The 100-plus Study of Dutch cognitively healthy centenarians: rationale, design and cohort description

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ABSTRACT:

BACKGROUND: Although the prevalence of dementia increases exponentially with age, some individuals reach >100 years with fully retained cognitive abilities. To identify the characteristics associated with the escape or delay of cognitive decline, we initiated the 100-plus Study (www.100plus.nl).

METHODS: The 100-plus Study is an on-going prospective cohort study of Dutch centenarians who self-reported to be cognitively healthy, and family members. We collect demographics, life-history, medical history, genealogy, neuropsychological data and blood samples. Centenarians are followed annually until death. PET-MRI scans and feces donation is optional. Almost 30% of the centenarians agreed to post-mortem brain donation.

RESULTS: The first 300 centenarians (25% males) included in the cohort came from higher socio-economic classes and had higher levels of education compared to their birth-cohort; alcohol consumption of centenarians was similar, and most males smoked during their lifetime.

At baseline, the centenarians had a median MMSE score of 25 points (IQR: 22.0-27.5); the large majority lived independently, retained hearing and vision abilities and was independently mobile. Mortality was associated with cognitive functioning: centenarians with a baseline MMSE score ≥26 and <26 points had a mortality-rate of respectively 0.19 and 0.54 in the second year after baseline (p=0.003).

The cohort was 2.1-fold enriched with the neuroprotective *APOE-ε2* allele relative to 60-80 year-old population controls ($p=4.8x10^{-7}$), *APOE-ε3* was unchanged and *APOE-ε4* was 2.3-fold depleted ($p=6.3x10^{-7}$).

CONCLUSIONS: The 100-plus Study cohort represents cognitively healthy centenarians. Comprehensive characterization of this cohort might reveal protective factors that explain the pathophysiology of long-term preserved cognitive health.

KEYWORDS :

100-plus Study, prospective cohort study, centenarians, cognitive health, longevity,

APOE

BACKGROUND:

Although increasing age is the strongest predictive factor for cognitive decline and dementia, it is not an inevitable consequence of aging. The incidence of overall dementia starts to increase exponentially from approximately 60 years and at age 100 years the annual dementia incidence reaches 40% per year [1, 2]. However, the mere existence of cognitively healthy individuals older than 110 years [3-6] leads to the intriguing suggestion that the incidence of dementia decelerates somewhere after 100 years (See **Box**). Factors that allow for the preservation of cognitive health may thus be enriched for in super-agers, individuals who reach extreme ages while enjoying full cognitive functions [7].

This raises several questions: which lifestyle behaviors influence the preservation of cognitive health until extreme ages? What are the unique molecular mechanisms that cause resilience against age related decline? Which hereditable factors are involved, and what is the role of the immune system? How can human neurons, which do not or rarely divide after birth, remain competent for more than 100 years? The answers to these questions are likely to provide novel insights in the effects of aging on the brain and they will be informative for the design of novel strategies that intervene in processes that lead to neurodegenerative diseases [8]. To answer these questions, we initiated the 100-plus Study, a cohort study of centenarians who self-reported to be cognitively healthy (www.100plus.nl). The study was initiated in 2013 at the Alzheimer center at the VU University Medical Center, Amsterdam, the Netherlands and to date, the cohort includes >300 centenarians.

The combination of extreme old age with maintained cognitive health is often observed in families [9-14], suggesting that beneficial factors involved in the long-term maintenance of both cognitive and overall health are heritable, and likely genetic [15, 16, 7, 17]. Indeed, results from the New England Centenarians Study indicated that siblings from centenarians are ~8-12 times more likely to reach 100 years compared to individuals with no centenarian siblings [18]. Children of centenarians also profit from

the advantage they inherited by their centenarian parent: they live longer, and have almost 90% lower risk of developing myocardial infarction, stroke and diabetes compared to age-matched peers whose parents have average life spans [16, 19, 20]. Together, this suggests that first-degree family-members of centenarians are also enriched for protective (genetic) factors and that efforts to identify protective factors should include targeting the families of centenarians [21]. For this reason, we extended the 100-plus Study with a second phase in 2017, in which we also include first-degree family-members of centenarians and their partners.

Here we present the rationale for the 100-plus Study (See **Box**), we describe the study design and procedures, and we introduce the 100-plus Study cohort based on demographic characteristics relative to their birth-cohort, and we describe the clinical presentation of the centenarians at baseline.

METHODS/STUDY DESIGN

Please find in the **electronic supplementary material** a complete compendium of participant inclusion procedures and current data collection procedures of the 100-plus Study.

Inclusion and exclusion criteria: The 100-plus Study includes (i) Dutch-speaking centenarians who can (ii) provide official evidence for being aged 100 years or older, (iii) self-report to be cognitively healthy, which is confirmed by a proxy, (iv) consent to donation of a blood sample and (v) consent to (at least) two home-visits from a researcher, which includes an interview and neuropsychological testing. In the second phase of the 100-plus Study (from September 2017 onwards) we include (i) siblings or children from centenarians who participate in the 100-plus Study, or partners thereof who (ii) agree to donate a blood sample, (iii) agree to fill in a family history, lifestyle history and disease history questionnaire. Study exclusion criteria are limited to subjects who are legally incapable.

Recruitment and research visits:

Recruitment: We regularly perform an online search for local newspaper articles that mention a centenarian. These reports commonly include the name of the centenarian, and sometimes a description of their well-being and living situation. We retrieve an address online and we approach a prospective study participant by letter. When they express their interest in study participation and inclusion criteria are met, we schedule two baseline visits. (**See supplementary material** for detailed recruitment procedures).

Baseline visit: A researcher, trained to perform standardized visit procedures, will visit the centenarian. The baseline visit (T0) consists of two visits. The first baseline visit takes approximately 2 to 3 hours, and comprises obtaining informed consent for study inclusion, a life-history interview, an assessment of genealogy, and an assessment of current health and medical history (**Table 1**). The second baseline visit, approximately

one week after the first, takes approximately 1.5 hours: during this visit we subject the centenarian to a battery of neuropsychological tests and we measure grip strength and blood pressure (**Table 2**). During the first baseline visit we inform participants of optional parts of the 100-plus Study: post-mortem brain donation, PET-MRI or PET-CT imaging and feces collection. Once a centenarian volunteers to participate in these parts of the 100-plus Study, we obtain informed consent for these study parts separately.

Follow-up visits: During yearly follow-up visits (T1, T2…), which take approximately 2 hours: we inform about possible changes in cognitive functioning that took place in the last year, we update the interview questionnaire and re-administer the complete cognitive test battery and physical measurements (**Table 1, 2**). Follow-up is continued until the participant is no longer willing/able to participate. When the MMSE score declines ≤20 there is evidence of clear cognitive impairment [22], and subjecting a centenarian to a neuropsychological testing battery becomes more complicated. Follow-up visits by a researcher may no longer be constructive, and in such cases, we follow-up by informant questionnaire. To ensure up-to-date cognitive health measurements of brain donors, we administer telephone and informant questionnaires 6 months after the annual visit (T0.5, T1.5…). For a diagram of procedures see **Figure 2**. We ask informants to inform us when a participant dies and about the events that preceded death.

Data collection

Centenarian presentation: During each visit, the researcher subjectively estimates the visual, hearing and mobility function as “good”, “moderate”, “poor” or “very poor”, according to the determinants listed in **Table 3**. We collect the following variables regarding centenarian presentation: the level of independence during activities of daily living (ADL) using the Barthel Index [23], an estimation of the total hours of care/assistance needed *per week*; category housing situation, (independent-dependent); grip strength, systolic and diastolic blood pressure; heartbeat; and napping habits and sleep quality (Pittsburg Sleep Quality Index questionnaire [24]). We assess

whether the centenarian suffers from symptoms of depression [25, 26] by administering the 15-item geriatric depression scale (GDS-15). We ask about recent weight loss, current weight and length and active infections.

Medical History: For each centenarian we obtain a General Practitioner (GP) summary report, which lists diagnosed conditions and prescribed medications. These conditions are categorized by a dedicated GP (**Table 4**). After the centenarian died, we request a second synopsis from the GP, describing the medical proceedings until death. In a self-report medical history questionnaire, we inquire about blood pressure, heart disease; stroke (CVA) or TIA; tumors, head injuries, incontinence, dental condition, mental health problems, hospital visits, surgeries/anesthesia. We estimate BMI at midlife by recording self-reported weight and length at middle-age (~50 years). For centenarian-females we inquire about age at menarche, onset of menopause, number of pregnancies and/or miscarriages.

Cognitive profiling: We objectively evaluate cognitive functioning using a comprehensive neuropsychological test battery that addresses memory, attention and/or concentration, pre-morbid intelligence, language, executive and visuo-spatial functions (**Table 2**). To assess overall cognitive functioning we administer the Mini-Mental State Examination (MMSE) [22]. To generate a total MMSE score for all centenarians, also when geriatric sensory impairments led to missing items on the MMSE, we adjust scores using multiple imputation (**see supplementary material**). Additionally, at every visit the researcher formulated a subjective impression of cognitive functioning of the centenarian (**see supplementary material**). During each research visit we ask a proxy to fill in the Dutch version of the abbreviated form of the Informant Questionnaire on Cognitive Decline (IQ-CODE) to indicate whether the centenarians experienced cognitive decline in the past ten years (or, in case of follow-up visits, during the past year) [27, 28].

Lifetime/demographic characteristics: To investigate the family genealogy and disease occurrence, we draw a pedigree including children, siblings, parents and grandparents, their (maiden) names, gender, birth years, age at death and cause of death, occurrence of dementia/cognitive decline. To determine socio-economic background (SEB) and socioeconomic status (SES) we inquire about the main occupation of the father and mother of the centenarian, the main occupation of the centenarian him/herself at adulthood and the main occupation of their partner(s). We inquire about the education level and the number of years education followed. These were classified according to (I) ISCED 1997 [29] and according to Dutch 1971 census [30].

Lifetime habits: We address smoking habits and alcohol consumption (**see additional data**). We administer the Cognitive Activity Questionnaire (CAQ) [31, 32] to investigate 1) cognitive stimulating experience during adult life (from childhood to 50 years) and 2) current cognitively stimulating experience.

Data-collection of first degree living centenarian-relatives and partners: For centenarian siblings and their partners, we administer the MMSE at the study inclusion visit and we record the genealogy at the level of the centenarian-generation. We will yearly monitor changes in physical well-being and in cognitive health using TICS-M and IQ-Code-N. We ask centenarian-children and partners to fill in an abbreviated version of the centenarian questionnaire; we record the genealogy of the centenarian-generation, no cognitive testing will be administered; we will not follow-up centenarian-children and their partners (**Table 1**).

Biomaterials

Biomaterial collection: During baseline visits, we collect a blood sample from all subjects for DNA isolation, peripheral blood mononuclear cells (PBMCs), plasma, serum, and when consent is given for generation of induced pluripotent stem cells (iPSCs). DNA samples are currently used for APOE genotyping, GWAS, whole exome sequencing (WES) and Sanger sequencing. Furthermore, all centenarians are informed about the

option for feces donation for gut microbiome analysis, PET-MRI or PET-CT brain scans for in vivo detection of amyloid beta presence and structural brain imaging. We also inform about the option of post-mortem brain donation. Brain autopsies are performed in collaboration with the Netherlands Brain Bank [33, 34].

Data storage: OpenClinica open source software (version 3.1 onwards) is used for data management [35]. Biomaterials are stored in the biobank of the VUmc.

RESULTS/COHORT DESCRIPTION:

Included centenarians: Between January 1st 2013 and June 21st 2017, 773 centenarians were approached for study-participation of which 302 (40%) met study-inclusion criteria and were included in the study (**Figure 4**). As soon as the data collection from the first 300 centenarians was complete, we generated a first data-freeze on June 21st 2017. Of these 300 centenarians, 83 agreed to brain donation (28%); 37 of these had come to autopsy. The mean age at inclusion of centenarians was 101.3±1.7 years (**Figure 5A**). The majority of centenarians were born between 1910 and 1917 (**Figure 5B**). For all cohort descriptives see **Table 5**.

