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Association of Cytomegalovirus Serostatus with ELOVL2 Methylation: Implications for Lipid Metabolism, Inflammation, DNA Damage, and Repair Capacity in the MARK-AGE Study Population

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Abstract

Cytomegalovirus (CMV) infection has been linked to accelerated biological aging, potentially increasing the risk of cardiovascular disease. DNA methylation of the gene Elongation Of Very Long Chain Fatty Acids-Like 2 (*ELOVL2*) is a molecular biomarker for aging, and its gene product is involved in polyunsaturated fatty acid synthesis, which impacts immune and inflammatory responses. This study, conducted in the MARK-AGE population, aimed to investigate the relationship between CMV infection and ELOVL2 methylation in adults aged 35-75, as well as the influence of CMV IgG levels on lipid metabolism, inflammation, DNA damage, and DNA repair.

Our data revealed a higher prevalence of ischemic heart disease, atrial fibrillation, hypertension, and diabetes in CMV-positive individuals. CMV IgG levels were positively associated with *ELOVL2* methylation at specific CpG sites and with increased expression of DNA methyltransferase-1 (DNMT1). CMV IgG was linked to lipid imbalances, such as increased BMI, VLDL-cholesterol, triglycerides, and HDL1-cholesterol. Additionally, *ELOVL2* methylation was associated with systemic inflammation markers and altered T-cell subsets. A negative correlation was observed between CMV IgG levels and both baseline DNA integrity and repair capacity.

These results suggest that CMV infection may promote cardiovascular disease through *ELOVL2* hypermethylation, lipid dysregulation, inflammation, and DNA damage.

Keywords: CMV, aging, *ELOVL2*, inflammation, lipid metabolism

1. Introduction

Chronic viral infections have been shown to accelerate biological aging through epigenetic changes. This phenomenon has been observed in patients with human immunodeficiency virus (HIV) infection, revealing an average age acceleration of 5.2 years (Horvath and Levine, 2015). The impact of cytomegalovirus (CMV) infection on epigenetic aging was first described by Kananen and coauthors (Kananen et al., 2015), who found accelerated aging in CMV-positive young individuals aged 20-30 years, as well as in the elderly aged 90 years and older. These findings have been confirmed by other researchers (Bacalini et al., 2017; Poloni et al., 2022).

One of the most robust molecular biomarkers for biological age is the methylation of the Elongation Of Very Long Chain Fatty Acids-Like 2 (*ELOVL2*) gene promoter, which increases linearly with age (Bacalini et al., 2017; Garagnani et al., 2012). The *ELOVL2* gene encodes a transmembrane protein involved in the elongation of long-chain fatty acids and the synthesis of polyunsaturated fatty acids (PUFAs), which play key roles in immune and inflammatory responses (Jakobsson et al., 2006). ELOVL2 may contribute to aging at the cellular level by altering lipid metabolism (Ali et al., 2023; Chao and Skowronska-Krawczyk, 2020; Li et al., 2022). Knockout of the *ELOVL2* gene in mice has been shown to cause male infertility, altered serum levels of PUFAs (Zadravec et al., 2011), insulin resistance, and dysregulated lipid metabolism (Cruciani-Guglielmacci et al., 2017; Pauter et al., 2014).

Increased *ELOVL2* methylation with age may reduce its expression, contributing to dyslipidemia, diabetes, or other cardiovascular risk factors (Cruciani-Guglielmacci et al., 2017; Li et al., 2022).

In fact, decreased *ELOVL2* expression leads to a decline in PUFA synthesis and the accumulation of short fatty acids, including PUFA precursors, in the endoplasmic reticulum (ER). This alteration affects mitochondrial energy metabolism and results in chronic ER stress and mitochondrial dysfunction (Li et al., 2022), which are involved in the development and progression of cardiovascular diseases (Navas-Madroñal et al., 2023). PUFA synthesis, regulated by ELOVL2, also modulates immune response and inflammation, which are critical factors in the aging process and cardiovascular health (Buckley et al., 2014; Oishi et al., 2017) .

