments that would interfere with study assessments. Participants received detailed medical history evaluation and cognitive testing at baseline as well as at annual follow-up visits. All participants signed written informed consents for clinical assessments and genetic testing before enrollment. The Einstein institutional review board approved the study protocol.

A total of 965 older individuals were enrolled in the LonGenity study between October 2008 and August 2017. We excluded 64 individuals who did not complete frailty assessments as well as 264 who did not complete genetic testing. Hence, the eligible sample for this analysis included 637 participants, who had been genotyped and completed frailty assessments.

Frailty Syndrome

The two common approaches to defining frailty clinically are as a clinical syndrome (5) or as a cumulative deficit score (44–46). The syndromic definition of frailty (see below) is widely adopted in research and clinical practice (5). While the cumulative deficit score approach has advantages in research settings, it is less intuitive in clinical settings in the community (47). Frailty diagnosis,

hence, was operationalized using the widely used Cardiovascular Health Study criteria (48) for this study. Frailty was operationally defined as meeting three or more of the following five attributes: *unintentional weight loss* (≥10 lb in past year), *muscle weakness* (objectively measured grip strength or self-report; described below), *exhaustion* [negative response to the question "do you feel full of energy?" on the Geriatric Depression Scale (49)], self-reported *low physical activity* levels [positive response to the question "Have you been less active physically?" on the Health Self-Assessment Questionnaire (5)] and *slow gait* (**Table 1**). A Jamar handgrip dynamometer was used to objectively measure dominant hand grip strength at baseline. Weakness was defined using a cut score of 1 SD or more below age and sex mean values (**Table 1**). Similar to previous reports (50–52), subjective grip strength ("do you feel as though your grip is weak?") was used on follow-up waves as a frailty criterion, since objective grip strength measures were not available for all our participants on follow-up. A previous study in this same cohort showed substantial agreement between the objective and subjective grip strength rating methods (53). Gait speed (cm/s) was measured using an 8.5 m long computerized walkway with embedded pressure sensors (GAITRite; CIR Systems, PA). The GAITRite system is widely used in clinical and research settings, and excellent reliability has been reported in our and other centers (54, 55). Participants were asked to walk on the walkway at their normal pace in a quiet well-lit room wearing comfortable footwear and without any attached monitors. Slow gait was defined as 1.5 or more SD below age and sex-appropriate mean values. In total, we had 206 individuals who were diagnosed with frailty; 118 prevalent cases and 88 incident cases of frailty.

TABLE 1 | Clinical characteristics of cohort.

Variables	LonGenity	Frailty	Normal
Participants	637	206	431
Age, mean ± SD, years	75.41 ± 6.55	77.72 ± 6.75	74.29 ± 6.16
Women, %	52.9%	55.3	51.6
Education, mean, years	17.47 ± 2.70	17.33 ± 2.73	17.55 ± 2.68
Gait speed, mean ± SD, cm/s	110 ± 20.1	100.9 ± 21.4	114 ± 17.6
Offspring of Parents with Exceptional Longevity/Offspring of Parents with Usual Survival (%)	43.6/56.4	40.7/59.3	44.8/55.2
Medical illnesses			
Cardiovascular disease, %	9.1	12.30	7.50
Stroke, %	3.6	6.90	2.10
Diabetes, %	9.2	11.80	8.20
Parkinson disease, %	1.4	2.50	0.90
Arthritis, %	40.9	56.90	34.10
Hypertension, %	43.6	62.00	40.80
Slow gait cuts, cm/s			
Men <75 years	88.55		
Men ≥75 years	76.44		
Women <75 years	87.4		
Women ≥75 years	71.28		
Low grip strength cuts, kg			
Men <75 years	32.05		
Men ≥75 years	24.21		
Women <75 years	17.67		
Women ≥75 years	14.27		

Selection of Gene Variants and Genotyping

We targeted 9p21-23 region spanning from chr9: 8743598 to 32586822 (NCBI build 37) for this analysis based on its functional significance and reported associations with major complex disorders (24, 30, 31, 34, 35). Genotyping was performed at the Center for Inherited Disease Research using Illumina HumanOmniExpress array (Illumina, San Diego, CA, USA), and the procedures have been described previously (40, 41).

Since the focus of our research was to explore complex disorder-associated alleles in this locus in regards to frailty; the few SNPs missing in the genotyping array were made available from imputation analysis. Imputation of un-genotyped autosomal SNPs were based on the 1000 Genomes data (worldwide reference panel of all 1,092 samples from the phase I integrated variant set) (v3, released March 2012) (56) using IMPUTE2, version 2.3.0. Poorly imputed SNPs with low imputation quality (info_metric < 0.3) were excluded from the analysis. For this study, we selected SNPs with minor allele frequencies of >0.10.

Statistical Analysis

Baseline characteristics of participants were compared using descriptive statistics (**Table 1**). The preliminary objective of this study was to identify the association of variants in the 9p21-23 region with frailty using logistic regression analysis. Prevalent and incident cases of frailty were examined together in this analysis to maximize sample size. In participants who did not have frailty at baseline or develop incident frailty, the wave at which the first non-frail status was diagnosed was used as baseline for comparing clinical characteristics. As previous studies have shown frailty to increase with age and in women (57), all analyses were adjusted for age and gender (Model 1). All SNP based association analyses were conducted using Plink v1.90.[1] All other statistical analyses were carried out using SPSS software (version 24; IBM Corporation). Presence or absence of diabetes, heart failure (including myocardial infarction, angina, or congestive heart failure), hypertension, strokes, Parkinson's disease, and arthritis was used to calculate a global health score (range 0–6) as previously described (58). To account for the LonGenity study design described above (40, 41) and health status, we conducted sensitivity analyses further adjusting the models for OPUS/OPEL status and global health score (Model 2).

A total of 5,556 variants were available for analysis in the selected region (chr9: 8743598 to 32586822) after removing SNPs that had minor allele frequencies < 0.10 (n = 1,856) and failed the Hardy–Weinberg exact test ($p \geq 0.01$) ($n = 79$). Linkage disequilibrium (LD) plots were generated using Haploview 4.2 (59). Haplotype blocks were defined based on the Gabriel criteria (60). Haplotype analyses were performed using SNPStats software (61). Functional prediction of the associated variants was carried out using various *in silico* approaches. Genotype-Tissue Expression portal (GTEx)[2] was used to determine the significant expression quantitative trait loci (eQTL) for SNPs associated

[1]https://www.cog-genomics.org/plink2.
[2]http://www.gtexportal.org/home/.

with frailty (62). Regulome DB[3] based on Encyclopedia of DNA Elements (ENCODE) project (63) was used to identify functional effects of the identified SNPs in the association and eQTL analyses. rVarBase,[4] updated database for regulatory features of variants was also used to find the effect of SNP of chromatin states, interacting regulatory elements and target genes (64). Functional Single Nucleotide Polymorphism; a web-based tool that integrates 16 databases and bioinformatic tools to uncover the functional effect of the SNPs (65) and FuncPred[5] were used to predict the functional effects of associated variants.

RESULTS

Study Population

Of the 637 eligible individuals with phenotype and genotype data in the LonGenity cohort, 356 were OPUS and 281 were OPEL. Of the eligible sample, 206 individuals (32.5%) received a diagnosis of frailty at baseline ($n = 118$) or at various time points over the study follow-up ($n = 88$), and 430 individuals (67.5%) remained non-frail throughout the study follow-up. The overall median follow-up time was 3.7 years (range 0–9 years). Demographic and clinical characteristics are summarized in **Table 1**. The mean age of the participants was 75.41 ± 6.55 years, and 52.9% were women. The mean years of education was 17.47 ± 2.70 years. A higher percentage of OPUS individuals met frailty diagnosis (34%) compared with OPEL (30.4%). All major medical illnesses were more prevalent in individuals who had frailty compared with normal individuals (**Table 1**).

Association and *In Silico* Functional Analyses

The strongest association with frailty was found with the G allele of rs518054 [odds ratio (OR): 1.635, 95% CI = 1.241–2.154; p-value: 4.81×10^{-04}] (**Table 2**; **Figure 1**) (Model 1). None of the SNPs studied survive Bonferroni correction for threshold for statistical significance. The associations remained similar after adjusting for longevity status (OPEL vs. OPUS) and global health score for all of these four SNPs (Table S1 in Supplementary Material) (Model 2). We also observed modest associations of three other SNPs (rs7019262, rs571221 and rs1324192) in this region with frailty (**Table 2**; **Figure 2**). LD plot of associated SNPs showed presence of SNPs in two LD blocks (rs518054–rs571221 and rs1324192–rs7019262) in frail and single LD block in normal individuals (Figure S1 in Supplementary Material). Haplotype analysis to investigate the combined effect of associated SNPs found significant association (p-value < 0.0005) with haplotype involving risk alleles (AGGC) at four loci combination (29.7 vs. 20.8%) (**Table 3**).

All four SNPs associated with frailty in this region were intergenic and located between LOC105375977 and C9orf146 (LINC00583) (**Figure 2**). The nearest well-characterized gene was *NFIB* coding for nuclear factor 1B (**Figure 2**). We assessed the functional significance of the associated SNPs in our study. The lead SNP rs518054 is located in the DNase 1 hypersensitive site. ENCODE data showed a *Regulome DB* score of 2b for rs518054, which predicted its role as likely to affect gene expression level, and the evidence includes transcription factor binding, any motif change, DNase footprint, and DNase peak (**Table 4**). The data show this region to be a binding site for CTCF, a transcriptional regulator. *rVarBase* data further suggest this SNP to be located in the chromatin interactive region with predominantly enhancer function in most tissues including muscle in both male and female. The available ENCODE data further showed this region harboring rs518054 interacted with the *NFIB* gene located downstream (**Table 4**). The associated SNP rs518054 located in the DNase hypersensitive site might play a role in the transcriptional regulation of *NFIB* gene through an enhancer effect. Furthermore, considering that these SNPs were located in the regulatory regions (e.g., enhancers), we used an *in silico* approach to determine whether they were local eQTL. Using GTEx portal, we could not find any significant eQTLs for SNP rs518054 in studied tissues.