Presentation at baseline: Subjective researcher estimates of geriatric sensory impairments indicated that 87% of the centenarians had moderate-good hearing abilities (**Figure 6A**), that 77% of the centenarians had moderate-good vision (**Figure 6B**), and that 80% of the centenarians were independently mobile (**Figure 6C**). The majority (52%) of the centenarians in the cohort lived independently (i.e. community dwelling without assistance, or independent in a residence with available services), 42% lived in private quarters in a residential care center, while only 1.7% of the centenarians lived in a nursing home (**Figure 6D**). Centenarians scored a median of 15 points (IQR: 12-18), on the Barthel index: 45% of the centenarians scored between 15-19, which indicates a need for minimum help with activities of daily living (ADL), while 32% scored 20 points which indicates they are fully independent in ADL (**Figure 6E**). The centenarians in the cohort have no or very few symptoms of depression: they scored a median of 2 points on the 15-items version of the Geriatric Depression Scale (IQR: 1-3), and scores <5 indicate no evidence for depression [25] (**Figure 6F**).

Disease prevalence and multi-morbidities: We analyzed the conditions listed in the GP medical records from 209 centenarians **(Table 4**). At baseline, centenarians were diagnosed with or had symptoms of on average 3.9±1.6 morbidities (**Figure 6G**). Musculoskeletal disease and hypertension were more prevalent in females (73% vs. 39% and 54% vs. 34%), while cardiovascular conditions were more prevalent in males (68%

vs. 56%). Most aging-associated diseases were first mentioned in the GP report when the centenarian was >90 years, suggesting a seemingly high age at onset. As we cannot correct for methodical differences in data collection by GPs, we were not able to perform a systematic comparison with disease incidence statistics from prospective cohort studies.

Cognitive function (MMSE): Overall cognitive functioning of 300 centenarians was tested by the MMSE, and scores were imputed to adjust for missing items due to hearing or vision impairments (see Methods). Of all centenarians, 206 (69%) had no missing items on the MMSE and scores were not imputed; for 10 centenarians (3%), hearing or vision impairments led to too many missing items: these were not adjusted by imputation and excluded from analysis. The MMSE included missing items for 84 centenarians (28%) and scores were imputed: imputation increased MMSE scores <1 point for 37 centenarians (12%), 1-2 points for 25 centenarians (8%), 2-3 points for 14 centenarians (5%), 3-6 points for 8 centenarians (3%). At study inclusion, the MMSE score of the 100-plus Study cohort was on average 24.3 ±4.23 points and (median score: 25, IQR: 22.0-27.5) (**Figure 7A**). For 287 centenarians, a trained researcher estimated cognitive health. The large majority (83%) of the centenarians was estimated to be cognitively healthy, and this group scored a median of 26 points on the MMSE (IQR: 23.5-28). This was significantly higher than the median MMSE score of 19 (IQR: 16.4-22) by the 41 centenarians for whom cognitive health was "doubted" ($p=4x10^{-3}$, two-tailed t-test with unequal variance), and the median MMSE score of 8 centenarians who were estimated to have "probable cognitive impairment" was 16.4, (IQR: 12.8-17) (**Figure 7B**).

MMSE and mortality rates: Within the group of 293 participants for which a baseline MMSE was available, there were 67 deaths that occurred before a next planned visit: the planning of a next visit was used to confirm which centenarians were still alive and who had died (for mortality rate calculation procedures, see **supplementary material**). The overall mortality rate in the first year after inclusion was 0.24 (95%CI: 0.17-0.32), specifically, the 135 centenarians who scored ≥26 on the MMSE at baseline had a

mortality rate of 0.19 (95%CI: 0.11-0.29), while the 158 centenarians with MMSE scores <26 had a mortality rate of 0.29 (95%CI: 0.19-0.43) (p=0.075). In the second year after baseline, the mortality rate increased to 0.32 (95%CI: 0.20-0.49); specifically, the mortality rate of the centenarians who scored ≥26 points at baseline remained at a low 0.19 (95%CI: 0.08-0.37), while the mortality rate of centenarians who scored <26 points increased to 0.54 (95%CI: 0.29-0.90) ($p=3.0.x10^{-3}$) (**Figure 7C**). For conversion to mortality percentages see **Table 4**.

Education: We retrospectively compared centenarian-education levels with 54-61 year-olds as reported in the Dutch population in the 1971 census [36], these individuals were from the same birth-cohort as the centenarians (1910-1916). Both male and centenarian-females attained significantly higher levels of education compared to their birth-cohort in the 1971 census [30] ($p<1x10^{-5}$, Mann-Whitney U test) (**Figure 8A**). Workers and self-employed persons with little education were overrepresented in the ~20% non-responders in the 1971 census, suggesting that this is a conservative estimate of the differences [30].

Socio-economic background and status: Based on *paternal* professions, centenarian-socio-economic background (SEB) was compared to 2,815 individuals born between 1910-1915 from the from the Historical Sample of the Netherlands (HSN) [37] ($p<1x10^{-5}$, Mann-Whitney U test) (**Figure 8B-left**). Centenarian-*fathers* were 3-fold more likely to have an elite-upper middle class occupation and >3-fold less likely to be an unskilled worker compared to their birth-cohort. Based on the professions of the 219 centenarian-males and centenarian-female-partners, centenarians *themselves* attained a significantly higher SES than the 408 males from the HSN sample born between 1910-1919 ($p<1x10^{-5}$, Mann-Whitney U test). Centenarians were >4-fold more likely to be elite-upper middle class, >2-fold more likely to be farmers, and >3-fold less likely to be unskilled or farm workers (**Figure 8B-right**). There was no difference between the socio-economic status (SES) attained as adults of 81 male centenarians and the male-partners of 138 female-centenarians (p=0.22, Mann-Whitney U test).

Smoking behavior and alcohol consumption: Retrospective comparison of smoking behavior suggests that centenarians smoked less than a representative sample of Dutch individuals born between 1909-1923, as indicated a 1958 survey [38, 39]. Of the centenarian-males 67% indicated to have smoked regularly or often during an extended period in their life, while 91% of the birth-cohort males reported to smoke in 1958. Of the centenarian females, 15% indicated to have smoked regularly or often while 32% of the birth cohort females smoked. Alcohol consumption was common: only 11% of the centenarian-males and 22% of the centenarian-females indicated to never consume alcohol, similar to 14% of male-abstainers and 21.8% female-abstainers among the birth cohorts in the 1958 survey [38], whereas 54% of the centenarian-males and 31% of the centenarian-females indicated to consume alcohol regularly or often.

Marriage and children: Centenarians had on average 3.9±2.2 children, which was more than the average 3.5±2.5 children from 860 Dutch parents born between 1910-1915 [40] ($p=0.03$, Mann-Whitney U) (**Figure 9C**). Overall, 91% of the centenarians was ever-married, and 86% had one or more children. Of the centenarian females, 16.5% remained childless (36/219), similar to the 16% childless females born between 1915-1919 [41]. Five males in the cohort (6%) remained childless (birth-cohort data not available [41]).

APOE allele frequency: APOE was genotyped for 266 centenarians (**Figure 9**). We observed that the centenarians were >2-fold more likely to carry an *APOE*-ε2 allele than to 2,233 relative to Dutch population controls aged 60-80 years [42]. Specifically, centenarians are 2.5-fold more likely to be genotyped *APOE*-ε2/ε3 (**Table 5**). In contrast, centenarians are >2-fold *less* likely to carry an *APOE*-ε4 allele compared to the Dutch population; specifically, centenarians are 2.8-fold less likely to be genotyped *APOE*-ε3/ε4 and 6.7-fold less likely to be genotyped *APOE*-ε4/ε4. The allele frequency of the *APOE* ε3 allele was identical for both cohorts.

DISCUSSION:

Here, we present the 100-plus Study cohort of cognitively healthy centenarians based on the first 300 centenarians included in the 100-plus Study.

On average, the centenarians in the 100-plus Study cohort have a high performance on the MMSE; the large majority is independent and retained hearing and vision: Our inclusion criteria of "*self-reported cognitive health, which is confirmed by a proxy*" sorted centenarians with a relatively high level of overall cognitive functioning. The cohort scored an average of 24.3 ±4.23 points on the MMSE, which is considerably higher than average MMSE scores of representative centenarian populations (16.2 ± 8.8 points, Georgia Centenarian Study [43]; 18.7 ± 7.4, centenarians from central Italy (Rome and surroundings) [44]; 17.7 ± 8.3 centenarians from Northern Italy [45]). The overall cognitive performance of the 100-plus cohort participants is similar to "*community- dwelling cognitively healthy centenarians*" from the Georgia centenarian Study [46], and "*cognitively healhy*" Japanese centenarians, who repectively scored a mean of 24.8 points and 22.3±3.32 points on the MMSE [47].

Next to their retained cognitive functioning, the large majority of the centenarians had moderate-good hearing and vision abilities, they were independently mobile, they enjoyed a relatively high level of independence in activities of daily living (ADL), and had no or few symptoms of depression. Centenarians were either community dwelling or lived independently in a residence or in a care center with available services. Together, these findings echo that the 100-plus Study cohort represents high-performing, independent Dutch centenarians.

Cognitive performance is associated with mortality: The high cognitive and overall performance of our cohort was related with a low mortality. The first year after baseline, the mortality of the centenarians in our cohort was 20.5%, which is 2-fold lower than the 40% mortality of the overall Dutch centenarian population [48]. In the second year after baseline mortality increased to 28%: specifically, centenarians with high cognitive functioning retained a low mortality of 17%, while centenarians with a

decline in cognitive functioning had a mortality rate of 42%. Our results confirm that there is an overlapping etiology of maintained cognitive and overall health, and cognitive functioning might be employed to predict overall decline and mortality [49, 50]. The longitudinal set-up of our study will allow us to monitor changes in cognition in combination with other factors of overall health that occur between baseline and death to identify to which extent centenarians escaped or delayed cognitive impairment.

The 100-plus cohort is 2-fold enriched with males: The fraction of centenarian-males is 27%, twice the fraction of males (14.4%) in the total Dutch centenarian population on January 1st 2017 [51]. Indeed, since dementia-prevalence in centenarian populations is consistently lower in males (~40%) than in females (~60%) [52, 53], we had on forehand expected that our inclusion criteria of "*self-reported cognitive health, which is confirmed by a proxy*" might sort relatively more centenarian males than females. Based on the fraction of centenarian males in the population (14.3% in 2017) and lower dementia prevalence in males (40% vs 60%), we estimated that the fraction of males in the 100-plus Study cohort should be approximately 20%. This suggests that dementia incidence does not fully explain the excess of males in our cohort, which leaves room for, for example, the influence of a participation bias and a better general well-being of centenarian males [54].

Disease in male and female centenarians: Despite the cognitive health of the centenarians in the 100-plus Study cohort, they were diagnosed with on average four morbidities at baseline. Previous studies have shown that females are more prone to develop chronic nonfatal conditions such as dementia, arthritis and osteoporosis [55], while males are more likely to develop fatal conditions, such as cardiovascular disease and cancer [56, 57]. In agreement with these studies, we found that the females in the 100-plus Study cohort had a higher prevalence of musculoskeletal diseases and hypertension while males had a higher prevalence of heart disease and CVA/TIAs.

The 100-plus Study cohort is equally depleted with the APOE-ε4 allele compared to other centenarian cohorts, but it is strongly enriched with the neuroprotective APOE-

ε2 allele: The 100-plus Study cohort was 2.3-fold *less* likely to carry the APOE-ε4 AD-risk allele compared to their birth cohort at 60-80-years (OR=0.44, $p=6.3x10^{-7}$) [42]. This is in complete concordance with the depletion observed in a meta-analysis of 2,776 (mostly Caucasian) centenarians and 12,000 controls (OR=0.43, $p<1x10^{-3}$) [58]. It is well established that carrying one or two *APOE*-ε4 alleles is associated with respectively a 3-5 and 10-30-fold *increased* risk of developing AD. Depletion of the *APOE*-ε4 allele in centenarians supports an association of the ε4 allele with mortality at younger ages: carriers dropped out of the population during aging [59], leading to a depletion of the *APOE*-ε4 allele in centenarians that is consistent across studies.