Based on these observations, the aim of this study is to investigate the relationship between CMV infection and *ELOVL2* methylation in community-dwelling adults aged 35-75 years enrolled in the MARK-AGE project, and the influence of *ELOVL2* methylation on lipid metabolism, inflammation, DNA damage and repair.

2. Materials and methods

2.1. Study population, recruitment, data and blood collection

This cross-sectional study included 1992 RASIG (Randomly recruited Age-Stratified Individuals from the General population) participants within the age bracket of 35 to 75 years. These subjects were recruited as part of the European MARK-AGE project (Bürkle et al., 2015; Moreno-Villanueva et al., 2015a).

The details of the recruitment procedures, along with the collection of anthropometric, clinical, demographic, and laboratory parameter data, have already been reported (Jansen

et al., 2015; Moreno-Villanueva et al., 2015b, 2015a). Health status and medical history were evaluated on the basis of detailed questionnaires (Moreno-Villanueva et al., 2015a). Plasma isolation procedure from blood, as well as shipment and distribution of biological samples have been described (Moreno-Villanueva et al., 2015a). Briefly, lithium heparin plasma was prepared from whole blood, obtained by phlebotomy after overnight fasting, and subsequently stored at −80 °C. Samples were then shipped from the various recruitment centers to the MARK-AGE Biobank located at the University of Hohenheim, Stuttgart, Germany. From the biobank, coded samples were subsequently sent to the IRCCS INRCA on dry ice, where they were stored at -80°C until use.

2.2. CMV IgG antibody titer

CMV-specific IgG in serum were measured using DRG Cytomegalie Virus (CMV) IgG ELISA Kit according to the manufacturer's specifications (DRG International Inc., U.S.A.). CMV IgG levels >11 U/mL were considered positive.

2.3. Measurement of *ELOVL2* Methylation

The EpiTYPER assay (Sequenom) was used to quantitatively measure the methylation level of individual CpG sites in *ELOVL2*, as previously described (Bacalini et al., 2017). The amplified regions were chr6:11,044,680-11,045,053 (GRCh37/hg19) within the *ELOVL2* locus. *ELOVL2* amplicons included probes: cg16867657, cg24724428, cg21572722, cg16323298 from the Infinium 450k design. PCR products were processed following manufacturer's instructions to measure the percentage of methylation of single CpG sites or of group of adjacent CpG sites (indicated as CpG units), depending on the genomic sequence. For each locus, CpG site/units with more than 20% missing values and samples with missing values in more than 20% of the CpG site/units were excluded from the analysis

(Fig 1S Supplementary material). It has been also calculated the mean methylation value of *ELOVL2* CpG sites assessed (from CpG_2.3 to CpG_34.35.36) (Bacalini et al., 2017).

2.4. Lipid profile analysis

The cholesterol and triglyceride content in serum lipoprotein fractions (HDL, LDL, VLDL) and subfractions (HDL2, LDL2, VLDL2) were measured using nuclear magnetic resonance (NMR) spectroscopy (Bruker Biospin), as previously described (Heijmans et al., 2006; Vaarhorst et al., 2011). Each measurement generates signal amplitudes from lipoprotein subclasses, enabling the estimation of both total lipoprotein particle concentration and subclass distribution, including small particles (HDL2, LDL2, VLDL2) (Giacconi et al., 2019).

2.5. Cell phenotyping

Lymphocyte subsets in PBMC samples from MARK-AGE biobank were analyzed by FACS as previously described (Dudkowska et al., 2017). Briefly, we used the BD Multitest™ IMK kit (340503) to identify and determine the percentage and absolute counts of the following mature human lymphocyte subsets in PBMC: T lymphocytes (CD3+), B lymphocytes (CD19+), T lymphocytes (CD3+CD4+ and, CD3+CD8+) and natural killer (NK) lymphocytes (CD3-CD16+ and/or CD56. Samples were recorded and analyzed on the FACS Calibur (Beckton-Dickinson, Warsaw, Poland) using the Cell-Quest software (BD) and Multiset software (Beckton-Dickinson), respectively.