Though none of the SNPs survived multiple corrections in this study, rs518054 emerged to be lead SNP with functional relevance in all models studied (**Table 2**; Table S1 in Supplementary Material). The unadjusted association analysis results are shown in Table S3 in Supplementary Material.

Sensitivity Analyses

The next objective of our study was to find out the risk conferred by specific complex disorder-associated SNPs in this region with frailty. A number of CVD-associated SNPs were observed in the 9p21-23 locus followed by SNPs for cancers

[3]http://regulomedb.org/.

[4]http://rv.psych.ac.cn/.

[5]https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html.

TABLE 2 | Logistic regression analysis of 9p21-23 locus with Frailty with genotyped SNPs adjusted for age and gender (Model 1).

CHR	SNP	Position	Allele	Frail	Normal	STAT	Odds ratio (95% CI)	*p*
9	rs518054	13689066	G	0.314	0.214	3.491	1.635 (1.241–2.154)	4.81×10^{-04}
9	rs10511667	18989696	G	0.164	0.106	3.411	1.855 (1.301–2.645)	6.48×10^{-04}
9	rs1855850	10480030	T	0.329	0.419	–3.401	0.635 (0.489–0.825)	6.73×10^{-04}
9	rs571221	13690235	C	0.314	0.219	3.341	1.597 (1.213–2.101)	8.35×10^{-04}
9	rs7019262	13614384	G	0.510	0.400	3.330	1.517 (1.187–1.938)	8.68×10^{-04}
9	rs7034231	28119512	G	0.186	0.115	3.254	1.780 (1.258–2.519)	1.14×10^{-03}
9	rs1324192	13612345	A	0.483	0.383	3.176	1.488 (1.164–1.902)	1.50×10^{-03}
9	rs7038172	16708269	C	0.147	0.087	3.125	1.802 (1.245–2.607)	1.78×10^{-03}

SNPs with p-value < 0.002 is shown in this table.

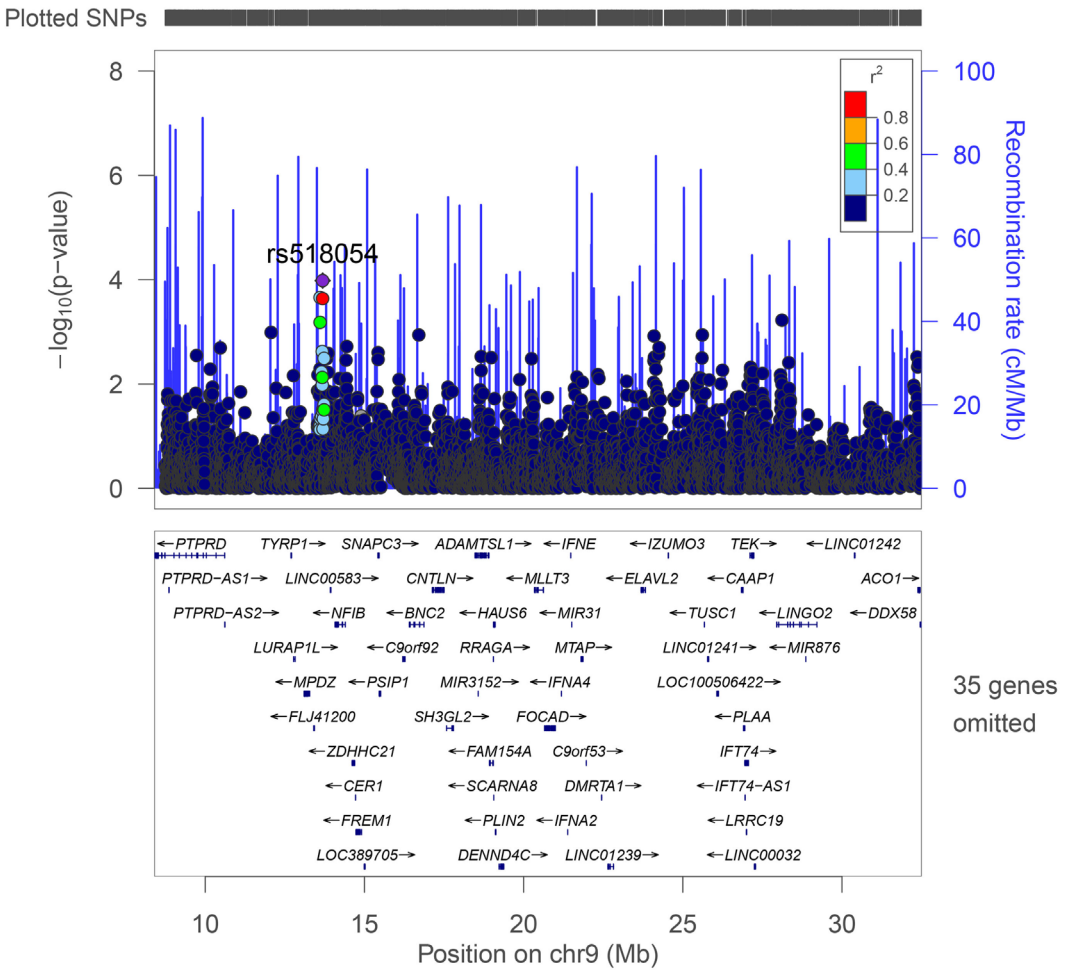

FIGURE 1 | LocusZoom plot of the region studied with frailty on chromosome 9p21-23. Genes and ESTs within the region are shown in the lower panel, and the unbroken blue line indicates the recombination rate within the region. Each filled circle represents the *p*-value for one SNP, with the top SNP rs518054 shown in purple and SNPs in the region colored depending on their degree of correlation (r^2) with rs518054 [as estimated internally by LocusZoom on the basis of CEU (Utah residents of Northern and Western European ancestry) HapMap haplotypes].

and many other complex disorders. Our analysis showed lack of association of these disease-associated SNPs with frailty (Table S2 in Supplementary Material). Interestingly, there was an increased prevalence of CVD-associated risk alleles [rs10757278 ($p = 0.116$), rs1333040 ($p = 0.133$), and rs1333049 ($p = 0.116$)] in frail individuals compared with non-frail individuals (Table S2 in Supplementary Material). SNPs associated with gait rhythm (rs71321217; *p*-value = 0.384) and physical activity (rs2811712; *p*-value = 0.205) in previous studies (38, 39) were not associated with frailty in our cohort (Table S2 in Supplementary Material).

Even though genetic studies have been carried out combining prevalent and incident cases (66–68), to check the possibility of survival bias arising from the possible systematic differences in allele frequencies between the prevalent and incident cases, we carried out case only analysis comparing allele frequency in incident and prevalent cases. There was slight difference in the allele frequency of rs518054 in incident and prevalent cases of frailty (*p*-value = 0.014) with associated G allele found to be 0.36 in prevalent cases and 0.25 in incident cases. The frequency of G allele in controls was 0.21. The overall association was mainly driven by cases of prevalent frailty (OR: 1.980, 95% CI = 1.426–2.749; *p*-value: 4.50×10^{-05}) than incident frailty (OR: 1.198, 95% CI = 0.808–1.776; *p*-value: 0.368) when each of them were compared independently to controls adjusting for age and gender.

DISCUSSION

This study attempted to delineate the role of the 9p21-23 region with frailty in a well-characterized AJ cohort as a strategy to understand healthy aging. We uncovered a novel association of SNPs at the 9p21-23 region with frailty, not implicated previously with any of the complex disorders associated with this locus. Using functional analyses, we found the lead variant to be located in the enhancer region and involved in the transcriptional regulation of the *NFIB* gene. The study further observed increased frequency of

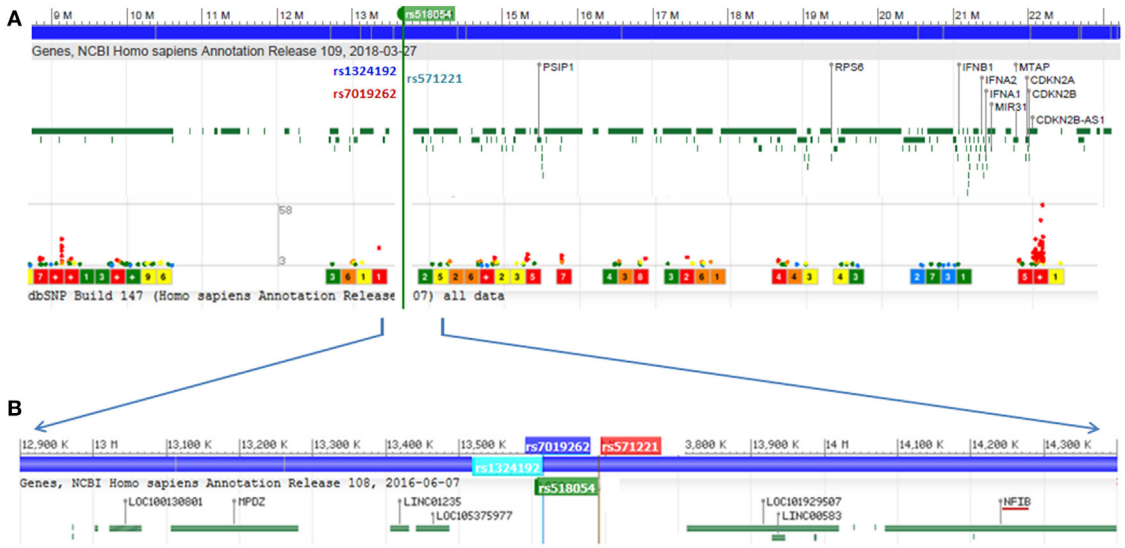

FIGURE 2 | **(A)** Genome wide association study hotspot locus of 9p21-23 region screened in this study. Frailty-associated SNPs are marked in rsIDs, and lower dots indicate disease-associated SNPs in this region and level of significance. **(B)** Localized view of associated SNPs showing its location between LOC105375977 and LINC00583 (C9orf146). *NFIB* is the nearest well-characterized gene to SNP rs518054. Genomic region data adapted from NCBI dbSNP database.