On the other hand, carrying one protective *APOE*-ε2 allele is associated with a 2-fold *decreased* lifetime risk of developing AD [60, 61]. But the large centenarian meta-analysis indicated that the protective aspect of the *APOE*-ε2 allele does not extend to an enrichment in centenarians (OR=1.08, p=0.66), although a weak enrichment of the *APOE*-ε2/ε3 genotype was observed (OR=1.4 $p=1.7x10^{-2}$) [58]. In contrast, we observed that the *APOE-ε2* allele was strongly enriched in cognitively healthy centenarians from the 100-plus Study cohort compared to their birth cohort at 60-80-years: centenarians were 2.1-fold more likely to carry the *APOE*-ε2 allele ($p=4.8x10^{-7}$), and 2.5-fold more likely to have an *APOE*-ε2/ε3 genotype ($p=3.4x10^{-7}$). This confirms previous suggestive findings in a cohort of Italian centenarians who were free of dementia or any other major age-related conditions, which had a similar enrichment of the *APOE-ε2* allele [62]. We speculate that this enrichment of the *APOE*-ε2 allele is not a consequence of our selection of extreme ages, but that it reflects our selection of individuals with retained (cognitive) health until extreme ages.

The specific enrichment of the *APOE-ε2* in the centenarians with high cognitive performance suggests that the etiology for reaching 100 years with maintained (cognitive) health may be distinct from the etiology of reaching 100 years in general. Our results indicate that while searching for (genetic) factors that maintain cognitive health, the *APOE* genotype should be taken into account.

Centenarians came, on average, from higher socio-economic classes and had higher levels of education: On average, centenarians came from a higher socio-economic background than their birth cohort. A high fraction of centenarian-fathers were farmers, mostly on their own farm, a common occupation in the Netherlands during the early twentieth century. As adults, centenarians attained a higher socio-economic status and they had significantly more children compared to their birth-cohort. Both male- and female-centenarians attained higher levels of education than the males and females from their birth-cohorts. These findings reflect the selective survival advantage of individuals from the higher/middle socioeconomic classes and farmers, during the majority of the twentieth century in the Netherlands [63]. Together, this is in agreement with results from several centenarian studies, which showed that socioeconomic background, educational attainment, and adult socioeconomic status influenced the chance to become a centenarian [64]. Likewise, having children associates with an increased chance of reaching extreme ages, likely due to the involvement of children in the care for their aged parent [65].

Alcohol consumption of centenarians was similar to birth cohort peers, and they smoked -but less: Two-thirds of the centenarian males and 15% of the centenarian females indicated to have smoked regularly or often during an extended period in their life. This was less than their birth-cohort peers, of whom almost all males and a third of the females smoked [38]. Alcohol consumption of the centenarians was similar to their birth cohorts. These results are partly in agreement with lifestyle behaviours from the American Ashkenazi Jewish centenarians, whose alcohol consumption and smoking behaviour was not different from the general population [66].

We note that comparisons of lifestyle habits such as alcohol consumption and smoking rely on recall of habits they had several decades ago, which may introduce recall bias. For this reason, we focused on investigating lifestyle factors that are manifest for a longer period during a lifetime. Habits that may be more variable throughout life, such as dietary or exercise habits, might be more difficult to recall and we chose to refrain

from investigating these. Despite limitations, the collected statistics can be applied in within-cohort analyses, such that they add to the rich phenotypic data available for this cohort.

Conclusions: The 100-plus Study cohort represents cognitively healthy Dutch centenarians. Compared to their birth-cohort peers, centenarians from this cohort attained significantly higher levels of education, were from a higher socioeconomic background, attained higher socioeconomic status, and they had more children, all of which confirms previous findings that these factors are associated with the chance of reaching 100 years in cognitive health. The combined contributions of these features, which are often concentrated within families, and the enrichment with the genetically heritable *APOE-ε2* allele, will most likely explain a considerable proportion of the high heritability of reaching 100 years in maintained cognitive health. However, these features do not apply to all centenarians, and only a third of the cohort carries the *APOE-ε2* allele. This suggests that additional protective factors may account for the cohort phenotype.

With the recent developments in biotechnology novel findings regarding the pathophysiology of exceptional longevity and cognitive function are emerging [67, 68]. To advance such findings, the availability of blood and brain tissues of extensively phenotyped centenarians with the best possible aging-outcome and their family members, provides the opportunity to acquire insights in the molecular constellations associated with the long-term maintenance of cognitive health. Ultimately, with this cohort we aim to contribute to the generation of novel hypotheses regarding the generation of novel therapeutic targets that offer resilience to cognitive decline.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE: The Medical Ethical Committee of the VU University Medical Center has approved the 100-plus Study (METc-VUmc registration number: 2016.440). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT: Informed consent was obtained from all individual participants included in the study.

AUTHORS' CONTRIBUTIONS: HH conceived and designed the study and wrote the manuscript; NB helped obtain ethical approval for the study and with the management of communication with participants and their proxies; TD is our dedicated GP and analyzed GP reports, KP, EW, KS, LT, DB and SR visited the centenarians and helped design/improve the study as it evolved; SS advised on the design of the neuropsychological testing battery; FvP provided expert knowledge on occupation classification and the demographics of the 1910-1920 birth cohorts, HMH contributed substantive support for study set-up; MH helped with the study design and manages the Open Clinica database for data storage; PS contributed substantive support for the study and aided with obtaining necessary funding. All authors read and approved the final manuscript.

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REFERENCES

1. Lobo A, Lopez-Anton R, Santabarbara J, de-la-Camara C, Ventura T, Quintanilla MA et al. Incidence and lifetime risk of dementia and Alzheimer's disease in a Southern European population. Acta psychiatrica Scandinavica. 2011;124(5):372-83. doi:10.1111/j.1600-0447.2011.01754.x.
2. Corrada MM, Brookmeyer R, Paganini-Hill A, Berlau D, Kawas CH. Dementia incidence continues to increase with age in the oldest old: the 90+ study. Annals of neurology. 2010;67(1):114-21. doi:10.1002/ana.21915.
3. den Dunnen WF, Brouwer WH, Bijlard E, Kamphuis J, van Linschoten K, Eggens-Meijer E et al. No disease in the brain of a 115-year-old woman. Neurobiol Aging. 2008;29(8):1127-32. doi:10.1016/j.neurobiolaging.2008.04.010.
4. Jeune B, Robine J-M, Young R, Desjardins B, Skytthe A, Vaupel JW. Jeanne Calment and her successors. Biographical notes on the longest living humans. 2010:285-323. doi:10.1007/978-3-642-11520-2_16.
5. Andersen SL, Sebastiani P, Dworkis DA, Feldman L, Perls TT. Health Span Approximates Life Span Among Many Supercentenarians: Compression of Morbidity at the Approximate Limit of Life Span. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2012;67A(4):395-405. doi:10.1093/gerona/glr223.
6. Robine JM, Jagger C. What Do We Know About the Cognitive Status of Supercentenarians? In: Christen Y, editor. Research and Perspectives in Longevity; Longevity and Frailty. Springer; 2003. p. 145-52.
7. Barzilai N, Atzmon G, Derby CA, Bauman JM, Lipton RB. A genotype of exceptional longevity is associated with preservation of cognitive function. Neurology. 2006;67(12):2170-5. doi:10.1212/01.wnl.0000249116.50854.65.
8. Harper AR, Nayee S, Topol EJ. Protective alleles and modifier variants in human health and disease. Nature reviews Genetics. 2015;16(12):689-701. doi:10.1038/nrg4017.
9. Barral S, Cosentino S, Costa R, Andersen SL, Christensen K, Eckfeldt JH et al. Exceptional memory performance in the Long Life Family Study. Neurobiol Aging. 2013;34(11):2445-8. doi:10.1016/j.neurobiolaging.2013.05.002.
10. Cosentino S, Schupf N, Christensen K, Andersen SL, Newman A, Mayeux R. Reduced prevalence of cognitive impairment in families with exceptional longevity. JAMA neurology. 2013;70(7):867-74. doi:10.1001/jamaneurol.2013.1959.
11. Haworth CM, Wright MJ, Martin NW, Martin NG, Boomsma DI, Bartels M et al. A twin study of the genetics of high cognitive ability selected from 11,000 twin pairs in six studies from four countries. Behavior genetics. 2009;39(4):359-70. doi:10.1007/s10519-009-9262-3.
12. Petrill SA, Saudino K, Cherny SS, Emde RN, Fulker DW, Hewitt JK et al. Exploring the genetic and environmental etiology of high general cognitive ability in fourteen- to thirty-six-month-old twins. Child development. 1998;69(1):68-74.
13. Petrill SA, Kovas Y, Hart SA, Thompson LA, Plomin R. The genetic and environmental etiology of high math performance in 10-year-old twins. Behavior genetics. 2009;39(4):371-9. doi:10.1007/s10519-009-9258-z.
14. Plomin R, Haworth CM. Genetics of high cognitive abilities. Behavior genetics. 2009;39(4):347-9. doi:10.1007/s10519-009-9277-9.
15. Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H et al. Life-long sustained mortality advantage of siblings of centenarians. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(12):8442-7. doi:10.1073/pnas.122587599.

16. Adams ER, Nolan VG, Andersen SL, Perls TT, Terry DF. Centenarian offspring: start healthier and stay healthier. Journal of the American Geriatrics Society. 2008;56(11):2089-92. doi:10.1111/j.1532-5415.2008.01949.x.
17. Mostafavi H, Berisa T, Day FR, Perry JRB, Przeworski M, Pickrell JK. Identifying genetic variants that affect viability in large cohorts. PLoS Biol. 2017;15(9):e2002458. doi:10.1371/journal.pbio.2002458.
18. Sebastiani P, Andersen SL, McIntosh AI, Nussbaum L, Stevenson MD, Pierce L et al. Familial Risk for Exceptional Longevity. North American actuarial journal : NAAJ. 2016;20(1):57-64. doi:10.1080/10920277.2015.1061946.
19. Bucci L, Ostan R, Cevenini E, Pini E, Scurti M, Vitale G et al. Centenarians' offspring as a model of healthy aging: a reappraisal of the data on Italian subjects and a comprehensive overview. Aging (Albany NY). 2016;8(3):510-9. doi:10.18632/aging.100912.
20. Atzmon G, Schechter C, Greiner W, Davidson D, Rennert G, Barzilai N. Clinical phenotype of families with longevity. Journal of the American Geriatrics Society. 2004;52(2):274-7.
21. Silverman JM, Smith CJ, Marin DB, Birstein S, Mare M, Mohs RC et al. Identifying families with likely genetic protective factors against Alzheimer disease. American journal of human genetics. 1999;64(3):832-8. doi:10.1086/302280.
22. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research. 1975;12(3):189-98.
23. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. Maryland state medical journal. 1965;14:61-5.
24. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
25. Yesavage JA, Sheikh JI. 9/Geriatric Depression Scale (GDS). Clinical Gerontologist. 1986;5(1-2):165-73. doi:10.1300/J018v05n01_09.
26. Bleeker JAC, de Winter FML, Cornelissen E. Geriatric Depression Scale. 1985.
27. Jorm AF, Jacomb PA. The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): socio-demographic correlates, reliability, validity and some norms. Psychological medicine. 1989;19(4):1015-22.
28. de Jonghe JF, Schmand B, Ooms ME, Ribbe MW. [Abbreviated form of the Informant Questionnaire on cognitive decline in the elderly]. Tijdschrift voor gerontologie en geriatrie. 1997;28(5):224-9.
29. UNESCO. International Standard Classification of Education 1997. http://www.unesco.org/education/information/nfsunesco/doc/isced_1997.htm. 1997.
30. Vliegen JM, de Jong U, Wesselingh AA, van der Kley P, CBS, SISWO. Education in the Netherlands. Monografiëën volkstelling 1971. The Hague s-Gravenhage: Centraal Bureau voor de Statistiek: Staatsuitgeverij; 1981.
31. Wilson R, Barnes L, Bennett D. Assessment of lifetime participation in cognitively stimulating activities. Journal of clinical and experimental neuropsychology. 2003;25(5):634-42. doi:10.1076/jcen.25.5.634.14572.
32. Ossenkoppele R. Cognitieve Activities Questionnaire. 2013.
33. Netherlands Brain Bank. http://www.brainbank.nl/.
34. Ravid R, Swaab DF. The Netherlands brain bank--a clinico-pathological link in aging and dementia research. Journal of neural transmission Supplementum. 1993;39:143-53.
35. Open Clinica. Copyright © OpenClinica LLC and collaborators, Waltham, MA, USA http://www.OpenClinica.com.
36. Statistics Netherlands. 14e Algemene Volkstelling annex woningtelling 28 februari 1971. The Hague: Centraal Bureau voor de Statistiek; 1980. p. 40.