2.6. Cytokine determination

BD CBA Human Flex set" and the "BD FACS Array" (BD Biosciences, USA) were used to quantify specifically and simultaneously concentration of the plasma cytokines (IL-1β, IL-6, IL-10, IL-12p70, TNF-α, and IFN-γ).

2.7. Quantification of DNA strand breaks and DNA repair

We used the automated FADU assay (Fieres et al., 2022; Morath et al., 2014; Moreno-Villanueva et al., 2009) a very sensitive in vitro method, to assess DNA strand breaks and DNA repair. The detection of DNA strand breaks and repair is based on progressive DNA unwinding (denaturation) under highly controlled conditions of alkaline pH, time, and temperature. The starting points for the unwinding process are DNA strand interruptions, such as replication forks or chromosome ends, but also DNA strand breaks induced by reactive oxygen species (ROS), irradiation, or chemical compounds. For monitoring alkaline DNA unwinding, a commercially available fluorescent dye (Sybr®Green, Invitrogen, Darmstadt, Germany) is used as a marker for double stranded DNA remaining after termination of the alkaline conditions. Fluorescence intensity is expressed as a percentage of fluorescence in control cells without induced DNA damage (P0). A decrease in the fluorescence intensity of Sybr®Green indicates an increase of DNA unwinding and, consequently, a higher number of DNA strand breaks. Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood according to the density gradient principle using Biocoll® (Biochrom AG, Berlin, Germany), counted using a cell counting device (Casy® counter), pelleted (5 min, 200 *g*), and resuspended in RPMI-1640 medium (Invitrogen) containing 100 U/ml penicillin (Invitrogen) and 100 mg/ml streptomycin (Invitrogen) at 5×10^5 cells/ml. Then several 100-µl aliquots of cell suspension were irradiated on ice with 3.8 Gy (dose rate 1.9 Gy/min) using an X-ray generator (C.H.F. Müller, Hamburg, Germany, 70 keV, 1-mm Al filter). To allow DNA repair, cells were incubated in a CO_2 incubator at 37°C for 40 minutes and subsequently transferred to the pipetting robot for the FADU assay. DNA repair was analyzed by the FADU assay.

2.8. Statistical analysis

Subject characteristics were reported as mean ± standard error of the mean (SEM) or percentages for continuous and categorical variables, respectively. For continuous

variables, normal distribution was verified by the 1-sample Kolmogorov–Smirnov test. All the variables not normally distributed were log-transformed. Differences among groups were checked by One-way Analysis of Variance for continuous variables and Pearson's χ^2 test for categorical variables. We performed stepwise linear regression analyses to examine the associations between ELOVL2 CpGs (from CpG_2.3 to CpG_34.35.36) and CMV IgG antibody levels, adjusting for age, gender, and country. Linear regression models were also performed to search for an association between CMV IgG antibody titer and *DNMT1* and *DNMT3* mRNA levels or lymphocyte subsets after adjusting for age, sex and country in the RASIG population. Multivariate stepwise linear regression analysis was performed to explore the associations between CMV IgG levels (dependent variable), lipid profile and laboratory parameters. The following variables were included in the analyses: age, sex, country, BMI, Charlson comorbidity index (CCI), total cholesterol, HDL-cholesterol, HDL1-cholesterol, HDL2-cholesterol, LDL-cholesterol, LDL1-cholesterol, LDL2-cholesterol, total triglycerides, HDL-triglycerides, HDL1-triglycerides, HDL2-triglycerides, LDL-triglycerides, LDL2-triglycerides, LDL1-triglycerides, VLDL-cholesterol, VLDL1-cholesterol, VLDL2-cholesterol, VLDL-triglycerides, VLDL1-triglycerides, VLDL2-triglycerides, leukocyte count, neutrophils, lymphocytes, monocytes, platelets, albumin, CRP, free fatty acids, uric acid, insulin, fasting glucose, HbA1c, ceruloplasmin, alpha-2 macroglobulin, creatinine, fibrinogen, adiponectin. A linear regression model, adjusted for age, sex, and country, was performed to examine the association between mean *ELOVL2* methylation and lymphocyte subsets in the RASIG population. All the analyses were performed using the SPSS/Win program (version 27.0; Spss Inc., Chicago, IL).

