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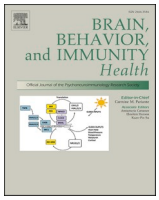

Systemic inflammation in relation to exceptional memory in the Long Life Family Study (LLFS)

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ABSTRACT

Background and objectives: We previously found a substantial familial aggregation of healthy aging phenotypes, including exceptional memory (EM) in long-lived persons. In the current study, we aim to assess whether long-lived families with EM and without EM (non-EM) differ in systemic inflammation status and trajectory.
Methods: The current study included 4333 participants of the multi-center Long Life Family Study (LLFS). LLFS families were classified as EM (556 individuals from 28 families) or non-EM (3777 individuals from 416 families), with 2 or more offspring exhibiting exceptional memory performance (i.e. having baseline composite z-score representing immediate and delayed story memory being 1.5 SD above the mean in the nondemented offspring sample) considered as EM. Blood samples from baseline were used to measure inflammatory biomarkers including total white blood cell (WBC) and its subtypes (neutrophils, lymphocytes, monocytes) count, platelet count, high sensitivity C-reactive protein, and interleukin-6. Generalized linear models were used to examine cross-sectional differences in inflammatory biomarkers at baseline. In a sub-sample of 2227 participants (338 subjects from 24 EM families and 1889 from 328 non-EM families) with repeated measures of immune cell counts, we examined whether the rate of biomarker change differed between EM and non-EM families. All models were adjusted for family size, relatedness, age, sex, education, field center, APOE genotype, and body mass index.
Results: LLFS participants from EM families had a marginally higher monocyte count at baseline ($b = 0.028$, $SE = 0.0110$, $p = 0.010$) after adjusting for age, sex, education, and field site, particularly in men ($p < 0.0001$) but not in women ($p = 0.493$) (p-interaction $= 0.003$). Over time, monocyte counts increased ($p < 0.0001$) in both EM and non-EM families, while lymphocytes and platelet counts decreased over time in the non-EM families ($p < 0.0001$) but not in the EM families. After adjusting for multiple variables, there was no significant difference in biomarker change over time between the EM and non-EM families.
Discussion: Compared with non-EM families, EM families had significantly higher monocyte count at baseline but had similar change over time. Our study suggests that differences in monocyte counts may be a pathway through which EM emerges in some long-lived families, especially among men.

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1. Introduction

Growing evidence has linked inflammation to cognitive health. Neuro-inflammation, specifically microglial activation, has been linked to the pathogenesis of mild cognitive impairment and Alzheimer's disease (AD) (Okello et al., 2009). In addition to neuroinflammation, strong evidence suggests peripheral systemic inflammation is involved in cognitive decline and related dementia (Gorelick, 2010; Holmes et al., 2009). Higher levels of peripheral inflammation, indicated by immune cell counts and cytokines, are associated with worse performance on cognitive abilities in cross-sectional studies (Baune et al., 2008; Marsland et al., 2015; Trollor et al., 2012), or faster cognitive decline in longitudinal studies (Marioni et al., 2009; Walker et al., 2019). In non-demented older adults, higher levels of inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL6) were found to be associated with brain health indices, including smaller brain volume (Satizabal et al., 2012) and cerebrovascular diseases (Gu et al., 2019). In addition, peripheral immune cells, such as white blood cell, neutrophil, monocyte, and lymphocyte, have also been associated with cognitive decline neuroimaging markers of AD (Li et al., 2023).

Interestingly, centenarians show features of the immune system that are similar to young adults, which may contribute their successful aging (Alonso-Fernández et al., 2008). Indeed, chronic systemic inflammation, rather than telomere length, was the most important physiobiological factor that predicted successful ageing among centenarians and semi-supercentenarians (Arai et al., 2015). Late nonagenarians and centenarians have also been shown to have improved or at least well-maintained levels of immune markers (Strindhall et al., 2007).