TABLE 3 | Haplotype analysis of the associated SNPs in the *9p21-23* region.

Sl. no.	rs1324192	rs7019262	rs518054	rs571221	Frailty	Normal	Odds ratio (95% CI)	*p*-Value
1	G	A	T	T	0.487	0.597	1	
2	**A**	**G**	**G**	**C**	**0.297**	**0.208**	**1.695 (1.266–2.273)**	**5×10^{-04}**
3	A	G	T	T	0.184	0.173	1.300 (0.934–1.818)	0.12
4	G	G	T	T	0.012	0.011	1.010 (0.312–3.226)	0.99

Significant difference in the haplotype (2) involving the risk alleles of associated SNPs was observed with 29.7% in individuals with frailty compared with 20.8% in normal. Haplotype analysis was adjusted for age and gender.

TABLE 4 | Details of putative regulatory functions of associated lead SNPs.

Variant	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	Chromatin state	Variant interacting gene	Frailty-associated cell line/tissue	Regulome Db score
rs518054	**T**	**G**	**0.20**	**–**	**10 tissues**	**10 tissues**	**CTCF**	**AIRE, Hoxb9**	**Enhancer**	**NFIB**	**Skeletal muscle**	**2b**
rs7019262	G	A	0.63	–	ESDR, LNG	MUS	P300	Pax-4, YY1	Enhancer	–	Skeletal muscle	4
rs571221	T	C	0.20	–	MUS	MUS, VAS	–	–	–	–	Skeletal muscle	5
rs10511667	A	G	0.89	–	Skin/lung	–	–	–	Enhancer	–	–	5
rs7034231	T	G	0.83	–	Neuron cells	–	–	–	Enhancer	–	–	5
rs7038172	T	C	0.94	–	Multiple tissues	–	GATA3 POLR2A	–	Enhancer	BNC2	–	6
rs1855850	C	T	0.67	–	–	–	–	–	–	–	–	–
rs1324192	A	G	0.66	–	–	–	–	–	–	–	–	–

MUS, skeletal muscle, male; VAS, HUVEC umbilical vein endothelial primary cells; ESDR, H1 BMP4-derived trophoblast cultured cells; LNG, lung.
Data are derived from HaploReg v4.1 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php), RegulomeDB (http://www.regulomedb.org/), and rVarBase (http://rv.psych.ac.cn/).
Lead SNP rs518054 is marked in bold.

CVD-associated alleles in individuals with frailty though failed to reach statistical significance with frailty phenotype.

The 9p21-23 region has emerged as a genetic hotspot for complex disorder associations in recent studies. With regard to the frailty-associated SNPs discovered in our study, the nearest well-characterized gene was *NFIB*, coding for the transcription factor Nuclear Factor IB, which plays a key role in the transcriptional regulation of a large number of genes in which our lead SNP rs518054 was found to be located in the enhancer region of this gene. *NFIB* has various functions ranging from promoting cell differentiation during development to maintaining stem cell populations in adult tissues and also possess antiapoptotic effect

(69–72). *In vivo* studies have shown a multi-potency restriction of adult hippocampal neuronal stem cells by Drosha–NFIB interactions (73). It plays an important role in lung maturation and brain development (74), mediates repression of the epigenetic factor ezh2 which regulates cortical development (75), and also has an important role in chromatin remodeling (76). *NFIB* alters and globally maintains hyper accessible chromatin state and an increase of chromatin accessibility at distal regulatory elements enacts a program of gene expression (76). Thus the association we observed in the enhancer region of *NFIB* gene seems clinically and functionally relevant. The wide spread binding of *NFIB* in open chromatin sites has been linked to its regulatory action in adipocyte differentiation (77) and cancer metastasis (76). *NFIB* is also associated with osteosarcoma (78) and sciatica (79) in GWAS. All these findings point toward possible tissue-specific as well as genome-wide effects mediated through *NFIB*.

There is a paucity of studies examining the role of epigenetic mechanisms in frailty (12, 80). Epigenetic mechanisms including chromatin remodeling plays a pivotal role in the aging process (81, 82). Genes in the 9p21-23 locus have an important role in chromatin remodeling (76, 83). For instance, non-coding RNA *ANRIL,* specifically binds two polycomb proteins: CBX7 (PRC1) and SUZ12 (PRC2) to regulate histone modification in the CDKN2A/B locus. Overexpression of this gene also causes the down regulation of several genes involved in important chromatin architecture and remodeling mechanisms in other chromosomal regions (83). These results point toward a possible role of this locus in mediating environmental factors influenced epigenetic mechanisms. This might explain why this locus is linked with various age and environmental risk-associated diseases such as CVD, strokes and diabetes (24, 27, 34). Our finding thus supports a possible role of epigenetic mechanisms in frailty pathogenesis. Though there was higher prevalence of CVDs-associated risk allele with frailty, the association was not statistically significant. This might be mainly due to the smaller sample size as well as multifactorial origin of these diseases and frailty. Larger studies need to validate the initial observations in this study. Furthermore, since dementia was an exclusion criterion for the cohort, the association of some dementia related risk alleles with frailty might have minimized.

The strengths of our study include the systematic clinical and frailty assessments as well as the well-characterized population (40, 41). Limitation of this study is inclusion of incident frailty for increasing statistical power. The allele frequency of associated rs518054 "G" allele was observed to be more in prevalent and incident cases of frailty when independently compared with individuals free from frailty during course of this study. But the association was mainly driven by the prevalent frailty. The inclusion of incident frailty in the model provides us healthy controls free from frailty throughout the course of study. The lack of significant association with incident frailty might be mainly due to smaller sample size as well as objective-subjective definition of frailty used in this study. Limitations also include the absence of functional studies to validate the effect of associated genotypes with gene expression and chromatin interaction as well as the relatively small sample size. We noted the lack of consensus regarding frailty definitions, and chose a widely used and clinically relevant syndromic definition of frailty. Further studies need to be carried out to find the relevance of these observations in case of other frailty definitions. Our findings were based in a relatively genetically homogenous AJ population with high levels of education, which was used successfully for other genetic discoveries (40–42, 84–86) that have then been cross validated in other heterogeneous cohorts. However, our findings need to be validated in other more diverse populations.

In conclusion, we found novel association of variants in the 9p21-23 locus with frailty with lead SNP located in the enhancer region of the *NFIB* gene. Further investigation of this region is required to gain insights into potential interventions to address biological derangements in these pathways to extend health span and to maintain functional independence in older adults. The dynamics of healthy aging are complex and maintaining functional ability in older age is multifactorial. Frailty is one of the most significant geriatric syndrome observed in elderly population. Studies have shown that complex disorders increases with age but whether aging is the cause or consequence of these diseases is controversial. Our study supports a role for the complex disorder GWAS-associated 9p21-23 locus in frailty and provides insights into healthy aging.

ETHICS STATEMENT

The study was approved by the Institutional Review Board at the Albert Einstein College of Medicine. Written informed consent was obtained from all the study participants in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

SS and JV contributed to the design of the study and interpretation of the data. SS, NB, SM, and EA contributed to the acquisition of data and writing of the manuscript. EA, SM, and GA contributed to the analysis of the data. SS, NB, GA, SM, EA, and JV contributed to the critical revisions of the manuscript. All the authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fmed.2018.00105/full#supplementary-material.

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Objective vs. Subjective Health in Very Advanced Ages: Looking for Discordance in Centenarians

Lia Araújo [1,2], Laetitia Teixeira [1], Oscar Ribeiro [1,3] and Constança Paúl [1]*

[1] Center for Health Technology and Services Research, Institute of Biomedical Sciences Abel Salazar (CINTESIS-ICBAS), University of Porto, Porto, Portugal, [2] School of Education, Polytechnic Institute of Viseu, Viseu, Portugal, [3] Department of Education and Psychology, University of Aveiro, Aveiro, Portugal

Background: Living beyond 100 years of age is associated with several functional and health constraints but their impact depends on one's perception of the situation. Associations between self-rated health (SRH) with sociodemographic and psychosocial variables have been explored in several studies, revealing that one's health appraisal depends of factors beyond the objective health condition. There is a large body of literature concerning SRH in later life but lack of evidence about centenarians' perception of health and its associated factors, which could increase the available knowledge on the strengths and resources individuals in very advanced ages have for facing daily life limitations.

Objective and Methods: This study aims to analyse the relationship between subjective and objective health status in a sample of centenarians ($n = 127$). Subjective health was assessed by a single-item health measure, and objective health by considering the number of reported diseases and a functional capacity scale (BADL and IADL). Main health characteristics are described as well as examined the association between objective and subjective health.

Results: 46.5% of the sample has a good, very good, or excellent appraisal of their own health. SRH was associated ($p < 0.05$) with BADL and IADL scores and with the total number of diagnosis; when analyzing SRH according to the level of functional capacity, results revealed that most individuals with severe and moderate dependence have a reasonable to excellent SRH ($p > 0.05$).

Conclusion: Having diseases and functional dependence at 100 years old may not mean to have a bad SRH. The high variability in SRH and the discordance between objective and subjective measures are a proof of centenarian's capacity of adaptation and the existence of individual resources, which may be decisive for one' perception and handling of health situation at such an advanced age.