3. Results

3.1. Characteristics of the RASIG population divided based on CMV positivity

The characteristics of the study population are summarized in Table 1. CMV positive subjects were older than CMV negative ones and showed a lower proportion of males. CMV positive subjects also displayed an increased BMI and lymphocyte count. The other laboratory parameters showed no significant differences. Moreover, the prevalence of ischemic heart disease (IHD), hypertension, atrial fibrillation and diabetes was significantly higher in the CMV positive group compared to the CMV negative group ($p<0.001$).

An adjusted logistic regression, accounting for age, sex, country, smoking, diabetes, hypertension, and dyslipidemia, confirms the association between CMV positivity and IHD (Supplementary Table 1S). Similar adjusted logistic regression models also confirm the association of CMV positivity with diabetes and hypertension (Supplementary Tables 2S and 3S).

3.2. Multivariate stepwise linear regression analysis for *ELOVL2* CpG site methylation independently associated with CMV IgG antibody levels in the RASIG population

Linear regression analysis using the stepwise method was carried out to explore the main predictors of CMV IgG antibody levels. The variables included were: age, sex, country and all *ELOVL2* CpGs from CpG_2.3 to CpG_34.35.36. A positive association was found with *ELOVL2* CpG_18.19.20.21 and *ELOVL2* CpG_6.7, while *ELOVL2* CpG_34.35.36 was negatively associated with CMV IgG levels (Table 2). An association was also observed between CMV IgG antibody titer, country, and sex (Table 2). In particular, the lowest CMV IgG titer was observed in Germany, while Poland, Italy, and Greece showed the highest values (Supplementary table 4S). Moreover, females had higher CMV IgG levels than males (Supplementary table 4S). After sex stratification, the association between CMV IgG and both *ELOVL2* CpG_18.19.20.21 and *ELOVL2* CpG_34.35.36 was confirmed in both sexes. Additionally, a negative association between CMV IgG and *ELOVL2* CpG_30.31.32 was

observed in females, while a positive association between CMV IgG and *ELOVL2* CpG_28.29 was found in males (Supplementary Table 5S).

3.3. Multivariate linear regression analysis between CMV IgG antibody levels and DNA methyltransferases in the RASIG population

A linear regression model, adjusted for age, sex, and country, identified a positive association between CMV IgG antibody levels and DNMT1 (β Coefficient = 0.336, $p<0.001$) and a negative association with DNMT3 (β Coefficient = -0.168, $p<0.05$) (Table 3).

3.4. Association between CMV IgG antibody levels, lipid profile and laboratory parameters.

Multivariate stepwise linear regression analysis was performed to explore the associations between CMV IgG levels (dependent variable) lipid profile and laboratory parameters.

We found a positive association with VLDL-cholesterol, HDL1-cholesterol, BMI, lymphocyte count, total triglycerides, α_2- macroglobulin, CCI. A negative association was observed between CMV IgG levels, VLDL-triglycerides, LDL2-triglycerides and HDL-cholesterol (Table 4).

3.5. Association between *ELOVL2* methylation with lymphocyte subsets and inflammatory markers in RASIG population

In a subgroup of 1,489 participants with a mean age of 55.1 ± 12.8 years, comprising 755 females and 734 males, we explored the association between the mean *ELOVL2* methylation of all CpG units (from CpG_2.3 to CpG_34.35.36) and lymphocyte subsets. Interestingly, a negative association with CD4 lymphocytes and a positive association with CD8 cells were observed. No association was found between *ELOVL2* methylation and NK cells or B cells (Table 5). Regarding the relationship between *ELOVL2* methylation and inflammation in the whole population, we found a positive correlation with the CRP to

albumin ratio (CAR), CRP, fibrinogen, homocysteine, and α_2-macroglobulin (Supplementary Table 6). In a subgroup of 207 RASIG subjects, we measured several cytokines (IL-1β, IL-6, IFN-γ) and the chemokine MCP-1, all of which showed a positive correlation with the mean *ELOVL2* methylation of all CpG units (Supplementary Figure 2S).