Cognitive aging is a key predictor for quality of life and mortality in older adults. With the rapidly aging population in U.S. and other countries, it is essential to identify key biological features that may contribute to the cognitive health and long lives with high quality. Findings from populations with successful ageing will help to gain insights into how to extend healthy life span for the wider population. The Long Life Family Study (LLFS) has previously examined five healthy aging phenotypes, (Barral et al., 2013; Singh et al., 2015) including cognition (i.e., episodic memory), blood pressure, pulmonary function, grip strength, and metabolism. We previously found that exceptional memory (EM) performance strongly aggregates in the LLFS families (Barral et al., 2013). The exact reason for such aggregation is unclear, but in addition to healthier metabolic and a physical/pulmonary profiles, (Barral et al., 2017a) lower levels of systemic inflammation may also contribute to the exceptional memory in families. Thus, the current study aims to examine whether systemic inflammation was associated with exceptional memory in LLFS. We hypothesized that LLFS participants who had better memory profiles would have lower circulating levels of CRP, IL6, and white blood cells, and less change in these immune markers over time.

2. Materials and methods

2.1. Study population

Details of the LLFS cohort have been published elsewhere (Barral et al., 2012, 2013; Cosentino et al., 2013; Newman et al., 2011). The cohort consists of 4953 participants from 539 families (Wojczynski et al., 2022). Families were selected for clustering of longevity at field sites in the United States (Boston, New York, and Pittsburgh) and Denmark. The following criteria were used to assess the eligibility of US families: (Okello et al., 2009) at least two living siblings over the age of 80; (Gorelick, 2010) at least one living offspring from one of the two living siblings; (Holmes et al., 2009) one living spouse of the offspring generation to serve as a control; and (Baune et al., 2008) evidence of exceptional survival as measured by the Family Longevity Selection Score (FLoSS) of seven or higher for members of the proband generation. FLoSS is a metric of familial longevity relative to what would be expected based on birth cohort specific life tables and the availability of living subjects for the study (Sebastiani et al., 2009). The Danish site identified individuals who would be ages 90 and above during the study recruitment period through the Danish National Register of Persons. Archived parish registers in Denmark were searched for information on the place of birth and the names were searched to locate the parents of the older adults to identify sibships. Based on the above information, 659 potentially eligible families were identified ranked by FLoSS. Contact was made with potential probands to further assess the family's eligibility for and willingness to participate in the LLFS using criteria parallel to that used in the United States.

2.2. Standard protocol approvals, registrations, and patient consents

Recruitment, informed consent, and study procedures were approved by the Institutional Review Boards of all participating sites.

2.3. Exceptional memory

Participants underwent cognitive testing during in-person visits. As described before (Barral et al., 2017b), LLFS families were classified as EM (556 individuals from 28 families) or non-EM (3777 individuals from 416 families) on the basis of a composite z-score representing immediate and delayed story memory measured using the Wechsler Memory Scale - Logical Memory test. The threshold for EM was defined in the offspring generation of the LLFS cohort (mean age $= 61 \pm 8.38$ years), as performance $\geq$1.5 standard deviations (SD) above the age, sex, and education adjusted mean scores in the normative cohort of non-demented LLFS offspring. Families were then defined as having EM if two or more offspring in the family performed above this threshold. Each individual was classified as belonging to an EM family or not regardless of their own cognitive condition.

2.4. Biomarkers

Blood samples used to measure inflammatory biomarkers at baseline were available in 4333 (556 from 28 EM families and 3777 from 416 non-EM families) LLFS study participants. A subset of 2227 subjects (338 subjects from 24 EM families and 1889 subjects from 328 non-EM families) also received a follow-up measurement approximately 7.7 years later. Detailed information about blood sample processing has been reported before (Sebastiani et al., 2016). Briefly, at in-person visits, 50 mL of fasting blood samples were obtained following a standardized venipuncture protocol by trained phlebotomists. The serum tubes were kept at room temperature for 30–45 min prior to centrifugation to allow for clotting and centrifuged on site at $3000 \times g$ for 10 min. The centrifuged serum tubes along with the other unprocessed EDTA blood tubes were shipped to the Advanced Diagnostics and Research Laboratory (ARDL) at the University of Minnesota. An unprocessed EDTA tube was used for the measurement of complete blood counts. All serum and plasma aliquots were stored at -80 °C until analysis (Sebastiani et al., 2016). Inflammatory biomarkers including counts of total white blood cell (WBC) and its subtypes (Neutrophil, Lymphocyte, Monocyte), and platelet counts were measured at baseline and at the follow-up visits. Among 4226 individuals who had at least one cell type counted, 25, 25, 30, and 7 had missing data on neutrophil, lymphocyte, monocyte, and platelet counts, respectively. High sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL6) were only measured at baseline. A total of 4138 and 4320 individuals had CRP and IL-6 below detection limit and were not included in the analysis.