Keywords: centenarians, longevity, physical health, self-rated health, well-being paradox

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Gil Atzmon,
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Paola Sebastiani,
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Francesco Prattichizzo,
MultiMedica (IRCCS), Italy

****Correspondence:***
Lia Araújo
liajaraujo@esev.ipv.pt

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INTRODUCTION

One of the great achievements of the Twenty-first century will undoubtedly be the increase in exceptional longevity. As projected by the Population Division of the United Nations (1), at the beginning of 2100 there will be more than 25 million of individuals reaching 100 years old, which is more than 50 times the size of the population of centenarians in 2015. Japan is the leading country in terms of number of centenarians, but also in Europe, due to low fertility rates and decreasing old age mortality, there is an emergence of oldest old groups (2). In 2011, the total number of centenarians in Europe was 89,156, with France, Italy, and Greece presenting ratios higher than 20 centenarians per 100,000 inhabitants (3).

Gerontology and geriatric research has been affirming the importance of distinguishing various age subgroups within old age, in the sense that there are striking differences among young-old and the old-old or very-old, i.e., between the third and the fourth ages (4). Centenarians' studies have been revealing that reaching 100 years old brings several challenges at an individual level, but also to formal and informal carers since age-related adversities and age-related needs can be particularly demanding. Functional decline (e.g., physical health/activities of daily living restrictions, mobility, sensory impairment), and psychological and social losses (e.g., loss of loved ones and appreciated activities, dependency have been reported as being often present in such an advanced ages (5, 6).

As long life and longevity increases, an interest toward positive aspects of aging is gaining strength. Due to the difficulty in preventing and avoiding major age-related constrains, there is an increased investment in knowing how individuals manage adversity and in discovering the resources and protective factors of positive functioning (7). The ability to maintain positive self-perceptions and well-being despite hardships is considered an important paradox in advanced life, closely related with one's psychological resilience (8).

Self-rated health (SRH) is one of the most recognized subjective measures in aging research, clinical settings, and population's surveys. With a single and simple question ("In general, would you say your health is…") it is possible to know if the person considers his/her health as "excellent," "very good," "good," "fair," or "poor" (9, 10). The importance of this question also relates to its predictive value and independent effect on mortality, which has been demonstrated in numerous studies and diverse populations (11, 12).

Despite being considered a measure to examine subjective health status, several studies have shown that SRH reveals much more information. SRH is related to disease and functional status (13), but also with mental health [e.g., depression; (14)], suggesting that SRH can reflect the states of the human body and mind (12). Also psychosocial variables (e.g., life satisfaction and social support) and socioeconomic status have shown correlation with SRH (11, 15, 16), as well as personality traits (17) and other internal (e.g., as optimism and perceived control) and external (e.g., education, financial status) resources (18).

Due to the observed relation between SRH and objective health aspects, a decrease of SRH with advancing age is to be expected. However, evidence does not irrefutably confirm this hypothesis. Two reasons have to be considered. First, the relationship between subjective and objective health is complex and not direct or independent. Second, in old and very-old age there is a paradoxical pattern of discrepancy between subjective and objective indicators of health. Although there are some contradictions across studies, it seems that SRH does not decline only due to age-related decrease in health status (19). In Pinquart's Pinquart's (10) meta-analysis, for instance, there was a larger association with physical illness and functional limitations in those aged 60–75 years when compared to those aged above 75. Also in the oldest group, an increased association between mental health and SRH was verified (10).

Henchoz et al. (19), French et al. (14), and Zikic et al. (20), have studied objective and subjective measures of health in individuals with 80, 85, and 90 plus years old, respectively. Their findings point to a weaker relationship between objective health measures (e.g., medical diagnoses) and SRH, and to a less rapidly decline of the perception of health with advancing years than the one occurring in physical and functional health status. This weakening relationship between subjective and objective measures of health can be accounted for by several factors, including the capacity to adapt and be resilient, and can be an important indicator of the presence or absence of external and internal resources that might influence life's appraisal.

Despite some differences across studies, the available evidence on the oldest-old show that is possible to reach 100 years old in a relatively good health condition (21, 22). Centenarians have been presented as robust and resistant individuals, since they tend to survive, delay, or escape to the major age-related diseases, such as cancer, and cardiovascular diseases among others (23, 24). But there are many centenarians living in a frail and morbidity situation as well (25, 26). Also great difficulties in sensory domains and basic and instrumental daily living activities (BADL and IADL) have been widely reported in this population [cf. (27–29)]. Nevertheless, several investigations are looking to this age group as a prototype of successful aging (30, 31) due to their ability to maintain a positive outlook about life.

Centenarians can be a very interesting group to examine objective vs. subjective health appraisals because they have to face several health and functional capacity problems. The study of this relation may reveal if SRH can serve as an indicator of centenarians' objective health status and if the discrepancy between the two dimensions of health still exists in such an advanced age. In this study, we sought to examine the association between centenarians' own subjective evaluations of health and their objective health status.

METHODS

Data Collection

Data came from two centenarians studies, the Oporto Centenarian Study (PT100) and the Beira Interior Centenarian Study (PT100 Beira Interior) which were conducted in two distinct geographical regions of Portugal, each one with an area of approximately 60 km. Individuals aged 100 years and older between December 2013 and December 2014 were identified

through voter registration files, churches, nursing homes, local media newspapers, and through snowball sampling. This first step of recruitment resulted in 291 potential participants; all of these were contacted, and a final sample of 241 participants was face-to-face interviewed. Fifty centenarians were excluded because they died in the interim or their relatives refused participation because of advanced dementia and other major health problems or due to lack of interest in the study. Since this study requires centenarians' own perceptions, information was only assessed if the individual was not affected by severe cognitive impairment and was willing to present information on these aspects ($n = 127$).

Data was collected during one or two sequential interview sessions directly with the centenarian and/or with a proxy respondent. Age was verified by following a protocol entailing personal identity document verification (e.g., birth certificate) and milestones assessments (e.g., wedding date, date of firstborn, subsequent birthdates of children) following best research practices in this field (32). An informed consent previously approved by the National Commission on Data Protection was used. More information about the methodological procedures of both centenarian studies can be found elsewhere (33).

Measures

Three variables were considered as objective health: the number of diseases, the functional capacity in basic activities of daily living (BADL) and in instrumental activities of daily living (IADL). Diseases were assessed with a list of common health problems in older ages: high blood pressure, heart condition, diabetes, chronic lung disease, ulcers or other serious stomach issues, cirrhosis or other liver problems, kidney condition, frequent urinary infections, incontinence, prostate problems, problems with vision or hearing, arthritis, osteoporosis, stroke, cancer, pneumonia, falls, and other. Conditions mentioned as "other" were later coded. Functional disability was assessed through the Older Americans Resources and Services (OARS) Multidimensional Functional Assessment Questionnaire (34, 35). The scale includes SEVEN items to assess basic daily living activities (BADL, e.g., the ability to talk on the phone, to travel, go shopping, prepare meals) and other seven items to evaluate IADL (e.g., the capacity for walking, bathing, eating, toileting). Respondents were asked how much difficulty they had performing each of these activities by rating them on a three-point scale (2 = no difficulty; 1 = do with some help; 0 = cannot do without help). Cronbach's alpha for this study was 0.909 for the BADL scale and 0.879 for the IADL scale. Information regarding these three variables was collected with the centenarian's proxies, in most cases a family member or in the case of institutionalized centenarians it was a professional (e.g., nurse) of the nursing home.

Self-rated health (SRH) was assessed directly with the centenarian through a single item: "In general, would you say your health is…?," with five response options labeled as excellent, very good, good, reasonable, and bad. Responses were scored in 1 indicating a bad SRH, 2 for a reasonable SRH, and 3 for a positive SRH (excellent, very good, and good).

According previous work (36, 37), BADL, IADL, and number of diagnoses were categorized in three categories each. For ADL, the categories considered were: 1—Mild (IADL dependence only); 2—Moderate (dependent in 1–2 BADL); 3—Severe (dependence in 3 or more BADL). For the number of diagnoses, the three categories considered were: 0–1; 2–3; ≥4. Additionally, SRH was also considered as a three-point scale: 1—bad; 2—reasonable; 3—good, very good, or excellent.

Sociodemographic data was obtained from structured questions about age, gender, current marital status, living arrangements, having children, income per month, and income management.

Statistical Analysis

Description of the sample was performed using frequencies (absolute and relative), mean and standard deviation. Mean differences of objective health measures according to self-perception of health were performed considering a one-way ANOVA. To evaluate the association between categorical variables (objective health and SRH), Chi-square test was used. In all analysis, a significance level of 0.05 was considered.

RESULTS

Sample Characteristics

The sample comprises 127 centenarians with a mean age of 101.1 years (sd = 1.5 years, range = 100–108). One hundred and twelve centenarians are female (88.2%) and only 15 are male (11.8%). The majority are widowed ($n = 108$, 85.0%), 13 (10.2%) never married, 5 (3.9%) are married, and only 1 (0.8%) is divorced. Forty-six (36.2%) lived in an institution, and 12 (9.4%) lived alone. One hundred and seven (84.3%) have children. Almost 50% of the sample never attended school ($n = 59$, 46.5%). Concerning income, 20 (16.9%) receive <250 €/month, 78 (66.1%) receive between 250 € and 500 €, 16 (13.6%) between 500 € and 750 €, and 4 (3.3%) more than 750 €. Forty-nine (40.5%) reveal that cannot make ends meet, 52 (43.0%) just manage to get by, 14 (11.6%) have enough money with a little extra, and only 6 (5.0%) refer that money is not problem (**Table 1**).

Subjective and Objective Health

Fifty-nine centenarians (46.5%) report their health as good, very good or excellent, 48 (37.8%) as reasonable, and 20 (15.7%) as bad (**Table 2**). Concerning objective health, the mean score of BADL and IADL is 8.6 (sd = 4.2) and 3.8 (sd = 3.3), respectively. The average number of diagnoses reported by the centenarians is 3.6 (sd = 2.0), ranging from 0 (minimum) and 9 (maximum).

Association Between Self-Perception of Health and Physical and Functional Health

Considering the three objective measures of health (continuous variables), and comparing the mean values according to the three groups of SRH, we can verify in **Table 3** that differences between groups were found ($p < 0.05$ for the three variables).

TABLE 1 | Sample characteristics.