37. Mandemakers K. Historical sample of the Netherlands HSN. Historical Social Research. 2001;26(4):179-90.
38. Gadourek I. Riskante gewoonten en zorg voor eigen welzijn (Hazardous habits and human well-being). Groningen: J.B. Wolters; 1963.
39. Nagelhout G. Trendpublicatie percentage rokers. Percentage rokers in de Nederlandse bevolking 1958 – 2012 2013.
40. Van Poppel F, Reher D, Sanz-Gimeno A, Sanchez-Dominguez M, Beekink E. Mortality decline and reproductive change during the Dutch demographic transition. Demographic Research. 2012;27:299-338. doi:10.4054/DemRes.2012.27.11.
41. Dykstra PA. Childless Old Age. In: Uhlenberg P, editor. International Handbook of Population Aging. Springer; 2009. p. 671-90.
42. Huisman M, Poppelaars J, van der Horst M, Beekman AT, Brug J, van Tilburg TG et al. Cohort profile: the Longitudinal Aging Study Amsterdam. International journal of epidemiology. 2011;40(4):868-76. doi:10.1093/ije/dyq219.
43. Miller LS, Mitchell MB, Woodard JL, Davey A, Martin P, Poon LW et al. Cognitive Performance in Centenarians and the Oldest Old: Norms from the Georgia Centenarian Study. Aging, Neuropsychology, and Cognition. 2010;17(5):575-90. doi:10.1080/13825585.2010.481355.
44. Bauco C, Borriello C, Cinti AM, Martella S, Zannino G, Rossetti C et al. Correlation between MMSE performance, age and education in centenarians. Archives of gerontology and geriatrics. 1998;26:23-6. doi:10.1016/s0167-4943(98)80004-6.
45. Baldelli MV, Salvioli G, Neri M, Pradelli JM. A survey of a centenarian population in Italy, focusing on self-sufficiency and cognition. Archives of gerontology and geriatrics. 1996;22 Suppl 1:345-54. doi:10.1016/0167-4943(96)86960-3.
46. Holtsberg PA, Poon LW, Noble CA, Martin P. Mini-Mental State Exam status of community-dwelling cognitively intact centenarians. International psychogeriatrics. 1995;7(3):417-27.
47. Inagaki H, Gondo Y, Hirose N, Masui Y, Kitagawa K, Arai Y et al. Cognitive Function in Japanese Centenarians according to the Mini-Mental State Examination. Dementia and geriatric cognitive disorders. 2009;28(1):6-12. doi:10.1159/000228713.
48. Central Bureau of Statistics: Prognosis period-life expectancy; gender and age, 2014-2060 [database on the Internet]. Netherlands Statistics. 2017. Available from: http://statline.cbs.nl/Statweb/publication/?DM=SLNL&PA=82690ned&D1=0&D2=a&D3=a&D4=0,16,l&VW=T. Accessed: 29 Aug 2017
49. Skoog J, Backman K, Ribbe M, Falk H, Gudmundsson P, Thorvaldsson V et al. A Longitudinal Study of the Mini-Mental State Examination in Late Nonagenarians and Its Relationship with Dementia, Mortality, and Education. Journal of the American Geriatrics Society. 2017;65(6):1296-300. doi:10.1111/jgs.14871.
50. Mossakowska M, Broczek K, Wieczorowska-Tobis K, Klich-Raczka A, Jonas M, Pawlik-Pachucka E et al. Cognitive performance and functional status are the major factors predicting survival of centenarians in Poland. The journals of gerontology Series A, Biological sciences and medical sciences. 2014;69(10):1269-75. doi:10.1093/gerona/glu003.
51. Centraal Bureau voor de Statistiek. Aantal 100-plussers verdubbeld in 20 jaar. Webmagazine CBS 2017.
52. Yang Z, Slavin MJ, Sachdev PS. Dementia in the oldest old. Nature reviews Neurology. 2013;9(7):382-93. doi:10.1038/nrneurol.2013.105.
53. Corrada MM, Brookmeyer R, Berlau D, Paganini-Hill A, Kawas CH. Prevalence of dementia after age 90: results from the 90+ study. Neurology. 2008;71(5):337-43. doi:10.1212/01.wnl.0000310773.65918.cd.

54. Hazra NC, Dregan A, Jackson S, Gulliford MC. Differences in Health at Age 100 According to Sex: Population-Based Cohort Study of Centenarians Using Electronic Health Records. Journal of the American Geriatrics Society. 2015;63(7):1331-7. doi:10.1111/jgs.13484.
55. Pinn VW. Past and future: sex and gender in health research, the aging experience, and implications for musculoskeletal health. The Orthopedic clinics of North America. 2006;37(4):513-21. doi:10.1016/j.ocl.2006.09.006.
56. Wingard DL, Cohn BA, Kaplan GA, Cirillo PM, Cohen RD. Sex differentials in morbidity and mortality risks examined by age and cause in the same cohort. American journal of epidemiology. 1989;130(3):601-10.
57. Roman-Blas JA, Castaneda S, Largo R, Herrero-Beaumont G. Osteoarthritis associated with estrogen deficiency. Arthritis research & therapy. 2009;11(5):241. doi:10.1186/ar2791.
58. Garatachea N, Marin PJ, Santos-Lozano A, Sanchis-Gomar F, Emanuele E, Lucia A. The ApoE gene is related with exceptional longevity: a systematic review and meta-analysis. Rejuvenation research. 2015;18(1):3-13. doi:10.1089/rej.2014.1605.
59. Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW. Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a "frailty gene," not a "longevity gene". Genetic epidemiology. 2000;19(3):202-10. doi:10.1002/1098-2272(200010)19:3<202::AID-GEPI2>3.0.CO;2-Q.
60. Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O et al. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Molecular psychiatry. 2011;16(9):903-7. doi:10.1038/mp.2011.52.
61. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA : the journal of the American Medical Association. 1997;278(16):1349-56.
62. Garatachea N, Emanuele E, Calero M, Fuku N, Arai Y, Abe Y et al. ApoE gene and exceptional longevity: Insights from three independent cohorts. Experimental gerontology. 2014;53:16-23. doi:10.1016/j.exger.2014.02.004.
63. Kunst AE, Looman CW, Mackenbach JP. Socio-economic mortality differences in The Netherlands in 1950-1984: a regional study of cause-specific mortality. Social science & medicine. 1990;31(2):141-52.
64. Fors S, Lennartsson C, Lundberg O. Childhood living conditions, socioeconomic position in adulthood, and cognition in later life: exploring the associations. The journals of gerontology Series B, Psychological sciences and social sciences. 2009;64(6):750-7. doi:10.1093/geronb/gbp029.
65. Modig K, Talback M, Torssander J, Ahlbom A. Payback time? Influence of having children on mortality in old age. Journal of epidemiology and community health. 2017;71(5):424-30. doi:10.1136/jech-2016-207857.
66. Rajpathak SN, Liu Y, Ben-David O, Reddy S, Atzmon G, Crandall J et al. Lifestyle Factors of People with Exceptional Longevity. Journal of the American Geriatrics Society. 2011;59(8):1509-12. doi:10.1111/j.1532-5415.2011.03498.x.
67. Zainabadi K. A brief history of modern aging research. Experimental gerontology. 2018;104:35-42. doi:10.1016/j.exger.2018.01.018.
68. Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S et al. Alzheimer's disease. Lancet. 2016;388(10043):505-17. doi:10.1016/S0140-6736(15)01124-1.

69. CBS/HMD. Unpublished data from the NIDI mortality database provided by Ewa Tabeau (NIDI) (1850-1949); Central Bureau of Statistics 1949- onwards (Data obtained through the Human Mortality Database, http://www.mortality.org, on 29-5-2017).
70. Poon LW, Woodard JL, Stephen Miller L, Green R, Gearing M, Davey A et al. Understanding dementia prevalence among centenarians. The journals of gerontology Series A, Biological sciences and medical sciences. 2012;67(4):358-65. doi:10.1093/gerona/glr250.
71. Kliegel M, Moor C, Rott C. Cognitive status and development in the oldest old: a longitudinal analysis from the Heidelberg Centenarian Study. Archives of gerontology and geriatrics. 2004;39(2):143-56. doi:10.1016/j.archger.2004.02.004.
72. Andersen-Ranberg K, Vasegaard L, Jeune B. Dementia is not inevitable: a population-based study of Danish centenarians. The journals of gerontology Series B, Psychological sciences and social sciences. 2001;56(3):P152-9.
73. Calvert JF, Jr., Hollander-Rodriguez J, Kaye J, Leahy M. Dementia-free survival among centenarians: an evidence-based review. The journals of gerontology Series A, Biological sciences and medical sciences. 2006;61(9):951-6.
74. Ansart S, Pelat C, Boelle PY, Carrat F, Flahault A, Valleron AJ. Mortality burden of the 1918-1919 influenza pandemic in Europe. Influenza and other respiratory viruses. 2009;3(3):99-106. doi:10.1111/j.1750-2659.2009.00080.x.
75. Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. Emerging infectious diseases. 2006;12(1):15-22. doi:10.3201/eid1201.050979.
76. Gompertz B. On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies. Philosophical Transactions of the Royal Society of London. 1825;115. :513–83.
77. Janssen F, van Poppel FWA. Roken veroorzaakte vroeger grote verschillen in levensverwachting. DEMOS: Bulletin over Bevolking en Samenleving. 2016:4-6.
78. Newman AB, Murabito JM. The epidemiology of longevity and exceptional survival. Epidemiologic reviews. 2013;35:181-97. doi:10.1093/epirev/mxs013.
79. James BD, Schneider JA. Increasing incidence of dementia in the oldest old: evidence and implications. Alzheimer's research & therapy. 2010;2(3):9. doi:10.1186/alzrt32.
80. Central Bureau of Statistics: Life expectancy per birth cohort. [database on the Internet]. Netherlands Statistics. 2017. Available from: http://statline.cbs.nl/Statweb/dome/?TH=26190&PA=80333NED&LA=nl. Accessed: 11 Aug 2015
81. Gavrilov LA, Gavrilova NS. Mortality Measurement at Advanced Ages: A Study of the Social Security Administration Death Master File. North American actuarial journal : NAAJ. 2011;15(3):432-47.
82. Kok RM, Verhey FRJ. Gestandaardiseerde MMSE. Altrecht GGZ. 2002.
83. Nelson HE, O'Connell A. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. Cortex; a journal devoted to the study of the nervous system and behavior. 1978;14(2):234-44.
84. Schmand B, Lindeboom J, van Harskamp F. Nederlandse leestest voor volwassenen. . In: Bouma A, Mulder J, Lindeboom J, editors. Neuropsychologische Diagnostiek. Lisse: Swets & Zeitlinger; 1992. p. A45-A52.
85. Schmand B, Bakker D, Saan R, Louman J. De Nederlandse Leestest voor Volwassenen: een maat voor het premorbide intelligentieniveau. Gerontologie en Geriatrie. 1991;22:15-9.
86. de Jager CA, Budge MM, Clarke R. Utility of TICS-M for the assessment of cognitive function in older adults. International journal of geriatric psychiatry. 2003;18(4):318-24. doi:10.1002/gps.830.

87. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-65.
88. Lindeboom J, Schmand B, Tulner L, Walstra G, Jonker C. Visual association test to detect early dementia of the Alzheimer type. Journal of neurology, neurosurgery, and psychiatry. 2002;73(2):126-33.
89. Wilson BA, Cockburn J, Baddeley AD. The Rivermead behavioural memory test. 2nd ed., rev., enl. and redesigned. ed. Thames Valley Test Company; 1991.
90. Van Balen HGG, Groot Zwaaftink AJM. Rivermead Behavioural Memory Test. Nederlandse bewerking. In Thames Valley test Company, Reading. 1987.
91. Lindeboom J, Matto D. [Digit series and Knox cubes as concentration tests for elderly subjects]. Tijdschrift voor gerontologie en geriatrie. 1994;25(2):63-8.
92. Wechsler D. Wechsler Adult Intelligence Scale–Revised. San Antonio, TX: Psychological Corporation; 1981.
93. Wechsler D. WAIS-III administration and scoring manual. San Antonio, TX: Psychological Corporation; 1997.
94. Army Individual Test Battery. Manual of Directions and Scoring. 1944.
95. Reitan RM. Validity of the Trail Making Test as an indicator of organic brain damage. Perceptual and Motor Skills. 1958;8:271-6. doi:10.2466/PMS.8.7.271-276.
96. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. Journal of the International Neuropsychological Society : JINS. 2006;12(1):80-9. doi:10.1017/S1355617706060115.
97. Lezak MD. Neuropsychological assessment. 2nd edition. ed.
98. Schmand B, Groenink SC, van den Dungen M. [Letter fluency: psychometric properties and Dutch normative data]. Tijdschrift voor gerontologie en geriatrie. 2008;39(2):64-76.
99. Snijders J, Luteijn F, van der Ploeg F, Verhage F. Handleiding Groninger intelligentie test. Lisse: Swets & Zeitlinger; 1983.
100. Benton AL, Hamsher KD. Multilingual Aphasia Examination. Iowa City, IA: AJA Associates; 1989.
101. Krabbendam L, Kalff AC. Handleiding Nederlandse vertaling BADS, (B.A. Wilson, N. Alderman, P.W. Burgess, H. Emslie en J.J. Evans). 1997.
102. Wilson BA, Evans JJ, Alderman N, Burgess PW, Emslie H. Behavioural assessment of the dysexecutive syndrome. Methodology of frontal and executive function. 1997:239-50.
103. Lindeboom J, Jonker C. Amsterdamse Dementie Screeningstest. Lisse, The Netherlands: Swets and Zeitlinger; 1988.
104. Luteijn F, van der Ploeg FAE. Groninger Intelligentie Test, Handleiding. 1982.
105. Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. The British journal of psychiatry : the journal of mental science. 1986;149:698-709.
106. Roth M, Huppert F, Tym E, Mountjoy CQ. CAMDEX-R boxed set: the revised cambridge examination for mental disorders of the elderly. 1998:81-8.
107. Shulman KI, Pushkar Gold D, Cohen CA, Zucchero CA. Clock-drawing and dementia in the community: A longitudinal study. International journal of geriatric psychiatry. 1993;8(6):487-96. doi:10.1002/gps.930080606.
108. Shulman KI. Clock-drawing: is it the ideal cognitive screening test? International journal of geriatric psychiatry. 2000;15(6):548-61.
109. Warrington EK, James M. The Visual Object and Space Battery Perception. 1991.

110. Collin C, Wade DT, Davies S, Horne V. The Barthel ADL Index: a reliability study. International disability studies. 1988;10(2):61-3.
111. de Haan R, Limburg M, Schuling J, Broeshart J, Jonkers L, van Zuylen P. [Clinimetric evaluation of the Barthel Index, a measure of limitations in dailly activities]. Nederlands tijdschrift voor geneeskunde. 1993;137(18):917-21.
112. Post MW, van Asbeck FW, van Dijk AJ, Schrijvers AJ. [Dutch interview version of the Barthel Index evaluated in patients with spinal cord injuries]. Nederlands tijdschrift voor geneeskunde. 1995;139(27):1376-80.

BOX: Study Rationale

The design of an intervention for neurodegenerative diseases requires not only the understanding of the neurodegenerative processes involved, but also a deep comprehension of the processes that *maintain* cognitive health during ageing. Although increasing age is the strongest predictive factor for cognitive decline and dementia, some people live to be over 110 years in great mental health. A Dutch woman, Hendrikje van Andel-Schipper (1890-2005), reached the age of 115 with full cognitive abilities [3] and showed that *it is possible to reach extreme ages without any symptoms of cognitive decline.* Her remarkable case became the source of inspiration for the initiation of the 100-plus Study at the VUmc Alzheimer Center in 2013. To investigate the pathophysiology of her extended cognitive health, it is necessary to compare her clinical characteristics with those from other individuals with the same extraordinary combination of phenotypes: extremely old and cognitively healthy. Below, we provide a rationale for researching protective factors against cognitive decline in cognitively healthy centenarians, based on the increase in mortality rate and dementia incidence in their birth cohort during its process of aging.

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The number of centenarians in the Netherlands is growing quickly: on January 1st 2013 there were 1940 centenarians in the Netherlands, which grew to 2,225 centenarians by 2017, and this number is expected to rise to 5,000 by 2035 [51]. Of the individuals born between 1910-1915, approximately 1:160 (0.6%) have reached ages ≥100 years [69]. Since 25-30% of all centenarians are estimated to be free of symptoms of cognitive decline [70-73], becoming a centenarian with retained cognitive health was reserved for only 0.2-0.3% of the 1910-1915 birth generation.

The participants of the 100-plus Study cohort were born in the Netherlands just before or during WWI (1914-1917), in which the Netherlands was impartial. The 20th century in the Netherlands was further characterized by a depression in the thirties, WWII between 1940-1945, a post-war period typified by a rebuilding phase in the 1950s and a

continuous increase in prosperity, health care improvements and technological developments. According to the Human Mortality Database [69] males born in the Netherlands between 1910-1915 had a mean lifespan of 58.7 years and females had a mean lifespan of 66.1 years.

Here we describe the 1910-1915 birth cohort by their mortality rates and dementia incidence from birth to >100 years (**Box-Figure**). For ages 0-60 years we represented mortality using the statistics from individuals born in 1912, and for ages >60 years we represented mortality using combined statistics from individuals born between 1910-1915. (**A**) In 1912, 170,000 babies were born, and they were exposed to a ~11% mortality rate in their first year of life (males 12%, females 10%), reflecting prevalent childhood diseases. (**B**) In 1918, the Spanish Flu made >40,000 casualties in the Netherlands [74], which was especially lethal among 20-40 year olds [75]. The Spanish Flu increased mortality among the 1912-born six-year olds by 2-fold. (**C**) At age 10, most childhood diseases were overcome and the mortality in the cohort was at a steady rate of ~0.2% per year, caused by incidental deaths (i.e. fatal accidents, drowning etc.). (**D**) When the 1912 birth cohort was 28 years, the onset of WWII in 1940 led to a peak in male mortality. (**E**) The ensuing Hunger Winter in 1945 led to a second mortality peak when the 1912 birth cohort was 33 years old, more so in males than in females. (**F**) When the cohort was 40 years old, the mortality resulting from natural decline rose above the rate of incidental deaths, and increased in the log scale according to Gompertz Law (1825) [76]. (**G**) During natural decline of the 1912 birth cohort, the males had a higher mortality rate than females, and this mortality gender gap ultimately resulted in a 1:7 male/female ratio at age 100 [51]. Approximately 70% of the mortality gender gap in these cohorts can be explained by the difference in smoking behavior between males and females [77]: an estimated 91% of all males born in 1912 smoked while only 30% of the females smoked, which ultimately led to a relative increased incidence of fatal smoking-related diseases in males. The remainder of the mortality gender gap may be explained by biological or environmental differences between males

and females [78]. (**H**) At 100 years old, the 1912 cohort has reduced to ±1,000 persons and the mortality year for both males and females is at ~40%.

(**I**) Individuals from the 1910-1915 birth cohorts were exposed to an increasing incidence of overall dementia from age 60 years onwards, of which the greatest proportion was (**J**) Alzheimer's Dementia (AD) [1]. (**K**) At approximately 100 years, the annual incidence of dementia reaches ~40%, and surpasses the mortality per year at that age (also ~40%), suggesting that after turning 100 years, a centenarian is exposed to greater odds of developing dementia than to die [6]. (**L_1**) If dementia incidence after 100 years continues to increase with the same exponential rate as before, following the Gompertz law of natural decline [76], then, a conservative estimation of dementia incidence (by concentrating mortality on incident dementia cases) suggests that all individuals who reach 108-110 years would have to be demented. (**L_2**) In contrast, reports of individuals who are older than 110 years indicate that the majority of such individuals has in fact retained their cognitive health [3-5]. Therefore, it is likely that the incidence of dementia decelerates or even declines at extreme ages [6]. Although the slope of the incidence rate suggested by Corrada et al. (red dots in Figure) is slightly smaller compared to the extrapolated incidence (dashed line), there currently is no clear evidence for this deceleration between 90 and 100 years [2, 79], it is most likely that this deceleration becomes evident somewhere after 100 years. This line of reasoning is consistent with findings in super-centenarians by Andersen et al. [5], who demonstrated the progressive compression of both disability and morbidity (in 6 diseases including dementia) with survival beyond 100 years. Together, this suggests that factors that preserve (cognitive) health may be progressively enriched for during healthy aging [7], providing a window of opportunity to search for such protective factors in a population of healthy (super-)centenarians.

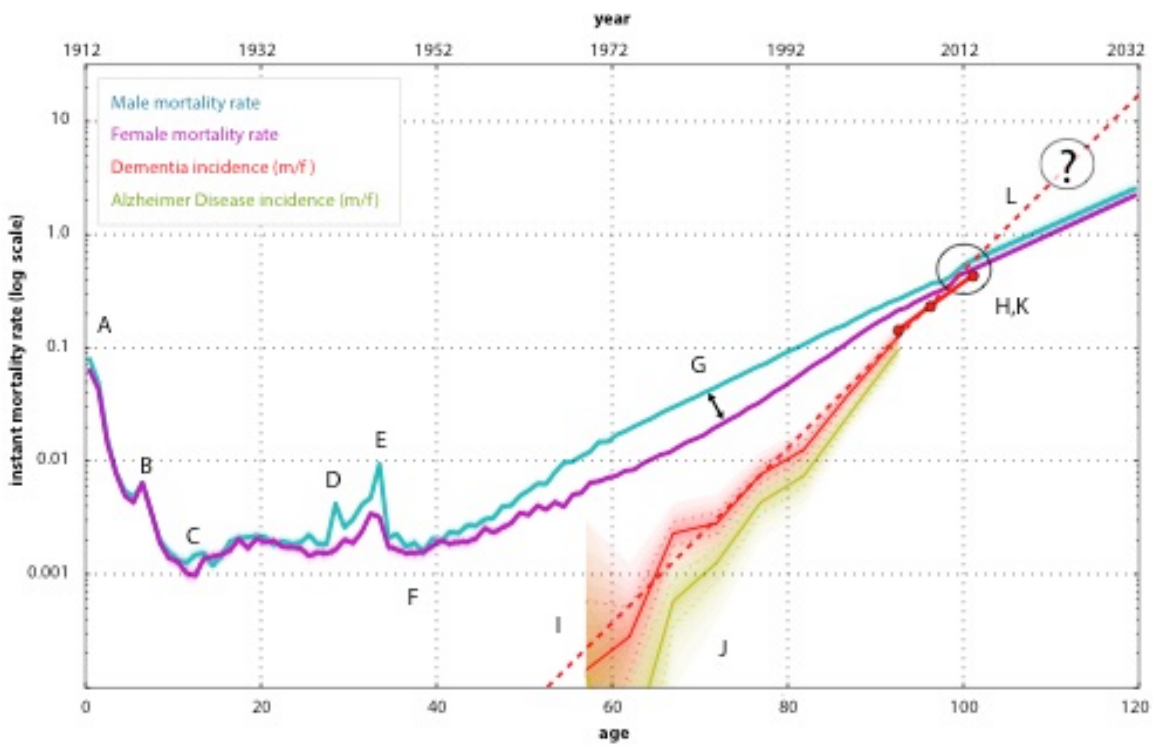

Figure Box: Mortality and dementia incidence in centenarian birth cohort.

Blue line*: Male mortality. Shades of blue represent mortality confidence intervals (CI) by 10-percentile increments [80]; For ages 0-59 years we used only the mortality statistics of individuals born in 1912 (as to avoid blurring specific mortality peaks), and for ages 60-100 years we combined statistics of the 1910-1915 birth cohorts, which reduced CI. Mortality after age 100 years was extrapolated according to Gompertz' law of mortality [81].* ***Purple line:*** *Female mortality with CIs [80].* ***Red line:*** *Median incidence of overall dementia with CIs for age groups 55-59, 60-64, 65-65, 70-74, 75-79, 80-84, 85-89 years [1]. To define mean age per age-group, we assumed that the ages of the individuals that constituted each age-group were distributed according to associated mortality statistics.* ***Red dots:*** *Dementia incidence for age groups: 90-94 years (mean age 92.7), 95-99 years (mean age 96.4) and 100+ (mean age 101.3) [2].* ***Green line:*** *Alzheimer's Disease (AD) incidence with CIs [1].* ***Dashed red line****: extrapolation of dementia incidence according to its exponential increase. To extrapolate dementia incidence, we fitted a Gompertz curve on available dementia incidence data [1, 2]. For the reported age ranges we compared*

the estimated dementia incidence with the reported incidence through a binomial distribution. This resulted in a log-likelihood, which was optimized.