Hemogram, differential, and platelet count were measured in EDTA whole blood using a Sysmex XE-2100 instrument (Sysmex America, Inc., Mundelein, IL 60060). The instrument directly measured white blood cell count, platelet count, eosinophil %, basophil %, lymphocyte %, and monocyte %. The remaining parameters that were calculated or derived are neutrophil % and differential absolute counts. The white blood cell

differential channel classified lymphocytes, monocytes, eosinophils, and granulocytes by cellular complexity and nucleic acid content.

High sensitivity CRP (hsCRP) was measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a two-reagent, immunoturbidimetric method (Roche Diagnostics, Indianapolis, IN 46250). The inter-assay CV is 4.5%.

IL-6 was measured in plasma using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit from R & D Systems (Minneapolis, MN). Commercially obtained controls and in-house controls are run daily with inter-assay CV's of 4.9–6.5%.

APOE alleles were genotyped using real time PCR and were defined based on the SNPs rs7412 and rs429358 as E2: rs7412 = T; rs429358 = T, E3: rs7412 = C; rs429358 = T, or E4: rs7412 = C; rs429358 = C (Du et al., 2021).

2.5. Statistical analysis

Descriptive analysis: Absolute cell counts, hsCRP, and IL6 had skewed distributions and were logarithm transformed. Correlations of these biomarkers with age were performed using Pearson's correlation analysis. Characteristics of the EM family members and non-EM family members were compared using T-test for continuous variables and chi-square test for categorical variables. Median levels of the inflammatory markers were compared using non-parametric Mann Whitney Wilcoxon Test.

Cross-sectional analyses: To examine whether baseline inflammatory biomarkers differ between EM and non-EM families, we used separate models for each biomarker. Specifically, we used General Linear Models with Generalized Estimating Equations (GEE) to adjust for differences in family size and relatedness among LLFS participants. The predictor was family type (EM or non-EM), and the inflammatory biomarkers were included as the outcome variable individually. Analyses were adjusted for age at enrollment, sex, education, and field site in Model 1, and additionally adjusted for body mass index (BMI) and APOE ε2 genotype (ε2ε2 or ε2ε3 vs others) in Model 2.

Longitudinal analyses: We calculated the annual relative change of each type of cell from baseline visit to follow-up visit as follows: Annual $\Delta Y = [\log(Y_{fu}) - \log(Y_b)]/\text{time} = [\log(Y_{fu}/Y_b)]/\text{time}$ in years, where Y_{fu} and Y_b represented the cell count at follow-up visit and baseline visit, respectively, and time was the duration (in years) between baseline and follow-up visits. We used one-sample *t*-test to examine whether individuals' annual change in biomarkers was significantly different from zero, by randomly selecting one family member from each family, repeating the procedure 1000 times to determine an average estimated annual change. Similar to the cross-sectional analyses, we used GEE to examine whether LLFS families with EM had a slower rate of change in biomarker levels over time compared to non-EM families, adjusted for age, sex, education, and field site in Model 1, and also for BMI and APOE ε2 status in Model 2. The dependent variable of the GEE model was the annual change of these cells as described above. The predictor of the GEE model was family type (EM or non-EM).

Sensitivity analysis: We examined the interaction of EM status with potential moderators [sex (females vs. males), *APOE* status (ε2 carriers vs. non-carriers), and BMI (BMI≥25 overweight or obese vs BMI <25 others)] on inflammatory markers that were significantly associated with EM status in the overall population. Stratified analyses by these potential moderators were also performed. We performed the analyses by limiting to the offspring generation only for both cross-sectional (n = 2964) and longitudinal (n = 1922) analyses. Post-hoc sensitivity analyses were performed to test the robustness of the significant findings on monocytes, including using the monocyte counts at the follow-up visit, and using the monocyte/high-density lipoprotein (HDL) ratio (Song et al., 2023). For longitudinal analysis, we also performed the multi-variable adjusted analyses in 2204 subjects after excluding 23 individuals who died within six months of the follow-up visits.

All statistical analyses were performed using SPSS 26 statistical software. Bonferroni correction was used to adjust for multiple comparisons, with $p < 0.007$ (0.05/7) and $p < 0.01$ (0.05/5) considered as meeting statistical significance for cross-sectional and longitudinal analyses, respectively.