	N	*n*	%
Gender	127		
Male		15	11.8
Female		112	88.2
Age			
Mean (sd)	127	101.1 (1.5)	
Marital status	127		
Never married		13	10.2
Married		5	3.9
Divorced		1	0.8
Widowed		108	85.0
Living arrangements			
Live in a institution	127	46	36.2
Live in community	127	81	63.8
Have children	127	107	84.3
Attended school	127	68	53.5
Income per month	118		
<250 €		20	16.9
250–500 €		78	66.1
500–750 €		16	13.6
750–1,000 €		1	0.8
>1,000 €		3	2.5
Income management	121		
Can't make ends meet		49	40.5
Just manage to get by		52	43.0
Enough money with a little extra		14	11.6
Money is not a problem		6	5.0

TABLE 2 | Subjective and objective health of the sample.

	N	*n*	%
Self-Rated Health (SRH)	127		
Bad		20	15.7
Reasonable		48	37.8
Good, very good, or excellent		59	46.5
BADL, mean (sd)	127	8.6 (4.2)	
IADL, mean (sd)	127	3.8 (3.3)	
Number of diagnosis, mean (sd)	127	3.6 (2.0)	

Both BADL and IADL scores increase with the improvement of self-perception of health (i.e., better functional capacity was related with better SRH). Considering the number of diagnoses, results revealed that the group with a reasonable self-perception of health presented a higher number of diseases than the group with a bad self-perception (**Figure 1**).

The **Table 4** presents the results obtained (the percentage presented are % of total). No significant association was found between ADL and SRH. The percentage of centenarians with some extent of agreement between the two measures was 42%. Additionally, 29% referred a worse SRH comparing with the ADL capacities, and 29% referred a better SRH comparing with the ADL capacities. A significant association was found between SRH and number of diagnoses. The percentage of some agreement of the two measures was 32.3%. Only 8.7% referred a worse SRH comparing with the number of diagnoses and 59% referred a better SRH comparing with the number of diagnoses.

TABLE 3 | BADL, IADL, and number of diagnoses (mean scores) according to SRH.

	Bad SRH	Reasonable SRH	Good, very good or excellent SRH	*p*
	Mean (sd)	Mean (sd)	Mean (sd)	
BADL	6.46 (3.73)	8.40 (4.23)	9.57 (4.03)	0.013
IADL	2.20 (2.34)	3.50 (2.73)	4.65 (3.76)	0.010
Number of diagnosis	3.40 (1.90)	4.17 (1.85)	3.22 (2.02)	0.041

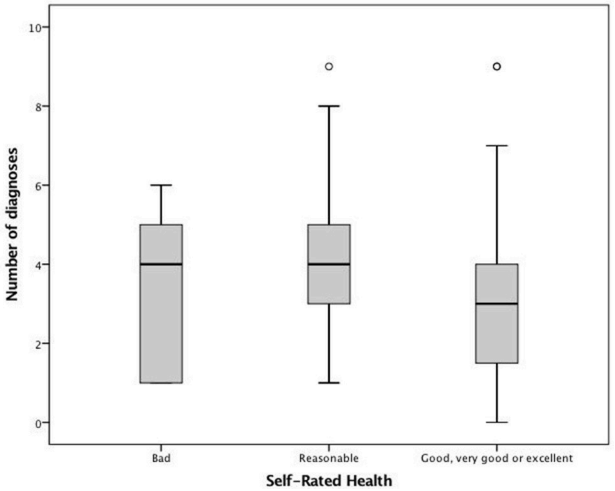

FIGURE 1 | Association between SRH and the number of diseases.

DISCUSSION

The present study analyzed different aspects of health in a sample of Portuguese centenarians, comparing their perceived health with measures of objective health status. Centenarians presented a mean number of diseases of 3.6, as well as several functional limitations. These results are in line with other international studies conducted with centenarians, such as the ones from Georgia and Fordham (USA), Denmark and Heidelberg (Europe) (6, 25, 28, 29). Nevertheless, almost half (46.5%) of our sample perceived their health positively (good/very good/excellent). This percentage is, however, lower than the results presented by Cho et al. (30), Jopp et al. (29), Tigani et al. (38), and Liu and Zhang (15) who revealed that 73, 67, 66.8, and 54.3% of the centenarians in their studies rated their health in similar positive ways. These results can be understood under the influence of age and culture in self-ratings of health (12).

TABLE 4 | Association between SRH and objective health measures (categorical variables).

	Bad SRH		Reasonable SRH		Good, very good or excellent SRH		*p*
	n	%	*n*	%	*n*	%	
ADL							0.264
Severe (dependence in 3 or more BADL)	8	6.5	14	11.3	12	9.7	
Moderate (dependent in 1–2 BADL)	4	3.2	8	6.5	10	8.1	
Mild (IADL dependence only)	6	4.8	26	21.0	36	29.0	
Number of diagnosis							0.006
≥4	12	9.4	31	24.4	15	17.3	
2–3	2	1.6	14	11.0	22	17.3	
0–1	6	4.7	3	2.4	22	11.8	

When examining the association between centenarians' own perception of health and their functional capacity, results of our study revealed that in overall BADL and IADL scores are associated with SRH, but that when further analyzing the different levels of dependence according to SRH, the association is no longer statistically significant. The majority of centenarians with mild dependence (IADL dependence only) have a positive (good, very good, excellent) SRH, but also the centenarians with moderate and severe dependence have higher rates of reasonable and good-excellent SRH (rather than a poor SRH). The association between the number of diagnosis and the SRH is statistically significant and the pattern is very similar to the previous one. The majority of centenarians with zero or one disease have a positive SRH, but the same happens for the ones with two or three diseases. Even the majority of centenarians with four or more diseases have a reasonable or good-excellent SRH. Together, these results allow us to state that having more diseases and dependence is not necessarily a sign of having a bad or negative SRH.

As verified with very old individuals [e.g., (10, 14, 17, 39)], it seems that also centenarians hold a weaker emphasis on their physical and functional status in the appraisal of their health condition. Previous studies that have shown discordance between objective and subjective health measures have associated it with the contribution of external and internal resources as optimism and perceived control (18, 40). A positive SRH may reflect the greater importance of psychological adaptation in very advanced age. Also the influence of downward social comparison has been reported. It seems that comparing oneself with others from the same age group who are in poorer health enables oldest old individuals (aged 80 plus) to maintain a positive SRH (19, 25). This is an important and common mechanism for the oldest old, since it is more frequent to find congeners in poor health at the age of 85 than at the age of 20, 40, or even 60 (19); in the cases of centenarians, however, since most individuals of the same generation are already dead, the comparison may elicit a more positive appreciation of one's health as it focuses on the exceptionality of still being alive. To date, there is limited information on the social comparison processes underneath SRH at such an advanced age, particularly the age group target for comparisons and its consequences for well-being. Such psychosocial process deserves, therefore, further attention.

Due to the great individual differences and disparities among oldest old individuals, especially in self-appraisals, several researches have been considering the role of risk and protective factors in explaining such variability. These studies have been presenting as a common trait of this age group the high weight of mental health aspects and psychosocial well-being factors when considering the correlates of SRH (10, 14, 17, 38). Puvill et al. (40) when analyzing the correlates of SRH in a representative population of 85-year olds found a weaker association with mortality and a stronger with mental health and life satisfaction. Therefore, an underrated subjective health condition may be indicative of psychosocial distress or burden of physical disability (14) and these health pessimists may be prone to depressive feelings (39). SRH has also been linked to frailty and anxiety in centenarians. Ribeiro et al. (26), for instance, found that SRF was the only predictor of depression in frail and pre frail centenarians and that a worse SRH increased the odds of experiencing clinical anxiety (41). When comparing centenarians with sexagenarians and octogenarians, Quinn et al. (42) found personality and levels of control as unique set of SRH's correlates in the oldest-old group. An apprehensive personality and low levels of control over health were more important than physical health variables in predicting a poor subjective health. Also Ruthig and Chipperfield (43), in a study on health incongruence in later life (ages 79–98 years) found that perceived control was weaker among pessimists; Tigani et al. (38) in a sample of Greek centenarians found that high optimism, adaptability and internal health locus of control were independently associated with good SRH.

CONCLUSION

The analysis of associations between objective and subjective health allowed to conclude that not all centenarians with moderate/severe constrains of ADL and diseases have a bad SRH, which may be related with the existence of other factors that are weighted in one's self-perception of health at such and advanced age. Being in the limit of longevity brings several challenges at the health level, and these may demand specific developmental regulation processes for fostering well-being. Research on these long-lived individuals regarding their difficulties but also the

variables promoting resilience (and inherent positive SRH) is a necessary investment in order to reach better years of life. Future studies should further examine the association between objective and subjective measures of health by considering the influence of adaptive resources, such as characteristics of personal disposition, and protective social comparison mechanisms which may be decisive for centenarians' perception and handling of health limitations.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Instituto de Ciências Biomédicas Abel Salazar (Universidade do Porto, Portugal) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Portuguese national data protection commission.

AUTHOR CONTRIBUTIONS

OR, LA, and LT were responsible for the study conception and design. OR supervised data collection and helped writing the manuscript. LA wrote the manuscript. LT performed the data analysis and CP critically revised the paper for important intellectual content.

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REVIEW
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Scoping Review of Neuroimaging Studies Investigating Frailty and Frailty Components

David López-Sanz[1,2†], Isabel Suárez-Méndez[1†], Raquel Bernabé[3], Natalia Pasquín[3], Leocadio Rodríguez-Mañas[3,4,5], Fernando Maestú[1,2,6] and Stefan Walter[3,5,7]*

[1] Laboratory of Cognitive and Computational Neuroscience (UCM-UPM), Centre for Biomedical Technology (CTB), Technical University of Madrid (UPM), Madrid, Spain, [2] Department of Experimental Psychology, Complutense University of Madrid (UCM), Madrid, Spain, [3] Fundación Para la Investigación Biomédica, Getafe University Hospital, Madrid, Spain, [4] Geriatrics Department, Getafe University Hospital, Madrid, Spain, [5] Centro de Investigación Biomédica en Red Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain, [6] Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain, [7] Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, United States

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Edited by:
Gil Atzmon,
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***Correspondence:**
Stefan Walter
swalter@psg.ucsf.edu

[†] These authors have contributed equally to this work and shared first authorship

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Background: Neuroimaging techniques are a cornerstone for diagnosing and investigating cognitive decline and dementia in the elderly. In frailty research, the physical as opposed to the cognitive domain of the aging process, neuroimaging studies are less common. Here we systematically review the use of neuroimaging techniques in frailty research.