Association between CMV IgG levels baseline DNA integrity and DNA repair capacity in PBMCs

Baseline DNA integrity and repair were assessed in PBMCs from RASIG participants (N=1,710 subjects, 862 females and 848 males, mean age: 55.3 ± 11.4 years) using the automated FADU assay. In Fig. 1 (A) it is shown that higher CMV IgG levels were associated with reduced intact DNA, reflecting increased baseline DNA damage (Linear regression, β = -0.063; p = 0.012, adjusted for age, sex, and country). In Fig. 1 (B), DNA repair capacity following 3.5 Gy X-irradiation is reported across percentiles of CMV IgG levels. Elevated CMV IgG levels were linked to impaired DNA repair (Linear regression, β = -0.058; p = 0.020, adjusted for age, sex, and country). The negative values of DNA repair are likely due to very high DNA damage after irradiation that overwhelms the repair machinery, preventing the expected increase in fluorescence as DNA is repaired. This suggests a reduced DNA repair capacity, particularly in individuals with higher CMV IgG levels.

4. Discussion

Recent studies indicate that chronic viral infections can accelerate epigenetic aging (Oltmanns et al., 2023; Poloni et al., 2022). The epigenetic age acceleration is linked to an increased risk of cardiovascular disease (CVD) and all-cause mortality (Ammous et al., 2021; Hillary et al., 2020; McCrory et al., 2021; Wang et al., 2021). Poloni (Poloni et al., 2022) found that CMV-positive adults have a higher average epigenetic age compared to CMV-seronegative individuals. Large population studies have also shown a significant

association between CMV seropositivity and an elevated risk of CVD (Gkrania-Klotsas et al., 2012; Savva et al., 2013; Spyridopoulos et al., 2016), though there are conflicting results regarding the increased risk of CVD mortality (Chen et al., 2021; Chu et al., 2022; Spyridopoulos et al., 2016). In our study, we observed a higher prevalence of ischemic heart disease (IHD), atrial fibrillation (AF), hypertension, and diabetes among CMV-positive subjects compared to CMV-negative ones. We found a positive association between CMV IgG levels and the global *ELOVL2* methylation (mean of all CpG sites) with a more significant positive association with *ELOVL2* CpG_18.19.20.21 and *ELOVL2* CpG_6.7.

ELOVL2, in addition to being a biomarker for chronological age (Bacalini et al., 2017; Garagnani et al., 2012), is involved in the synthesis of PUFAs. PUFAs influence inflammation by acting as precursors of eicosanoids. Notably, eicosanoids derived from n-3 PUFAs have demonstrated anti-inflammatory effects and can lower the production of inflammatory cytokines, (Darwesh et al., 2019) modulate lipogenesis, ER stress, and mitochondrial dysfunction in chronic diseases (Okada et al., 2018). In addition, a recent meta-analysis demonstrates the beneficial effects of n-3 PUFAs on coronary heart disease (Xiao et al., 2024). Accordingly, in our cohort we found a positive association between *ELOVL2* methylation, markers of systemic inflammation (CRP/albumin ratio CRP, fibrinogen and homocystein) and certain plasma cytokines and chemokines (IL-1β, IL-6, IFN-γ, MCP-1).

CMV infection remodels the host lipidome, regulating fatty acid elongation and balancing SFAs, MUFAs, and PUFAs (Xi et al., 2019), also inducing several lipogenic enzymes involved in fatty acid synthesis and elongation, which are crucial for CMV replication (Koyuncu et al., 2013; Purdy et al., 2015; Spencer et al., 2011).