3. Results

3.1. Characteristics of the study population

The study subjects were on average 71 years old, had 12 years of education, and 55% were women. Compared with LLFS members from non-EM families, those from EM families were younger, had more years of education, and had higher estimated family exceptional longevity as measured by FLoSS scores ($p < 0.001$ for all). There were no statistically significant differences in the proportion of women or distribution of the APOE-ε4 allele or APOE-ε2 allele between EM and non-EM families (Table 1). Compared with non-EM family members, EM family members had lower levels of WBC count, neutrophil count, hsCRP, and IL6 levels (Table 1). At baseline, lymphocytes and platelet counts were negatively correlated with age, while all other biomarkers were positively correlated with age, in all subjects and in the non-EM families ($p < 0.0001$ for all, Supplementary Table S1). In EM families, though, lymphocytes and platelet counts were not correlated with age, but all other biomarkers were positively correlated with age (Supplementary Table S1). The participants were followed up at an average of 7.78 (SD = 1.34) years. Those who completed follow-up visits were younger at enrollment, had higher FLoSS scores and higher education, but otherwise similar in sex, APOE status, BMI, compared to those who did not have follow-up visits (Supplementary Table S2).

3.2. Cross-sectional association between baseline inflammatory biomarkers and EM status

Compared to LLFS participants from non-EM families, LLFS participants from EM families had a marginally higher monocyte count at baseline (b = 0.028, SE = 0.0110, p = 0.010) after adjusting for age, sex, education, and field site (Table 2). The results were attenuated (b = 0.027, SE = 0.0114, p = 0.016) after additionally adjusted for BMI and *APOE-ε2* (Table 2).

We found the association between EM status on baseline monocyte counts differed by sex (p-interaction = 0.003) but not by *APOE* status or BMI (data not shown). Stratified analysis showed that, after adjusting for age, education, field site, BMI, and *APOE*, male EM family members had higher baseline monocyte counts than male non-EM family members ($p < 0.0001$, Table 2), but among females, there was no difference between EM and non-EM family members on their baseline monocyte counts (Table 2).

Limiting analyses to the 2964 Offspring generation subjects found similar results (b = 0.031, SE = 0.0111, p = 0.006 in Model 1 and b = 0.028, SE = 0.0117, p = 0.016 in Model 2 for monocytes; not significant for other inflammatory biomarkers). The association between EM family status and baseline monocyte counts was significant for males (b = 0.050, SE = 0.0127, $p < 0.0001$ in Model 1, and b = 0.052, SE = 0.0127, $p < 0.0001$ in Model 2, Table 2) but not for females (Table 2). We found similar significant associations among men using monocyte count at follow-up visit (b = 0.042, SE = 0.012, $p < 0.001$) or using monocytes/HDL ratio (b = 0.032, SE = 0.016, p = 0.047), but not in women.

3.3. Longitudinal change of the inflammatory biomarkers and EM status

Over time, lymphocyte and platelet counts decreased significantly ($p < 0.0001$ for both) in the non-EM families but not in non-EM families, but the monocyte count increased in both non-EM and EM families (Table 3, Fig. 1). No significant change over time was observed for total WBC or neutrophil counts.

Table 1
Demographic and inflammatory characteristics of the EM and Non-EM families.