Methods: We searched PUBMED for any publication reporting the association between neuroimaging markers and frailty, following Fried's original definition, as well as its determining phenotypes: gait speed, grip strength, fatigue and recent weight loss in the non-diseased population older than 65 years.

Results: The search returned a total of 979 abstracts which were independently screened by 3 reviewers. In total, 17 studies met the inclusion criteria. Of these, 12 studies evaluated gait speed, 2 grip strength, and 3 frailty (2 Fried Frailty, 1 Frailty Index). An association between increased burden of white matter lesions, lower fractional anisotropy, and higher diffusivity has been associated consistently to frailty and worse performance in the different frailty components.

Conclusions: White matter lesions were significantly associated to frailty and frailty components thus highlighting the potential utility of neuroimaging in unraveling the underlying mechanisms of this state. However, considering small sample size and design effects, it is not possible to completely rule out reverse causality between frailty and neuroimaging findings. More studies are needed to clarify this important clinical question.

Keywords: frailty, neuroimaging (anatomic and functional), review, gait speed, grip strength

INTRODUCTION

The number of older people in the global population is rapidly growing. From 2013 to 2060 the percentage of the population aged over 65 years is projected to increase from 18 to 28% and the proportion of those aged over 80 years will rise from 5 to 12% (1). Increased longevity raises social and economic challenges and has deep implications for the planning and delivery

of healthcare. Indeed, as the number of older people rises, so does the number of people with age-related disability and dependence that require support with daily activities, healthcare services and/or institutionalization.

The transition from a robust status to one of age-related disability is usually preceded by a physiological state termed frailty (2, 3). Although frailty can be characterized using classical clinical phenotypes and laboratory-based biomarkers, a universally accepted definition of frailty remains to be agreed upon (4, 5). The most widely accepted definition of frailty is "an age-associated biological syndrome, characterized by a decrease of the biological reserve and resistance to stress due to a decline in several physiological systems. This places the individual in a special risk category when facing minor stressors and is associated with poor outcomes (disability, hospitalization and death)" (6). The most prominent approach used to assess frailty is using Fried's Frailty Criteria (7). Following this model, frailty is diagnosed based on the presence of at least three of the five physical attributes and capabilities of an individual. These include: weight loss (unintentional weight loss of 4.5 kg or more in the last year), exhaustion (self-reported), physical inactivity, slow walking speed, and weakness (low grip strength).

Many research initiatives, including the large scale European FRAILOMIC initiative, investigate OMIC factors associated to frailty (4, 8). In a recent seminar published in the Lancet (6), the authors discuss under the subheading ≪The Frail Brain≫ only the structural and physiological changes taking place in the brain that are known to be associated with chronological age but not with frailty specifically. They reference the relationship between frailty and cognition as an example of the frail brain rather than answering which specific structural and physiological changes in the brain are associated with frailty.

In this scoping review the objective is to summarize the use of neuroimaging techniques in investigating Fried Frailty in the non-diseased, elderly population. In addition, we want to narratively outline whether current knowledge supports an overlap with dementia research.

METHODS

Following PRISMA methodology, for this scoping review we searched PubMed looking for works published prior to February 2018 (9–11).

We used the following query:

Neuroimaging [MESH] AND (Frailty OR (gait velocity OR gait speed) OR (grip strength OR muscle strength) OR fatigue OR weight loss)

We restricted the result set to those investigating humans using the PubMed filter functionality and adults older than 65 years. Nine Hundred and Seventy-Nine abstracts were reviewed independently by three researchers (SW, RB, and NP) with the help of abstrackr software without using the prediction algorithm (12). We excluded 958 papers, including those that investigated the relationship between neuroimaging markers and frailty parameters such as gait speed or grip strength only in diseased populations (e.g., Parkinson's Disease, Stroke, etc.) after reviewing the abstract. Of the remaining 21 papers that passed through full text screening, 13 were excluded for different reasons: not investigating frailty or its components ($n = 6$), study design not restricted to a population of 65 years of age or older ($n = 5$), inadequate study design ($n = 1$) and not including a neuroimaging marker ($n = 1$). When reviewing the references from the 21 articles originally deemed eligible after abstract screening, 9 studies were further considered eligible (**Figure 1**).

RESULTS

Of the 17 studies that fulfilled the inclusion criteria (**Table 1**), 3 studied frailty (2 Fried Frailty, 1 Frailty Index), 2 studies investigated grip strength, and 12 studies investigated gait speed or gait parameters. All but three studies were cross-sectional in nature. **Table 2** lists the details of the outcome assessment, the imaging risk factors studied, the application of confounder control, and the conclusions for each of the studies included in this review.

In total, a maximum of 7,026 independent individuals participated in the studies evaluated, with the median study size

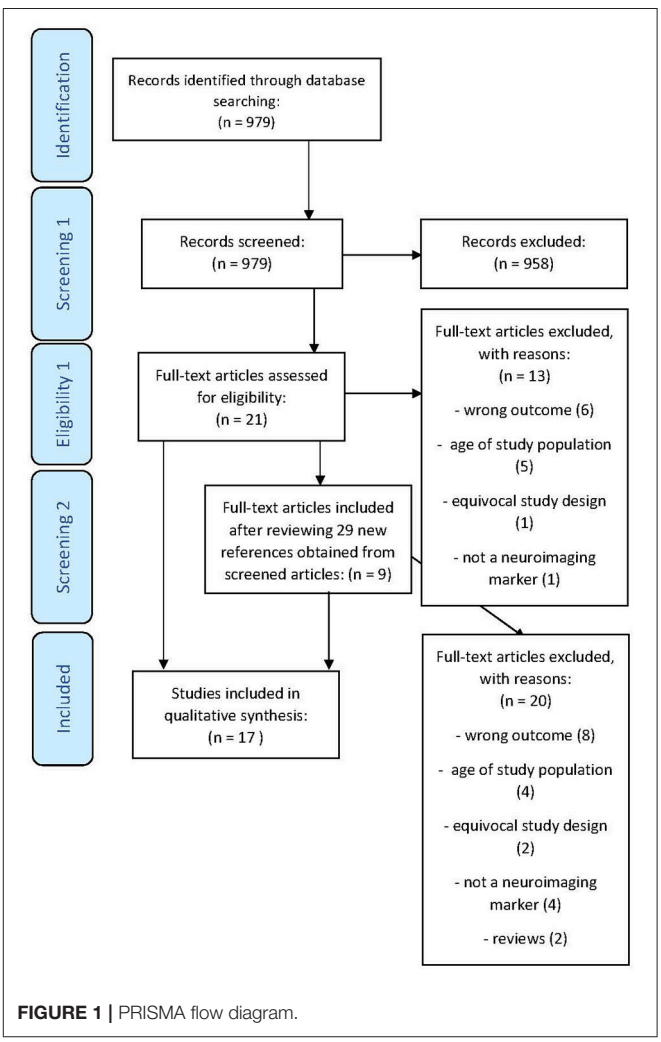

FIGURE 1 | PRISMA flow diagram.

TABLE 1 | Descriptive overview of reviewed studies.

References	Year	N (% female)*	Age (mean, SD)	Design	Outcome	Imaging technique
FRAILTY						
(13)	2017	176 (40.0%)	75.0 (5.2)	Cross-sectional	Frailty	Structural (T1-weighted MRI incl. DTI)
(14)	2014	87 (62.1%)	Median 78 (IQR 74-83)		Frailty	Structural (T2-weighted MRI)
(15)	2001	4735 (42.8%)	72.7 (4.33)	Cross-sectional	Frailty	Structural MRI (Image weighting not specified)
GRIP STRENGTH						
(16)	2015	191 (53.4%)	70.3 (4.8)	Cross-sectional	Grip Strength	Functional MRI (Resting State)
(17)	2016	165 (51%)	70.15 (4.50)	Cross-sectional	Grip Strength	Structural (T1-weighted MRI incl. DTI) and Functional (Resting State)
GAIT SPEED						
(18)	2010	148 (56.1%)	79 (IQR 76 - 83)	Cross-sectional	Gait Speed	Structural (T1-weighted MRI)
(19)	2012	214 (64.5%)	72.82 (3.77)	Cross-sectional	Gait Speed	Structural (T1-weighted MRI)
(20)	2010	795 (58.9%)	75.6 (5.5)	Cross-sectional	Gait Speed	Structural (T1-weighted MRI)
(21)	2016	265 (57%)	82.9 (2.7)	Cross-sectional	Gait Speed	Structural (T2-weighted MRI incl. DTI)
(22)	2015	30 (55.17%)	72.5 (5.22)	Cross-sectional	Gait Speed	Functional MRI (Resting State)
(23)	2008	104 (61.5%)	85.1 (5.6)	Longitudinal	Gait Speed	Structural MRI (T1 and T2-weighted)
(24)	2009	1702 (60.6%)	72.4 (4.1)	Longitudinal	Gait Speed	Structural MRI (T1 and T2-weighted)
(25)	2007	327 (56.5%)	78.2 (3.9)	Cross-sectional	Gait Speed	Structural (T1 and T2-weighted MRI)
(26)	2005	2450 (57%)	74.4 (4.7)	Longitudinal	Gait Speed	Structural (T1 and T2-weighted MRI)
(27)	1999	50 (62%)	85.1 (7.2)	Cross-sectional	Gait Speed	Structural (T1-weighted MRI)
(28)	2003	97 (40.2%)	78–79	Cross-sectional	Gait Speed	Structural (T2-weighted MRI)
(29)	2000	390 (0%)	72.37 (2.96)	Cross-sectional	Gait Speed	Structural MRI (Image weighting not specified)

**The n refers to the source population for which the descriptive statistics % female and mean age are reported.*

being of 191 participants, ranging from as low as $n = 30$ for the functional MRI study of gait speed (22) to $n = 2{,}450$ for the study with participants from the Cardiovascular Health Study (CHS) (26) published in 2005. None of the studies estimated a population effect by reweighting to the source or even general population (30).