FIGURES AND TABLES:

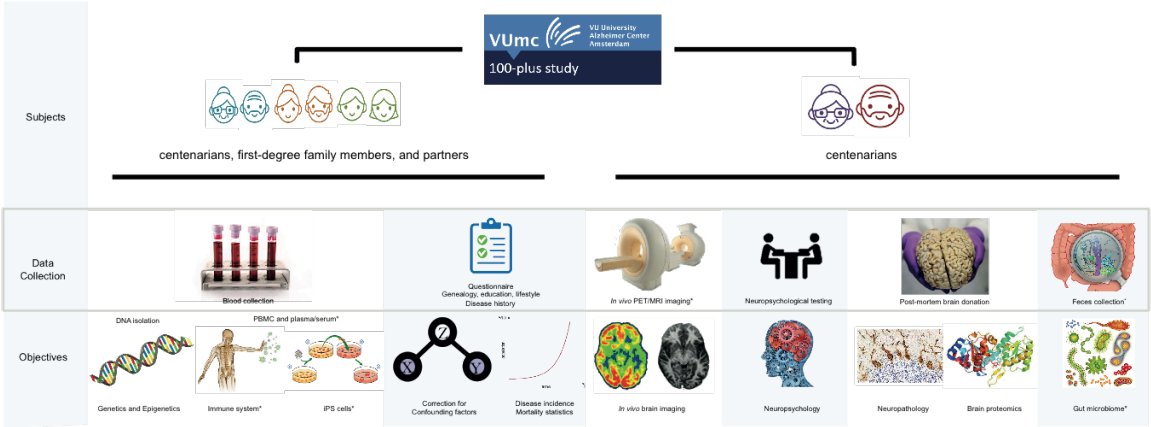

Fig. 1 Overview of the 100-plus Study, Phase 2

*During home visits we inquire about life-history of the centenarians, their family history, medical history, and current health. We assess their performance on neuropsychological tests, measure blood pressure and grip strength and we collect a blood sample, for blood testing and genetic analyses. Optional parts of the study are: a visit to the VUmc clinic for PET-MRI and/or PET-CT imaging, feces donation to investigate the gut microbiome, and the generation of iPS cells from peripheral blood. Furthermore, all participants are informed about the option of post-mortem brain donation in collaboration with the Netherlands Brain Bank [34]. This is optional and not required for study participation. We evaluate changes in general well-being and in neuropsychological test performance during (half-)yearly follow-up visits. Next to the centenarians, we also include their first-degree family members and their partners. * Collected in Phase-2 of the 100-plus Study, started in September 2017.*

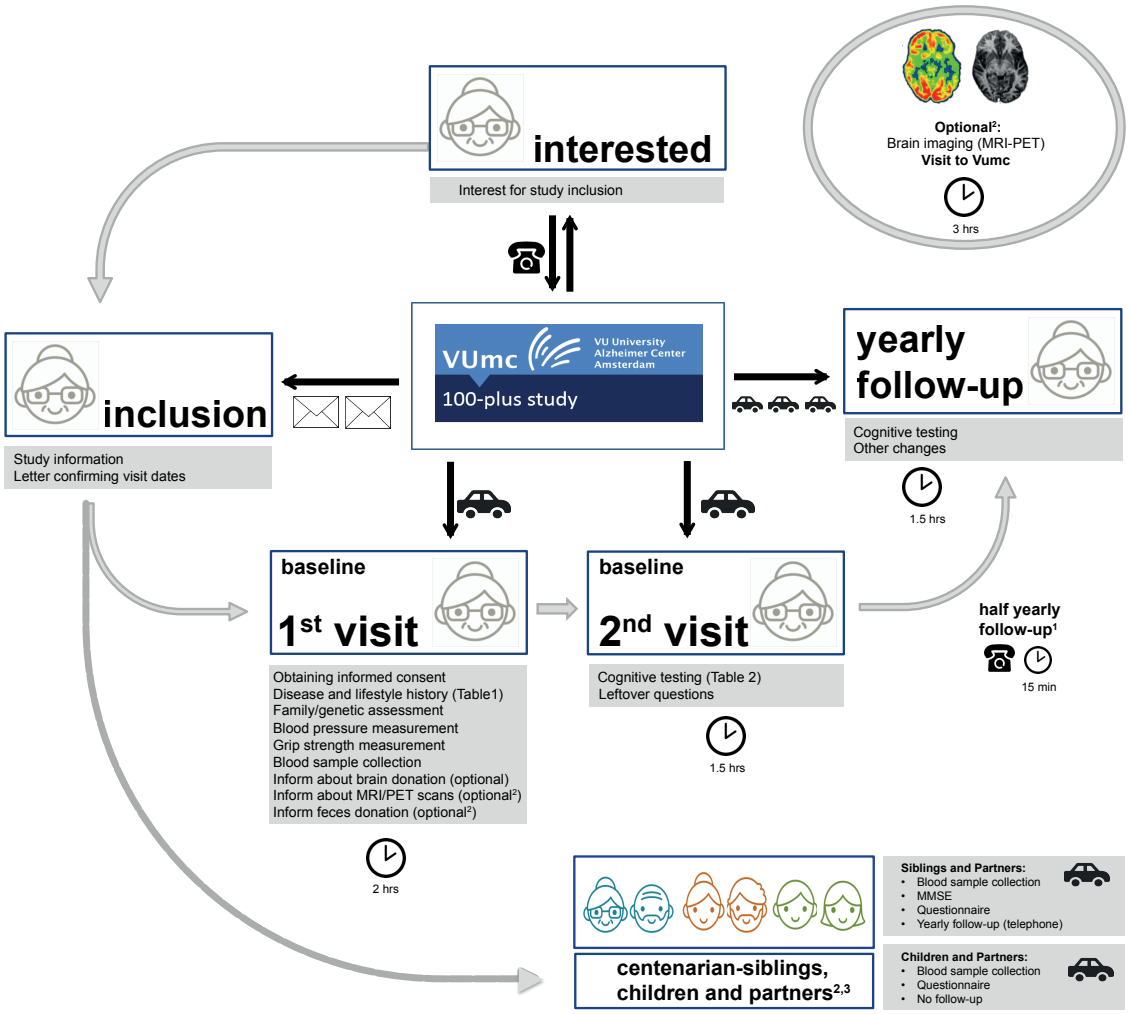

Fig. 2 Diagram of visit procedures of 100-plus Study

[1]Half yearly follow-up by telephone is performed for centenarians who agreed to brain donation. [2]Collected in phase-2 of the 100-plus Study, started in September 2017. [3]Data from centenarian-children and children in-laws will be obtained during the visit with the centenarian.

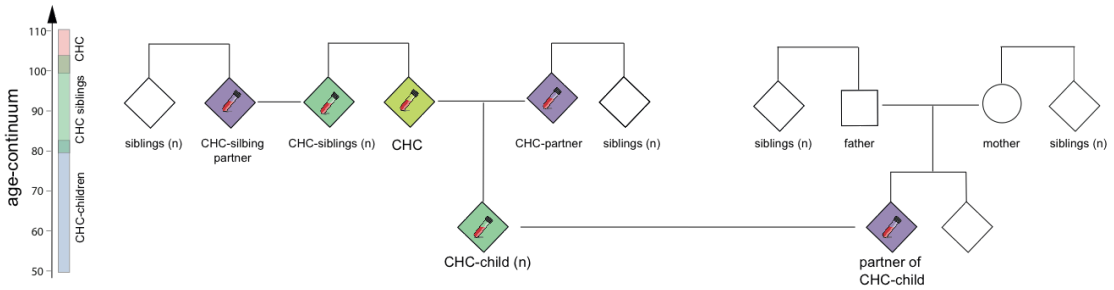

Fig. 3 Data collection from centenarians and their family-members

In Phase-2 of the 100-plus Study (September 2017), we obtain blood-samples from centenarians (yellow), and when willing, their siblings, their children (green) and their respective partners (purple). We will inquire about longevity and incidence of dementia in relatives from the same generation as the centenarian (white). Square: male, circle: female, diamond: both genders are possible.

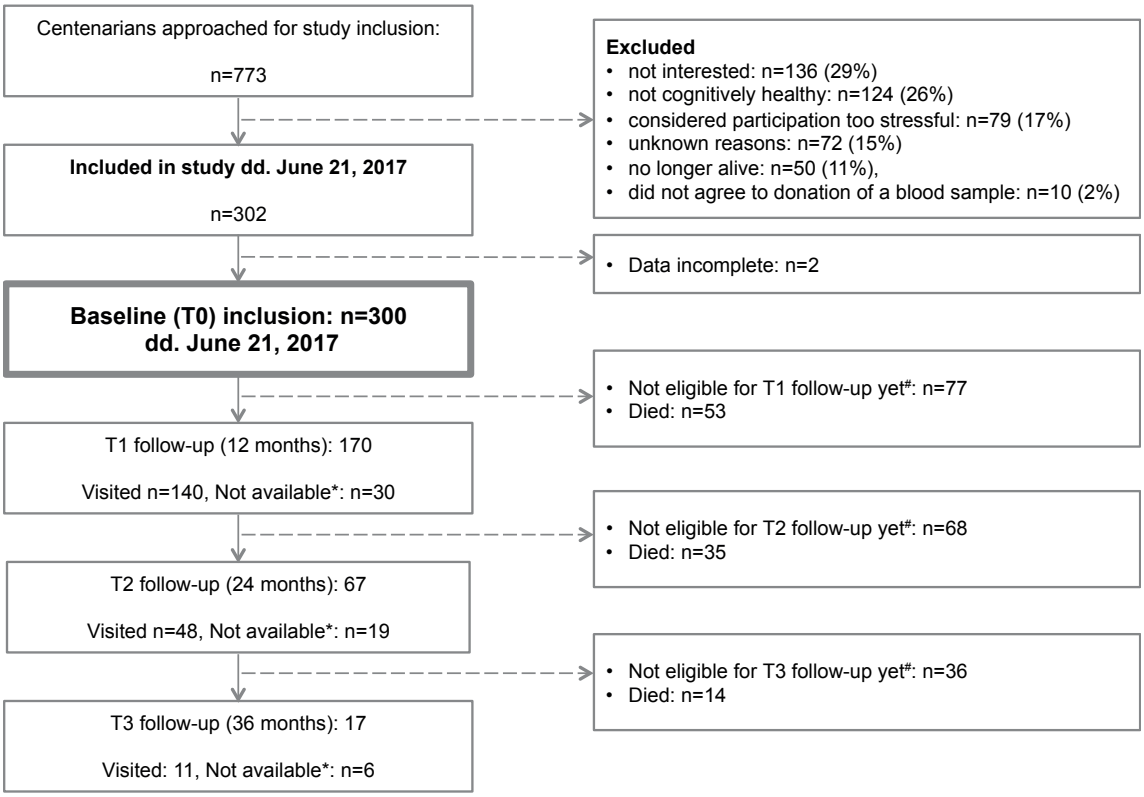

Fig. 4 Flowchart of study inclusion

**Not available: centenarians were on vacation, not interested or too frail for a follow-up visit. When possible, follow-up was performed by telephone and/or informant questionnaires. In several cases, centenarians were available for follow-up one year later, such that this 'unavailable' group was formally kept in the study until death. #Not eligible: centenarians were not yet included in the study long enough to be eligible for the next follow-up visit.*