	Non-EM	EM	Total	*p* value*
Number of families	403	27	430	/
Number of subjects	3777	556	4333	/
Age, years, mean ± *SD*	71.72 (15.93)	66.26 (15.28)	71.02 (15.95)	<.001
Education[#], years, mean ± *SD*	11.57 (3.59)	12.05 ± 3.74	11.63 (3.61)	<.001
Females, N (%)	2081 (55)	305 (55)	2386 (55)	0.915
APOE-ε4 carriers (ε3/ε4 OR ε4/ε4)[#], *N* (%)	646 (18)	100 (19)	746 (18)	0.471
APOE-ε2 carriers (ε2/ε3 OR ε2/ε2)[#], *N* (%)	560 (15)	97 (19)	657 (16)	0.07
FLoSS score, mean ± *SD*	8.77 (7.71)	11.77 (7.45)	9.16 (7.74)	<.001
BMI[#], kg/m^2, mean ± *SD*	27.15 (4.9)	26.74 (4.4)	27.1 (4.84)	0.067
White blood cell count 10e9/L, mean ± SD; median (interquartile range)	6.27 (2.23); 6 (5–7.2)	6.10 (2.24); 5.8 (4.9–6.9)	6.25 (2.23); 5.9 (5.0–7.2)	0.090 0.013
Neutrophil count 10e9/L, mean ± SD; median (interquartile range)	3.59 (1.47); 3.4 (2.6–4.3)	3.41 (1.48); 3.2 (2.4–4.1)	3.57 (1.47); 3.4 (2.6–4.3)	0.009 0.001
Lymphocyte count 10e9/L, mean ± SD; median (interquartile range)	1.92 (1.52); 1.8 (1.4–2.2)	1.93 (1.56); 1.8 (1.4–2.2)	1.92 (1.52); 1.8 (1.4–2.2)	0.905 0.522
Monocyte count 10e9/L, mean ± SD; median (interquartile range)	0.54 (0.28); 0.5 (0.4–0.6)	0.55 (0.23); 0.5 (0.4–0.7)	0.54 (0.27); 0.5 (0.4–0.6)	0.324 0.058
Platelet count 10e9/L, mean ± SD; median (interquartile range)	236.47 (63.29); 230 (194–271)	233.68 (55.17); 229 (196–266)	236.11 (62.32); 230 (195–270)	0.333 0.587
hsCRP, mean ± SD; median (interquartile range)	3.50 (7.63); 1.46 (0.75–3.33)	3.24 (10.25); 1.27 (0.70–2.98)	3.46 (8.00); 1.74 (0.43–3.28)	0.499 0.014
IL6, mean ± SD; median (interquartile range)	2.25 (6.04); 1.00 (0.57–1.96)	2.04 (6.75); 0.81 (0.46–1.50)	2.22 (6.14); 0.97 (0.56–1.91)	0.458 <0.001
Longitudinal data	Non-EM	EM	Total	p value
Family number	328	24	352	/
Subjects	1889	338	2227	/
Duration from baseline to follow-up	7.78 (1.34)	7.98 (1.06)	7.81 (1.30)	0.01

*The p values were from *t*-test for continuous variables and chi-square test for categorical variables. Median levels of the inflammatory markers were compared using non-parametric Mann Whitney Wilcoxon Test. # Five and 92, 184 subjects had missing data on education, APOE status, and BMI, respectively.

In the multi-variable adjusted GEE models, we found EM status was not associated with the change of cell count for any cell type (Table 4). Limiting analyses to the Offspring generation only or excluding subjects who died within a half year after the follow-up visits did not change the results (data not shown).

Table 2
Cross-sectional association between inflammatory biomarkers and EM status.

All subjects	Model 1*			Model 2*		
Counts	B	SE	p	B	SE	p
WBC	−0.0003	0.0075	0.972	0.004	0.0074	0.609
Neutrophil	−0.003	0.0096	0.778	0.002	0.0099	0.845
Monocyte	0.028	0.0110	0.010	0.027	0.0114	0.016
Lymphocyte	−0.004	0.0107	0.727	−0.002	0.0111	0.888
Platelet	−0.007	0.0064	0.294	−0.004	0.0070	0.554
CRP	−0.028	0.0237	0.231	0.001	0.0206	0.956
IL6	−0.0117	0.0232	0.463	−0.003	0.0257	0.918
Stratified analysis by sex #	Model 1*			Model 2*		
Monocyte	B	SE	p	B	SE	p
Male	0.050	0.0127	<0.0001	0.052	0.0127	<0.0001
Female	0.010	0.0143	0.493	0.007	0.0149	0.663

Results from GEE models. Model 1, adjusted for age, sex, education. Model 2, adjusted for age, sex, education, field center, APOE ε2 status, and BMI.
In model 2: Interaction between sex and EM status was significant (p = 0.001).

Table 3
Longitudinal change of the inflammatory biomarkers from baseline to follow-up visit.

Annual change	Subjects	Mean	SD	P value
WBC	Non-EM	0.001	0.015	0.022
	EM	0.002	0.014	0.021
	All	0.001	0.015	0.003
Neutrophil	Non-EM	0.001	0.023	0.018
	EM	0.001	0.024	0.347
	All	0.001	0.023	0.011
Lymphocyte	Non-EM	−0.003	0.017	<0.0001
	EM	−0.001	0.014	0.148
	All	−0.003	0.016	<0.0001
Monocyte	Non-EM	0.006	0.020	<0.0001
	EM	0.005	0.019	<0.0001
	All	0.006	0.020	<0.0001
Platelet	Non-EM	−0.003	0.012	<0.0001
	EM	−0.001	0.011	0.171
	All	−0.002	0.012	<0.0001

P-values were from one sample *t*-test with null hypothesis of mean = 0.