Findings by Frailty and Frailty Components

Frailty

In total, 3 studies were identified that investigated the association of structural brain parameters with frailty using MRI (13, 15, 31). Two of these, Avila-Funes et al. and Newman et al., analyzed directly the frailty phenotype originally proposed by Linda Fried (7), and Jung et al. reported in a letter the association between white matter abnormalities and a Frailty Index conceptualized as a combination of basic and instrumental activities of daily living, physical performance, cognitive function and serum albumin level. This index showed a significant correlation (Spearman′s $= 0.49$, $p < 0.001$) with Fried Frailty (14). All studies conclude that a higher burden of White Matter Lesions (WML) volume was associated with the prevalence of frailty. In addition, the original study from Newman et al. in participants from the above-mentioned CHS found evidence for a higher number of infarct lesions and increased ventricular size in frail participants but no association with sulcus size. Furthermore, Avile-Funes et al. found that white matter integrity assessed using diffusion tensor imaging was less preserved in frail participants from the AMIage study (13). This study investigated the relationship between fractional anisotropy (FA, lower in frail vs. non-frail participants), axial diffusivity (AD, higher), radial diffusivity (RD, higher), and mean diffusivity (MD, higher) across white matter tracts including the corpus callosum, anterior limb of internal capsule, external capsule, and posterior thalamic radiations.

All these studies adjusted for major confounders such as age, gender, and major age-associated diseases and were nested in longitudinal cohort studies.

Grip Strength

Hirsiger et al. evaluated the association between grip strength and structural/functional connectivity in the cingulum during resting state as obtained from DTI and fMRI respectively in 165, cognitively normal older participants (mean age 70.15) from the longitudinal healthy aging brain (LHAB) project of the University of Zurich, Switzerland (17). They found that an increase in FA in the cingulum bundle was positively associated with grip strength ($p = 0.022$) while an increase in mean diffusivity was negatively associated with grip strength ($p = 0.018$) in models adjusted for age, gender, education, and diastolic blood pressure. Resting state functional connectivity in the cingulum, more concretely the correlation between posterior cingulate cortex and medial prefrontal cortex BOLD signals, was not associated to grip strength ($p = 0.270$).

Seidler et al. evaluated the same study sample as Hirsiger et al. but looking at individual regions-of-interest (ROIs), using left primary motor cortex, left putamen and right cerebellum lobules V and VIII, all of them associated to hand motor performance

TABLE 2 | Outcome, Imaging Risk Factors, and Conclusions from reviewed studies.

References	Year	Study acronym or name (location)	Outcome assessment	Imaging risk factor	Confounder control	Conclusion
FRAILTY						
(13)	2017	AMImage	Fried Frailty	White Matter Hyperintensities and Integrity: Fractional Anisotropy (FA), Axial Diffusivity (AD), Radial Diffusivity (RD), and Mean Diffusivity (MD).	Yes	Frail people have higher white matter hyperintensity volume and loss of white matter integrity.
(14)	2014	Seoul National University	Frailty Index estimated from: Score in daily activies, cognitive function, physical performance and serum albumin test.	White Matter Lesions (WML).	Yes	Higher frailty score in those subjects with more WML, thus they conclude both variables to be associated.
(15)	2001	CHS	Fried frailty.	WML, Infarct-like Lesions, Sulcal Prominence and Ventricular Size.	Yes	Frail subjects showed more infarct lesions, increased white matter abnormalities and increased ventricular size, no effect on sulcus size was found.
GRIP STRENGTH						
(16)	2015	LHAB	Grip Strength with hydraulic hand dynamometer.	Functional Connectivity between Left Motor Cortex, Left Putamen, Right Lobule V, R Lobule VIII.	No	Sensorimotor cortex connectivity is positively associated with grip strength.
(17)	2016	LHAB	Grip Strength with hydraulic hand dynamometer.	White Matter Integrity: FA, MD, RD, AD in Cingular Bundle; approximated Default Mode Network Connectivity	Yes	RD was significantly associated to grip strength, resting state functional connectivity was not.
GAIT SPEED						
(18)	2010	MCSA	Gait Speed using a 4.88 m digitized walkway system.	White Matter Hyperintensities.	No	Higher white matter intensity volumes across all regions were associated to lower gait speed.
(19)	2012	CHS	Gait Speed using a 4.57 m course and the average of 2 measurements.	Gray Matter Volume of the Prefrontal Area.	Yes	Smaller prefrontal area gray matter volume is associated with slower gait speed.
(20)	2010	AGES	Gait Speed using a 6 m course and the average of 2 measurements.	Magnetization Transfer Ratio, White Matter Hyperintensities, Brain Athrophy and Brain Infarcts.	Yes	Lower magnetization transfer ratio, higher white matter intensity volume and generalized brain atrophy but not brain infarcts were associated to slower gait speed.
(21)	2016	HealthABC	Gait Speed using an 8 m computerized walkway.	White Matter Hyperintensities and FA.	Yes	Higher white matter lesion volume was associated with slower gait speed, a significant interaction was observed between white matter hyperintensities and FA. In high FA individuals, the association was non-significant.
(22)	2015	CCMA	Gait Speed on 6.10 m computerized walkway.	rs-FMRI and ICA Decomposition.	No	Gait Speed associated with well-established sensorimotor, visual, vestibular, and left fronto-parietal resting-state networks in older adults.
(23)	2008	Oregon Brain Aging Study	Gait Speed using a 9 m course.	Periventricular, Subcortical and Total WMH, Total Brain Volume, Hippocampal Volume, CSF Volume.	Yes	Higher baseline total and periventricular white matter hyperintensities was related to more pronounced change in gait speed and number of steps during follow-up. Higher rate of periventricular white matter hyperintensities accumulation was associated with increased gait slowing.
(24)	2009	3C study France	Gait Speed using a 6 m course.	White Matter Lesions.	Yes	Periventricular WML volume was associated with slow gait speed in those subjects above 90th percentile of WML volume, deep WML volume was not. Baseline total WML volume predicted walking speed decline in follow-up.

(Continued)

TABLE 2 | Continued

References	Year	Study acronym or name (location)	Outcome assessment	Imaging risk factor	Confounder control	Conclusion
(25)	2007	CHS	Gait Speed using a 4.57 m course and Balance checking the ability to hold semitandem position for at least 10 s.	Gray Matter Volume of ROIs known to be associated with mobility.	Yes	Smaller gray matter volumes remained associated with slow gait and poor balance after cofounder control in LH smaller cerebellum and dorsolateral prefrontal regions (slower gait) and RH basal ganglia, superior posterior parietal cortex and cerebellum (balance difficulty).
(26)	2005	CHS	Gait Speed using a 4.57 m course.	Ventricular Enlargement, White Matter Hyperintensities, Subcortical and Basal Ganglia Small Brain Infarcts.	Yes	Presence of structural brain abnormalities was associated with greater risk of incident functional impairment and greater risk of gait speed decline after cofounder control.
(27)	1999	Oregon Brain Aging Study	Gait Speed using a 9.14 m course.	Total Brain Volume, Intracranial Volume, Ventricular Volume, Periventricular High Signal, Deep High Signal.	Yes	Ventricular volume and periventricular white matter high signal volume, but not total brain volume or deep white matter high signal, were correlated gait speed independent of age.
(28)	2003	ABC 1921 Study	Gait Speed using a 6 m course.	WML, Periventricular Lesions and Brain Stem Lesions.	No	Decreased gait speed correlated significative with an increased grade of brain stem lesions.
(29)	2000	NHLBI Twin Study	Gait Speed using a 2.43 m course (faster of two walks).	White Matter Hyperintensities, Total Cranial Brain Volume (TCB).	Yes	Above the median total brain volume but not white matter hyperintensity volume was associated with higher gait speed.

(16). They found that resting state connectivity between the motor cortex, bilateral sensorimotor cortex and supplementary motor area was greater in participants with higher grip strength. They also found stronger connectivity between the putamen region, medial frontal cortex and precuneus, as well as between the cerebellar seeds, the frontal cortex and temporal regions associated with higher grip strength. In addition, cerebellar lobule V showed increased connectivity with lobules VIIIa and VIIIb with greater grip strength.

Gait Speed

Twelve studies using data from 9 population studies investigated gait speed. 11 studies used structural MRI imaging for testing, among other aspects, the association between WML ($n = 9$) (18, 20, 21, 23, 24, 26–29) and gray matter ($n = 2$) (19, 25) with gait speed, while one study evaluated resting state networks using fMRI and their association with gait speed (22).

In each study relating gait speed to neuroimaging markers, gait speed was assessed differently. Nevertheless, all but one study used velocity in units of distance (m or cm) per second as outcome measure rather than time in seconds for walking a predefined distance.

Nine studies investigated the relationship between gait speed and WML. Generalized measures of WML were associated with slower gait in models adjusted for major confounders in eight out of nine studies. Only the NHLBI Twin Study, one of the earliest neuroimaging studies from the year 2000, did not report a significant effect, although the tendency was consistent with the other works. Some studies (23, 24, 27) also analyzed the effect of periventricular WML burden coming to the same conclusions. In these studies, deep WML volume was not associated with gait speed. In addition, Silbert et al. examined the effect of change in WML volume and concluded that the accumulation of WML was associated with increased gait slowing during follow-up. Rosario et al. additionally investigated the possibility of an interaction effect between WML and white matter integrity measured by FA in participants from The Health, Aging and Body Composition Study (HealthABC) and found that the association between WML volume and gait speed was not significant in high FA individuals.