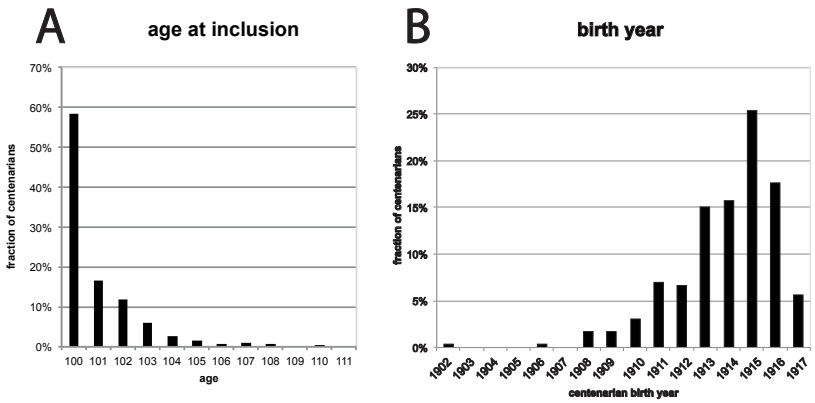

Fig. 5 Demographics of first 300 centenarians from 100-plus Study cohort: A. *Age at study inclusion of centenarians;* ***B:*** *Birth year of centenarians included in the cohort;*

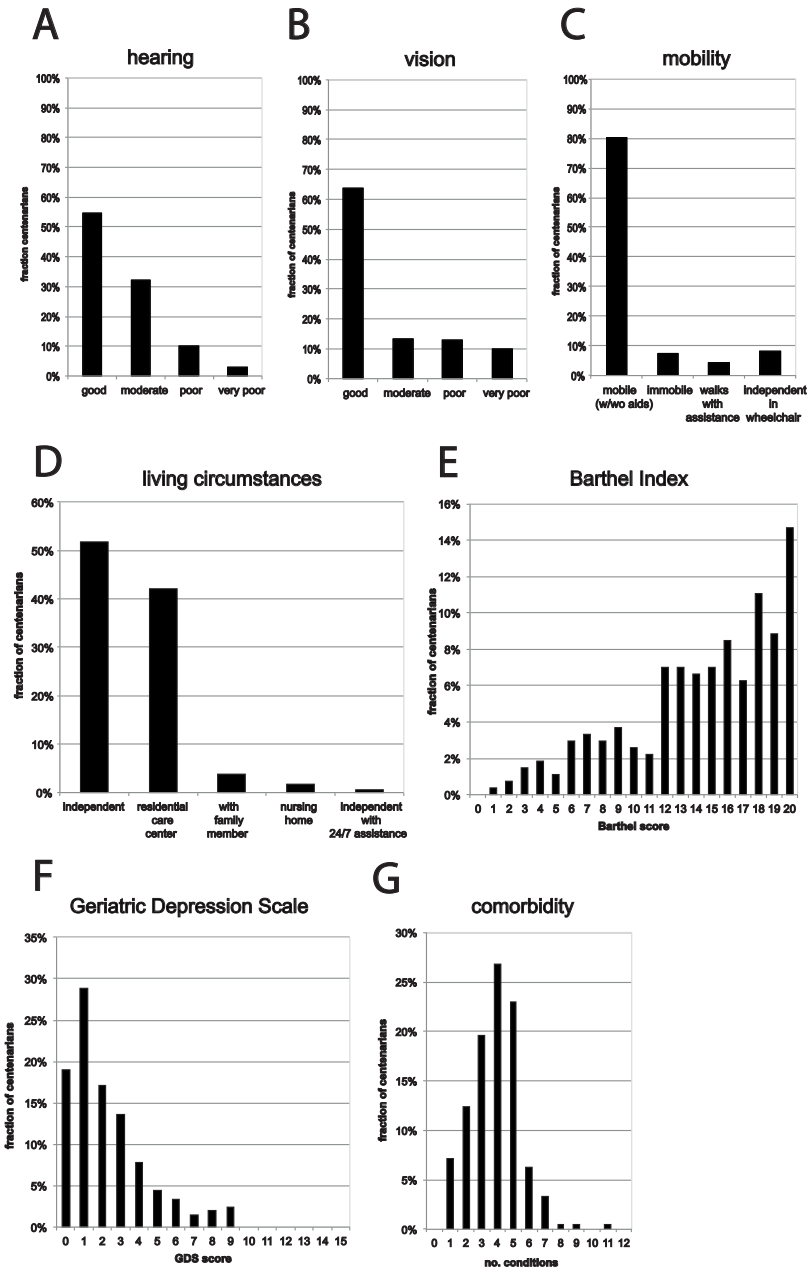

Fig 6 Baseline Presentation. *Researcher subjective impression of:* ***A.*** *hearing;* ***B.*** *vision;* ***C.*** *mobility;* ***D****. independence in living circumstances;* ***E.*** *independence in activities of daily living* ***(****Barthel Index scores: 15-19 minimal help necessary, 20 fully independent).* ***F.*** *Geriatric depression scale (GDS) scores of centenarians (score 0: no symptoms of depression, scores >6 is suggestive of a prevalent depression).* ***G.*** *Multi-morbidities: fraction of centenarians whose GP reports mention diseases that belong to a specific number of separate disease categories.*

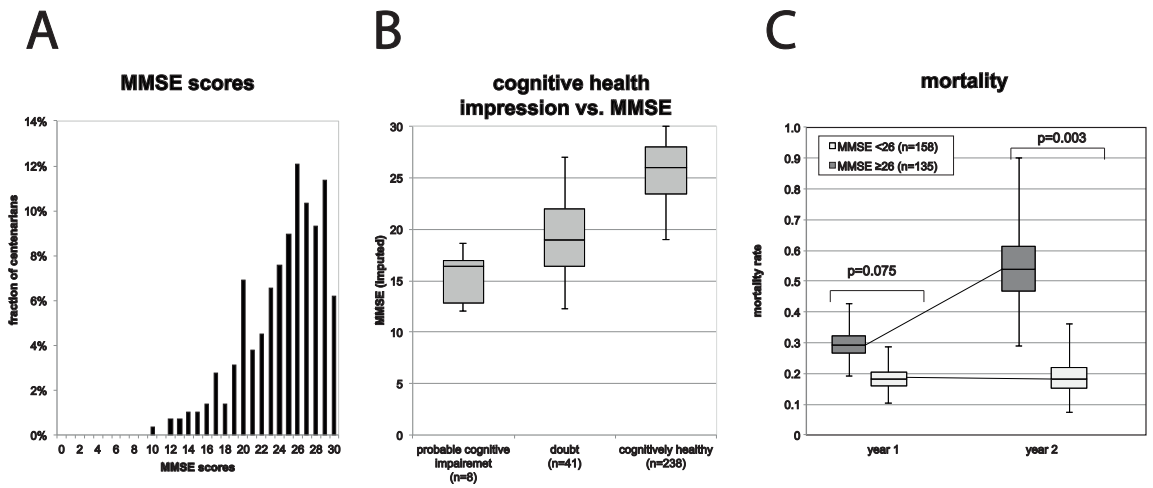

Fig 7 Overall cognitive functioning (Mini-Mental State Examination) A*. Mini-Mental State Examination (MMSE) scores****. B.*** *Researcher impression of cognitive health at first visit, compared to MMSE score.* ***C.*** *Mortality rate of centenarians with high and low performance on the MMSE.*

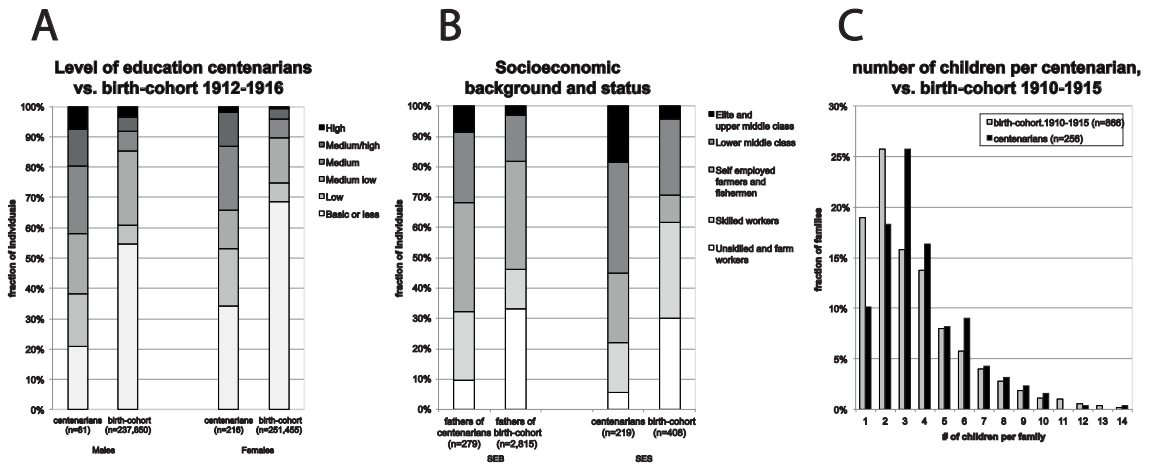

Fig 8 Lifestyle and socioeconomic characteristics of centenarians. A. *The level of education of male and females was compared to the population born between 1912-1916; basic or less (primary school or less); low (1 to 2 years secondary, unfinished); medium low (3 or 4 years secondary, or lower technical school); medium (5 to 6 years secondary, or medium technical/vocational); semi-high (applied sciences or first year university); high (university or alike).* ***B.*** *Social-economic class of centenarian-fathers compared to fathers of individuals born between 1910-1915; and social economic class of centenarians as adults compared to males born between 1910-1919.* ***C.*** *Number of children parented by centenarians, compared to individuals born between 1910-1915.*

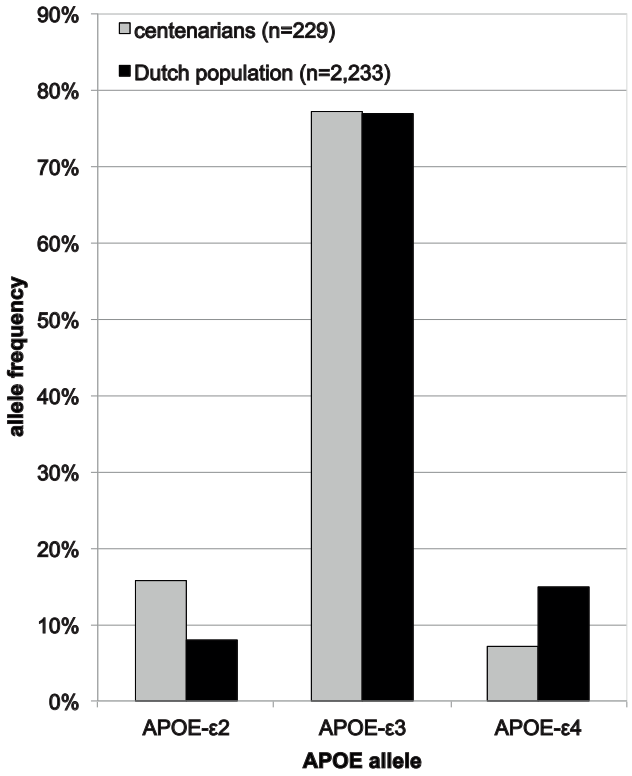

Fig 9 APOE allele distribution in 100-plus Study cohort.