4. Discussion

Our study adds to the growing body of research on the relationship between cognition and inflammation. We found that compared to non-EM families, EM families had higher monocyte counts at baseline in men, after controlling for multiple factors. The longitudinal change of the inflammatory markers did not differ by EM status.

Chronic inflammation leads to a wide array of health problems that comprise the leading causes of mortality, including cardiovascular diseases, cancer, and diabetes. Some risk factors for chronic inflammation include DNA damage, oxidative stress, diet, and psychological stressors (Furman et al., 2019). Aging also contributes to chronic systemic inflammation even without presence of an infection, a concept known as inflammaging. Some sources of inflammaging include the accumulation of damaged macromolecules and cells, higher gut permeability, continued cellular senescence, immunosenescence, and increased activation of the coagulation system (Franceschi and Campisi, 2014). Inflammaging is related to immune system aging and interacts with the nervous system, producing both neuroinflammation and systemic inflammation (Liang et al., 2017). In addition, the rate of progression of inflammaging has been shown to be a risk factor for morbidity and mortality in older adults (Fulop et al., 2018). Interestingly, long-lived individuals exhibit anti-inflammaging, which may help them counter inflammaging with an anti-inflammatory response (Franceschi et al., 2007).

In the LLFS cohort, EM families had a significantly higher monocyte

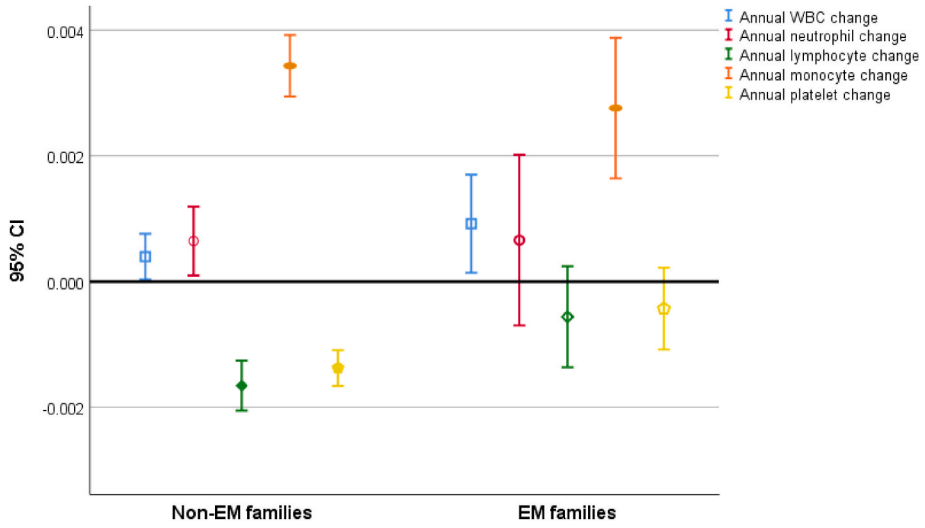

Fig. 1. Annual change of inflammatory biomarkers by exceptional memory status. Solid marks indicate statistically significant change over time. EM: exceptional memory.

Table 4
Longitudinal association between inflammatory biomarkers and EM status.

Annual change	Model 1			Model 2		
	B	SE	P	B	SE	p
WBC	0.001	0.0007	0.105	0.001	0.0007	0.070
Neutrophil	0.000	0.0013	0.460	0.001	0.0014	0.544
Lymphocyte	0.001	0.0009	0.276	0.001	0.0010	0.424
Monocyte	−0.001	0.0011	0.187	−0.001	0.0011	0.224
Platelet	0.001	0.0008	0.089	0.002	0.0009	0.075

Results from GEE models. Model 1, adjusted for age, sex, education. Model 2, adjusted for age, sex, education, field center, APOE ε2 status, and BMI.