Two studies by Rosano et al. (19, 20) in participants from the CHS and Age, Gene/Environment Susceptibility (AGES) study investigated the association between gray matter volume and gait speed. Using a ROIs approach of areas a priori known to be associated to mobility, they identified an association between small volumes in cerebellum and dorsolateral prefrontal regions (25) and prefrontal gray matter volume (19) with slower gait. In addition, brain atrophy—defined by an atrophy index computed as (intracranial volume–brain volume)/intracranial volume—but not cerebral infarcts were associated with reduced gait speed in the AGES study.

The only study investigating resting state connectivity via functional MRI in participants from the Central Control of Mobility in Aging (CCMA) study confirmed an association between well-established sensorimotor, visual, vestibular, and left

fronto-parietal resting state networks and gait speed in older adults.

DISCUSSION

Neuroimaging techniques and in particular functional neuroimaging, a cornerstone for diagnosing and investigating cognitive decline and dementia in the elderly, are hardly used to identify biomarkers and risk factors associated to frailty. This is surprising given the close link between frailty and cognitive decline which has led to ≪cognitive frailty≫ becoming a major research topic (32–34). As of end of January 2018, only 3 studies directly assessed the association between frailty and neuroimaging markers identifying a relationship between an increased burden of white matter hyperintensities, lower fractional anisotropy and higher diffusivity with a higher prevalence of frailty. None of these studies evaluated connectivity or any other functional metric. Furthermore, among these studies, the different frailty components have received uneven attention with many more studies focusing in the relationship between neuroimaging markers and gait speed compared to handgrip strength. A higher burden of white matter hyperintensities has been associated to lower gait speed. Furthermore, lower fractional anisotropy and an increase in mean diffusivity were associated to low gait speed and grip strength.

More white matter hyperintensities and lower white matter structural integrity were found to be associated with an increased prevalence of frailty, lower grip strength and slower gait in all studies that investigated this neuroimaging risk factor and were considered for this review. These results support investigative efforts into the role of the central nervous system and vascular damage as possibly being implicated in the pathophysiology of frailty. Findings supporting these results highlight the association between structural changes and WML with physical fitness and activity (35). In fact, white matter hyperintensities, possibly the result of arteriosclerotic processes, are almost ubiquitous in the elderly (36) and their presence is facilitated by the exposure in mid-life to well-known risk factors such as smoking, hypertension, diabetes mellitus, and chronological age (37). They are also consistently associated with cognitive impairment (37). However, to be associated with global cognitive decline, the presence of other lesions is required and by themselves they cannot be used as an indicator of dementia (38). As such, it is problematic to infer the role of WML in the development of frailty from the knowledge available to date, particularly since most of the studies reviewed here and all that directly investigated the frailty phenotype are cross-sectional and the reported findings could be a result of reverse causation. Accumulating longitudinal evidence in the fields of stroke, dementia and mortality, supports the role of white matter hyperintensities as a risk factor for these endpoints. But the associations reported for frailty, whether causal or not, might not be sufficient to back the classification of WML as a risk factor useful in the diagnosis or prognosis of frailty. Whether or not WML can provide useful information in combination with other biomarkers from the brain or OMICs remains to be evaluated.

White matter microstructure has been associated to frailty and its defining phenotypes in this review. DTI has emerged as a technique allowing the study of white matter changes occurring at a microscopic level before its macroscopic manifestations are visible on a structural MRI (39). DTI seeks to evaluate the loss of white matter microstructure integrity by characterizing the degree of restriction to movement across different ellipsoid axis (AD, RD, MD) as well as the relative degree of anisotropy in a region of interest indicative of a preferential diffusion path. DTI's sensitivity to subtle abnormalities has encouraged its application to the study of the aging brain under both healthy and pathological conditions, yet only two of the studies considered in this review deal directly with the microstructural alterations—as extracted from the exploration of DTI parameters—regarding frailty condition (13) or frailty-related components (17). The first study informed of a greater loss of WM integrity (lower FA and higher diffusivity values) in frail participants. Local decreases in FA have been also observed in normal aging—involving frontal WM and anterior cingulum—while DTI abnormalities found in participants undergoing cognitive decline (MCI) or neurodegenerative disease (AD) are also significant in posterior regions signaling a loci of irregularities that could be related to an Alzheimer's disease type pathology [for a systematic review, see (40)]. One of the regions reported in Avila-Funes et al. to exhibit a lower FA in frail older adults is the anterior limb of the internal capsule. This region has been subjected to some discrepancy in the MCI and AD literature. Some authors do not find significant reductions in FA (41, 42) while others do (43). The later suggest that motor dysfunction is part of the incipient process of AD but as this is not often clinically supported is thought to represent an uncommon subgroup within AD patients (40) that could be related to those individuals manifesting both a cognitive decline and a frailty condition. The anterior limb of the internal capsule, pinpointed in the study of Avila-Funes et al., is involved in the connection of frontal regions with different brain regions. Interestingly, frontal structural disconnection has been linked to cognitive decline in older adults, which seems to support the link between frailty and cognition. In Hirsiger et al., reduced grip strength was associated to the loss of WM microstructural integrity in the cingulum, a region whose fibers have been reported to present a significant FA reduction in MCI and even more in AD (44).

Many of the studies covered in this work agreed on the finding that brain volume reductions — manifested as either ventricular volume increase (15, 26, 27) or a diffuse reduction in total brain volume (20, 29)—are associated to classical phenotypes of frailty. However, the specific cortical atrophy pattern associated to physical frailty is yet to be fully established as very little work has addressed this question. In this vein, two of the studies reported significant reductions in prefrontal volume linked to slower gait speed (19, 25), which could shed some light in this regard. Gray matter atrophy is a hallmark of dementia progression and is closely linked to cognitive dysfunction (45). Interestingly, Silbert et al. (23) failed to find any significant relationship between gait speed and hippocampal volume, which is one of the first

structures showing volume reduction in Alzheimer's Disease dementia (46). Nevertheless, it is important to bear in mind that the specific pattern of gray matter atrophy is highly dependent on the dementia cause. Interestingly, the comorbidity between physical frailty and cognitive deterioration leading to dementia observed in epidemiological studies, seems to be supported by the fact that frailty has been consistently linked to gray matter atrophy in the few neuroimaging studies available to date, which is solidly known to be also a major risk factor for dementia development (47).

Functional connectivity estimates the reciprocal interactions between distant brain regions as a function of the statistical dependence between their respective activity time courses. Synchronous activity has been reported to be consistently associated to cognitive (48) and even motor performance (49). However, although its influence in cognitive deterioration and dementia is receiving increasing attention, its role has been very scarcely studied in the context of frailty. From the reviewed literature only three works reported functional connectivity metrics. Hirsiger et al. (17) failed to find any statistical relationship between posterior cingulate cortex-medial prefrontal cortex connectivity and grip strength. This particular link represents one of the major features of the default mode network (DMN), which is associated to internal processing states and is a critically associated to dementia progression (50). However, the other two studies employing FC metrics (16, 22) included a larger set of regions in their analyses obtaining in both cases similar results, highlighting a significant hyposynchronization affecting particularly sensorimotor areas and prefrontal regions. Although sensorimotor network is not one of the key networks in dementia progression, fronto-parietal network disruption (as reported by Yuon et al.) has been extensively linked to cognitive deterioration, particularly in attention and executive functions. This particular pattern of alterations could underlie the observed relationship between frailty and dementia risk. In general, functional neuroimaging techniques, such as MEG, have shown great utility in detecting the initial stages of dementia and its associations with amyloid-beta [for a review see (51)], which could be an important factor in explaining the link between frailty and dementia.

LIMITATIONS

This scoping review has important limitations. First, the restrictions to the Fried phenotype and the non-diseased, elderly (65+ years) population, might have significantly reduced the study base. However as frailty phenotype is both more prevalent and potentially impactful in the older population we focused our review in that specific segment of the population. Nonetheless, to the best of our knowledge, this is the first review addressing the use of neuroimaging markers in frailty research, thus making it important to focus on research that approaches frailty from a broad perspective and in the non-diseased population to avoid coming to conclusions biased by results from specific diseased groups. Furthermore, although frailty definitions different from the Fried phenotype model exist (6, 52), it is still the most commonly employed. Second, as most of the studies reviewed are cross-sectional, reverse causality cannot be excluded, and the results reported here should be considered as mere statistical associations. Third, as in all observational research, residual confounding that artificially creates a statistical association between neuroimaging markers and frailty due to a common, unknown factor cannot be excluded. Fourth, as this review was restricted to the general, non-diseased population, we did not include different studies pinpointing a link between frailty and beta-amyloid accumulation in AD-related regions in at-risk population (53, 54). However, these studies could also be considered a very promising direction for future research into the relationship between dementia or cognitive dysfunction and frailty.

In conclusion, current literature supports the association between increased burden of white matter lesions, lower fractional anisotropy, and higher diffusivity with frailty and an overall worse performance in the different frailty components (i.e., gait speed and handgrip strength). However, the overall study base contributing to these findings is very small, mostly cross-sectional and does not allow for generalizations. Representative, longitudinal neuroimaging studies, structural and functional, investigating frailty and the subgroup of people that exhibit frailty and cognitive decline as comorbidity are urgently needed to identify processes that are specific to frailty or common to both frailty and cognitive decline and dementia to facilitate the differential diagnosis in the clinical setting.

AUTHOR CONTRIBUTIONS

SW had the original idea of the manuscript, participated in the literature search and selection, and authored the initial draft of the manuscript. DL-S, IS-M, RB, NP participated in the literature review, the abstract screening, and the results extraction, and authored sections of the manuscript. FM, LR-M provided important intellectual input to the manuscript. All authors read, contributed, and approved the final version of the manuscript.

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