Study participants	Actions
Centenarians Phase 1	**<u>First baseline visit: Interview</u>** • Formalities for study inclusion: ICF; Proof of age • Childhood living environment; Education; Marriage/Partners; Number of children, Religion, Occupation; Occupation of parents and partner • Genealogy of first degree family members and partners; Disease history in family • Lifestyle Questionnaire: Smoking habits; Drinking habits; Lifetime cognitive activity scale; situation during WWII • Disease history (self-report): weight/length; incontinence; medication intake, dental condition (stopped); hospital visits/anesthesia • Researcher subjective estimate of sight, hearing, mobility, cognitive status; • Centenarian presentation: current housing situation, total hours of care; ADL (Barthel index); sleep quality (PSQI); Geriatric Depression Scale (GDS); cognitive well-being judged by informant (IQ-CODE) • Collection of biomaterials and biomarkers: blood sample* **<u>Second baseline visit:</u>** • Neuropsychological test battery: **Table 2** • Measurement of grip strength and blood pressure **<u>Follow up:</u>** • MMSE at last visit >20: Yearly visit: update of general well-being, disease history, and missed items at baseline interview; Researcher subjective estimate of sight, hearing, mobility, cognitive status; • Neuropsychological testing battery (**Table 2**) Barthel index; GDS; IQ-CODE , grip strength measurement; blood pressure measurement • MMSE at last visit ≤20; phone interview: update of general well-being, disease history, and missed items at baseline interview; IQ-CODE (by mail), ADL (Barthel index) • For brain donors: Half yearly follow-up: TICs-M (by telephone); IQ-CODE (by mail) **<u>GP:</u>** • At baseline inclusion: Request for summary of medical events • Post mortem: request medical events leading to death **<u>Optional in Phase-2:</u>** • MRI-PET or PET-CT scan • Feces donation • iPS cell generation • Post mortem brain donation
Centenarian children & Partners Phase-2	**<u>Baseline visit:</u>** • Formalities for study inclusion: ICF; • Collection of blood sample • Mail: Questionnaire on lifestyle, general well-being, education and occupation, disease history and genealogy **<u>Follow up:</u>** • No follow-up **<u>GP:</u>** • For specific cases: Request for summary of medical events
centenarian-siblings & partners, centenarian-	**<u>Baseline Visit:</u>** • Blood sample, MMSE, Barthel index; IQ-CODE; grip strength and blood pressure measurement, estimation of sight, hearing, and mobility; • Researcher subjective estimate of sight, hearing, mobility, cognitive status;

partners Phase-2	• Mail: Questionnaire on lifestyle, general well-being, education and occupation, disease history, and genealogy **Follow up:** • Yearly: TICs-M (by telephone); IQ-CODE (by mail) • Update lifestyle questionnaire, current health, disease history and general well-being **GP:** • For specific cases: Request for summary of medical events

Table 1 Overview of 100-plus Study data-collection*. *Blood sample collection may occur at a different occasion, close to first baseline visit; Phase-2 of the 100-plus Study started in September 2017.*

Domain or goal	Assessment/Questionnaires	Duration (min)
Cognitive functioning		
Overall cognitive functioning	• Researcher subjective impression of cognitive health (see methods)	0
	• Mini-Mental State Examination [22, 82]	5
	• National Adult Reading Test[a] [83-85]	3
	• Telephone Interview Cognitive Status –Modified (TICS-M)[d][86]	
Memory	• CERAD 10-word list – immediate and delayed recall [87]	15
	• Visual Association Test - Memory [88]	5
	• Rivermead Behavioral Memory Test (RBMT)[b] immediate and delayed recall [89, 90]	6
Attention	• Digit Span – forwards [91-93]	3
	• Trail Making Test A [94, 95]	
Executive functions	• Digit Span – backwards [91-93]	3
	• Letter Fluency – DAT [96-100]	2
	• BADS – subtest Key Search [101, 102]	3
	• BADS– subtest Rule Shift Cards [101, 102]	3
	• Trail Making Test B [94, 95]	10
	• Amsterdam Dementia Screening Test - Meander figure [103]	2
Language	• Category Fluency – Animals [96, 104, 97]	2
	• Visual Association Test - Naming [88]	1
Visuo-spatial functioning/construction	• CAMDEX-R/N CAMCOG - Figure Copying [105, 106]	3
	• Clock Drawing Test [107, 108]	2
	• Visual Object and Space Perception (VOSP) Battery[b] - subtest Number Location [109]	3
Depression, ADL, Sleep, Lifestyle, geriatric impairments		
Depressive symptoms	• Geriatric Depression Scale-15 (GDS) [25, 26]	4
(Instrumental) Activities of daily living	• Informant Questionnaire on Cognitive Decline in the elderly short form (IQ-CODE) [27, 28]	3
	• Barthel Index [23, 110-112]	3
Lifetime cognitively stimulating experience	• Lifetime Cognitive Activity Scale[a] [31, 32]	5
Sleep quality	• Pittsburgh Sleep Quality Index[a] (PSQI) [24]	5
Geriatric impairments	• Researcher subjective impression of sight, hearing, mobility	0

(methods)

Table 2 ***Neuropsychological tests and questionnaires.***

a: o*nly administered at baseline; b: in 100-plus Study-phase 1 only. c: Included with the confirmation letter of study-inclusion, collected during the first baseline visit.* d: Only administered during half yearly-follow up of brain donors and yearly follow-up of siblings

	Vision	Hearing	Mobility
Good	Able to read newspapers and watch television	Able to have and follow a conversation in a group of people	Able to walk independently (with or without help of a walking stick or walker)
Moderate	Able to read large texts with large letters and watch television	Able to have a conversation with one person/questions do not have to be repeated	Able to walk with help of another person
Poor	Not able to watch television/vision problems cause some difficulties in ADL	Limited ability to have a conversation with one person/questions need to be repeated multiple times	Able to move independently in a wheelchair
Very poor	Limited or complete loss of vision which causes severe difficulties in ADL	Not able to have a conversation with one person; this does not improve when speaking loud and clearly	Not able to move independently in a wheelchair

Table 3 Categorization of vision, hearing and mobility capacities

Category fraction of centenarians with ≥1 condition in this category (%)	**Conditions** fraction of centenarians with at least one mention of this condition in GP report (%)
musculoskeletal (64.1%)	osteoporosis, osteopenia (4.8%); coxarthrosis, gonarthrosis (23.4%); fractures (20.1%); osteoarthritis (gout, joint replacements) (7.7%); hernia, collapsed vertebrae (6.2%); amputations (1.9%);
cardiovascular (58.9%)	atrial fibrillation, congestive heart failure (25.4%); angina pectoris (10.0%); heart attack (6.7%); cardiac dysrhythmia, pacemaker (5.3%); hypercholesterolemia, atherosclerosis, coronary sclerosis, bypass surgery (4.8%); deep venous thrombosis & pulmonary embolism (3.8%); heart valve disease, hypertension (2.9%);
hypertension (48.8%)	hypertension (48.8%);
vision (41.6%)	vision impairment, glaucoma, cataract (with/without surgery); macular degeneration (41.6%);
hearing (30.6%)	hearing impairment, sudden deafness (30.6%);
cancer (27.8%)	skin cancer (14.8%); breast cancer (3.8%); colon cancer (3.3%); cancer other: vocal chord, bladder, palate, stomach, thyroid (2.9%); prostate cancer (1.9%); uterus cancer (1.0%);
autoimmune (22.5%)	hyperthyroidism, hypothyroidism (8.6%); diabetes mellitus (7.2%); rheumatoid arthritis, polymyalgia rheumatica (4.3%); Sogren's disease (1.4%); asthma (0.5%); hypopituitarism (0.5%);
CVA/TIA (18.7%)	Cerebrovascular Accident (CVA) or transient ischaemic attack (TIA) (18.7%);
bladder/prostate (17.7%)	urinary tract infections (6.7%); urge incontinence, catheter (6.2%); prostate hypertrophy benign (with/without surgery) (4.8%);
neurological/psychiatric disease (15.8%)	epilepsy, insomnia, migraine (5.3%); balance problems, dizziness (3.8%); depression, loneliness (2.9%); delirium after infection or fracture (1.9%); Parkinson's disease (1.9%), starting cognitive decline (1.9%);
gastrointestinal (14.8%)	kidney stones (removed), renal failure (6.7%); diverticulitis, (bleeding) ulcers, polyps, stomach bleeds, appendectomy (5.7%); resection gall bladder, removal gall stones (2.4%);
lung disease (10.5%)	pneumonia (5.3%); COPD (5.3%);
Other	erysipelas, chronic leg ulcer, pericarditis (4.3%); hysterectomy, prolapse, cysts removal (4.3%); cholesteatoma (0.5%); anemia (1.0%); restless legs (1.0%);

Table 4. Categories of conditions analyzed in the GP medical files of 209 centenarians

Cohort statistics		
100-plus cohort, June 2017 (N available, %)	300	
Age at inclusion (mean, stdev)	101.3 ± 1.7	
Birth years (median, IQR)	1914 (1913-1915)	
Brain donors (n,%)	81 (27%)	
Follow-up visits		
T0 Baseline visits	300	
T1 possible visits (visited, died, missed)	223 (140,53,30)	
T2 possible visits (visited, died, missed)	155 (48, 88, 19)	
T3 possible visits (visited, died, missed)	119 (11, 102, 6)	
Mortality		
Whole cohort:	**T0-T1**	**T1-T2**
Mortality rate (95% CI)	0.24 (0.17-0.32)	0.32 (0.20-0.49)
Mortality percentage (95% CI)	21% (16%-27%)	28% (18%-39%)
MMSE <26 at baseline (95% CI)		
Mortality rate (95% CI)	0.29 (0.19-0.43)	0.54 (0.29-0.90)
Mortality percentage (95% CI)	25% (17%-35)	42% (25%-59%)
MMSE ≥26 at baseline (95% CI)		
Mortality rate (95% CI)	0.19 (0.11-0.29)	0.19 (0.08-0.37)
Mortality percentage (95% CI)	17% (10%-25%)	17% (8%-31%)
Cognitive functioning at baseline		
Mini Mental State Examination (MMSE)		
100-plus cohort (median MMSE, IQR)	25 (22.0-27.5)	
MMSE >22 (fraction of cohort, %)	72.4%	
MMSE ≥26 (fraction of cohort,%)	47.2%	
Estimated by trained researcher *(n=287)*		
Cognitively healthy (fraction of cohort, %; median MMSE (IQR)	83%; 26 (23.5-28.0)	
Doubt	14%; 19 (16.4-22.0)	
Cognitively impaired	2.8%; 16.4 (12.8-17)	
Baseline presentation		
Geriatric impairments		
Mobile: without aids	80.2%	
Hearing: Moderate-Good	86.8%	
Vision: Moderate-Good	77.1%	
Maintained Continence	56.3%	
Number of comorbidities (avg ± stdev)	3.9±1.6	
Geriatric depression scale: ≤5 (no depression)	91.5%	
Living independence:		
Community dwelling/private residence with care available	51.9%	
Private quarters in residential care center	42.0%	
Independence in Activities of Daily Living (Barthel Index)		
Needs minimal assistance (15-19)	45.1%%	
Fully independent (20)	32.4%%	
Lifestyle characteristics		
Smoking: regularly/often		
Males	67%	
Females	15%	
Alcohol consumption: regularly/often		
Males	54%	
Females	31%	
Demographic characteristics		
Education >basic	**centenarians vs. population[a, b]**	
Males	79% vs. 45%	
Females	66% vs. 31%	

Socioeconomic status:		
SEB: Social Class Father: ≥ lower-middle class		31.2% vs. 17.9%
SES: Social Class-centenarian or –partner: ≥ lower-middle class		55.5% vs. 29.4%
number of children parented: (mean ± stdev)		3.9±2.2 vs. 3.5±2.5
APOE genotypes		
APOE genotypes:	Genotype frequency (%); centenarians vs. population[c]	**Odds Ratio (95%CI); p value[d]**
ε2/ε2	0.9% vs. 0.7%	1.30 (0.3-5.7); $p=9.6x10^{-1}$
ε2/ε3	24.9% vs. 11.7%	2.49 (1.8-3.5); $p=3.4x10^{-7}$
ε2/ε4	4.8% vs. 3.0%	1.63 (0.8-3.1); p=2.1x10-[1]
ε3/ε3	60.3% vs. 60.5%	0.99 (0.8-3.1); $p=8.9x10^{-1}$
ε3/ε4	8.7% vs. 21.3%	0.35 (0.2-0.6); $p=5.7x10^{-7}$
ε4/ε4	0.4% vs. 2.9%	0.15 (0.0-1.1); $p=3.2x10^{-3}$
APOE alleles:		
ε2	17% vs 10.7%	2.1 (1.6-2.8); $p=4.8x10^{-7}$
ε3	86.1% vs 87.1%	1.0 (0.8-1.3); p=1.0
ε4	3.2% vs 7.5%	0.44 (0.31-0.63); $p=6.3x10^{-7}$

Table 5. Descriptive statistics of 100-plus Study cohort.

a: Centenarian education levels were compared with 54-61 years olds reported in the Dutch population in the 1971 census [36]; b: socio-economic background was compared with 2,815 individuals born between 1910-1915 from the from the Historical Sample of the Netherlands (HSN) [37]; c: APOE genotypes were compared with 2,233 ~50-80-year olds from the Longitudinal Aging Study Amsterdam (LASA) [42]; d. P-values were calculated using a two-sided Fisher's Exact test.