count at baseline compared to non-EM families after adjustments for age, sex, education, and field site. Macrophages play a role in neurological repair but can also be harmful in response to various pathological states (Minogue, 2017). In the brain, macrophage polarization, or their production of functional phenotypes in response to a stimulus, impacts their activity and determines whether they will be involved in the inflammatory response or the resolution of inflammation (Minogue, 2017). The peripheral system may also play an essential role in clearing Aβ from the brain, and it has been estimated that about 40–60% of Aβ generated in the brain is actually cleared in the periphery (Xiang et al., 2015). As the counterparts of microglia in the periphery, blood monocytes seem to be even more effective in neuroinflammation regulation and Aβ clearance than microglia in AD. (Simard et al., 2006; Koronyo et al., 2015) Recent studies further found that Aβ uptake by monocytes in the periphery decreased during aging and further decreased in AD. (Chen et al., 2020) In AD transgenic mice, restriction of monocyte migration into the brain or monocyte ablation resulted in increased Aβ burden while addition of monocytes into the blood resulted in decreased Aβ pathology (Zuroff et al., 2017). Overall, monocytes might play a critical role in the clearance of brain-derived Aβ in the periphery, thus contributing to a more preserved cognitive performance by preventing pathological deposition of Aβ.

Our finding that EM families have higher monocyte counts thus is consistent with the evidence, (Minogue, 2017) suggesting that higher peripheral monocytes might play a positive role in maintaining cognition in older adults. Interestingly, we found the association between monocyte counts and EM status was mainly observed in men but not in women. A pooled analyses from five large cohorts including more than 26,000 individuals found sex differences in cognitive decline, with women having faster decline in global cognition and executive function but similar memory decline compared to men. (Levine et al., 2021) however, few studies have examined the sex difference in the association between monocytes and cognition. Differences in monocyte subsets have been reported between men and women, which may be due to the effect of sex hormones such as estrogen (Patel and Yona, 2019). Gender differences have also been observed in cytokine production and monocyte cytotoxic activity (Patel and Yona, 2019). Further studies are needed to confirm and explain the results.

Consistent with the report from a large Italian study with 40,987 individuals [46.2% males, mean (±SD) age: 50.7 (±17.5) years], (Biino et al., 2013) we found platelets were negatively correlated with age, especially in non-EM families. Furthermore, our longitudinal data also suggest a continued decline of platelet count during the follow-up among the non-EM families, although it remained stable in the EM families. Platelet granules carry molecules such as epidermal growth factor, vascular endothelial growth factor, transforming growth factor-β, as well as histamine and serotonin, which promote neurogenesis (Leiter and Walker, 2019). Reduction in neurogenesis is seen in neurodegenerative diseases such as AD and Parkinson's disease (Ma et al., 2017). Thus, the stable count of platelets in EM families but a decreased count of platelets in non-EM families may suggest cognitive benefits of more stable, better maintained platelet count among older adults. Further studies are warranted to clarify the role of platelets in cognition.

We did not find significant difference on the status or change of other inflammatory biomarkers in the study. There have been mixed findings on the relationship between these inflammatory biomarkers and cognition. A study examining peripheral immune profiles in 81 amyotrophic lateral sclerosis patients [48 males; mean (±SD) age: 54.9 (±11.2) years] showed decreased T lymphocytes, $CD4^+$ T lymphocyte, $CD8^+$ T lymphocyte, and B lymphocyte in patients with cognitive impairment compared to those without cognitive impairment (Yang et al., 2021). However, in 43 Parkinson's Disease patients [31 males; mean (±SD) age: mean (±SD) age: 68.9 (±8.4) years], cognitive impairment was associated with increased circulating lymphocytes (Magistrelli et al., 2020). A recent study with 161,968 participants [49% males; mean (±SD) age: 62.14 (4.07) years] from the UK Biobank found higher CRP and neutrophils, but not any other cell types, were associated with increased risk of incident dementia (Zhong et al., 2023). In mouse models, extravasated neutrophils contributed to cognitive impairment and AD pathogenesis while depletion of neutrophils led to memory improvements (Zenaro et al., 2015). Neutrophil percent has been found to be higher in AD and MCI patients compared to controls, and neutrophil phenotype may be related to rate of cognitive decline

(Dong et al., 2019). However, we did not observe any difference in neutrophil counts between those with and without EM. In 329 cognitively normal older Mexican-American participants [19% males; mean (±SD) age: 58.7(6.5) years] of the HABLE cohort, high CRP levels were associated with worse performance on the verbal fluency, while no association was found with performance on other cognitive measures including logical memory (Vintimilla et al., 2019). As logical memory was used to classify families by EM status in LLFS, our finding that there was no difference in hsCRP between EM and non-EM families is somewhat consistent with the HABLE study findings. The Whitehall II study, with 5217 participants [72% males, age 52–79 years] from mid-aged British civil service employees, showed that IL6 but not CRP was associated with worse cognitive score measured by Mini-Mental State Examination (Singh-Manoux et al., 2014). Overall, there are inconsistent results regarding each individual inflammatory biomarkers and cognition and future studies are needed to clarify their role in cognition among older adults.

Our study has several strengths. While there is a growing body of studies on the relationship between cognition and inflammation, the LLFS cohort allowed us to examine this association cross-sectionally and longitudinally in families with exceptional survival with a large sample size. Our analyses were adjusted for age, sex, education, field site, BMI, and APOE genotype. There are a few limitations in the present study. The threshold for EM status was based strictly on a $z = -1.5$ SD cutoff, so families would have been categorized differently with either a less stringent or more conservative cutoff (Barral et al., 2017b). EM status was also based on performance on a logical memory test and therefore did not incorporate other domains of cognition. Additionally, individuals were classified as belonging to an EM family or not regardless of their cognitive status to maintain the classification used in prior LLFS analyses (Barral et al., 2013). LLFS studies have found that different families have different healthy aging endophenotypes, and our analyses focused on the memory endophenotype clustering specifically (Marron et al., 2019). Performance may have been affected by deficits not related to episodic memory, e.g., hearing or attentional impairment. Additionally, the FLoSS score was used to recruit LLFS families and does not reflect the ultimate survival of participants in the study (Sebastiani et al., 2009). However, a previous LLFS study did find a better survival of LLFS participants compared to age- and sex-matched sporadic long-livers (Galvin et al., 2020). Nevertheless, still a large proportion of participants did not have follow-up visits due to death or other events, and those who did not have follow-up visits were older and had lower FLOSS and education. Therefore, our longitudinal analysis results may have been biased. Evidence on the relationship between the novel biomarker glycoprotein acetyls (GlycA) and cognition show mixed results (Slaney et al., 2023). Additionally, different subtypes of lymphocytes, as well as the balance between subtypes may affect cognitive outcomes (Yuan et al., 2023). We can't rule out the possibility that some subtypes of lymphocytes may be associated with EM status. Thus, our study is limited by the available biomarkers measured, and future studies may want to further investigate the relationship between other biomarkers such as GlycA or subtypes of lymphocytes and cognition. The results of our study may have limited generalizability, as the LLFS cohort participants are mostly non-Hispanic whites and participants were from families with exceptional longevity. While the current study supports the importance of systemic inflammation in cognitive health among older adults, further studies are warranted about evaluating and confirming whether monitoring peripheral immunity may help early detection of cognitive decline in clinical settings. Our study was also limited by having only two time points for the longitudinal analyses of cells and no repeated measures of CRP and IL6. Future studies may want to extend this longitudinal analysis by adding repeated measurement of these biomarkers.

5. Conclusion

In the current study, we found that compared to than non-EM families, EM families had higher monocyte counts at baseline, especially in men. The cell counts change over time were not different between EM and non-EM families, though. Further larger size and longitudinal studies are needed to better understand the relationship between inflammation and cognition.

CRediT authorship contribution statement

Ruhee Patel: Writing – original draft, Investigation, Conceptualization, Validation. **Stephanie Cosentino:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing, Data curation, Methodology, Validation. **Esther Zhiwei Zheng:** Data curation, Project administration, Writing – review & editing, Investigation. **Nicole Schupf:** Writing – review & editing, Investigation, Methodology, Resources. **Sandra Barral:** Investigation, Methodology, Resources, Writing – review & editing. **Mary Feitosa:** Investigation, Writing – review & editing, Methodology, Resources. **Stacy L. Andersen:** Investigation, Methodology, Writing – review & editing, Resources. **Paola Sebastiani:** Investigation, Methodology, Writing – review & editing, Resources. **Svetlana Ukraintseva:** Investigation, Writing – review & editing, Methodology, Resources. **Kaare Christensen:** Investigation, Writing – review & editing, Funding acquisition, Methodology, Resources. **Joseph Zmuda:** Investigation, Writing – review & editing, Funding acquisition, Project administration, Resources, Methodology. **Bharat Thyagarajan:** Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing, Project administration. **Yian Gu:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing – review & editing, Methodology, Validation, Visualization, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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