In vitro experiments have further demonstrated that knockdown of *ELOVL2* inhibits virus replication (Koyuncu et al., 2013). CMV induces *ELOVL7* to synthesize the saturated VLCFA required for membrane biogenesis and efficient virus replication (Purdy et al., 2015), while there is no evidence on the effect of CMV on *ELOVL2* expression.

In our study, we have demonstrated an association between CMV IgG levels, *ELOVL2* methylation and the expression of *DNMT1* and *DNMT3* genes, with implications for lipid profile modulation and inflammation. Some evidence shows that overexpression of DNMT1, a key enzyme in DNA methylation maintenance (Howell et al., 2001), is linked to reduced PPAR-γ and increased proinflammatory cytokines in peripheral blood monocytes of atherosclerosis patients (Yu et al., 2016). Additionally, disturbed blood flow upregulates DNMT, leading to genome-wide DNA methylation changes that alter endothelial gene expression, regulate vascular biology, and promote atherosclerosis (Dunn et al., 2015).

Other findings support the idea that DNMT1 may be upregulated by viral infections (Ksiaa et al., 2014; Youngblood and Reich, 2008), though specific studies on CMV are lacking.

Consistent with other research, we observed that CMV IgG levels were positively associated with BMI, VLDL-cholesterol, and total triglycerides, known risk factors for coronary atherosclerosis, while there was a negative association with HDL-cholesterol (Di Giorgi et al., 2021; Feng et al., 2023; Pita López et al., 2023).

Interestingly, we found a positive association with the HDL1-cholesterol subfraction, which has been reported to increase with age due to reduced hepatic catabolism (Bravo et al., 1994). Moreover, VLDL- and LDL2-triglyceride subfractions showed a negative correlation with CMV IgG levels. These findings strengthen the evidence for CMV's involvement in lipid metabolism, as highlighted by several studies (Gao et al., 2023; Xin and Zhou, 2024; Xi et al., 2019).

Previous evidence suggests that *ELOVL2* deficiency accelerates aging by disrupting metabolism, leading to the accumulation of short-chain fatty acids, ER stress, mitochondrial dysfunction, and increased inflammatory mediators (Li et al., 2022), all of which can cause DNA damage (Bauer and De la Fuente, 2016; Schank et al., 2021; Xiang et al., 2024). Notably, we observed a negative association between CMV IgG levels and both the baseline DNA integrity and DNA repair capacity. This may be attributed to low ELOVL2 expression, which increases ER stress and inflammation, but could also be a direct consequence of CMV infection.

Indeed, CMV infection has been shown to induce specific types of DNA damage (Smolarz et al., 2015), and an *in vitro* study demonstrated that infected fibroblasts have a reduced ability to repair cellular DNA damage (O'Dowd et al., 2012). This could explain the observed negative association between CMV IgG levels and DNA repair capacity. Other findings in our study include a negative correlation between the mean *ELOVL2* methylation at all CpG sites and CD4 lymphocytes, and a positive correlation with CD8 cells. These results partially align with previous research showing that *ELOVL2*-deficient mice exhibit a dysregulation in cytotoxic CD8+ T cells, which produce higher levels of pro-inflammatory cytokines (Talamonti et al., 2023).

Our study has some limitations, the most significant being its cross-sectional design, which only allows us to identify associations between CMV infection, *ELOVL2* methylation, markers of lipid metabolism, inflammation, and DNA damage. Consequently, we cannot establish causality or determine the direction of these associations. Longitudinal studies or experimental models are needed to confirm whether CMV infection directly triggers epigenetic modifications in the *ELOVL2* gene, contributing to lipid dysregulation and cardiovascular disease. Additionally, despite adjusting for multiple confounders, unmeasured factors may still have influenced our findings.

In conclusion, elevated CMV IgG levels are associated with *ELOVL2* hypermethylation, plasma lipid alterations, increased inflammation, heightened DNA damage, and reduced DNA repair capacity, all of which may contribute to the onset and progression of cardiovascular disease. Further studies are needed to clarify the mechanisms by which CMV influences *ELOVL2* methylation and lipid metabolism.

Ethics Approval and Consent to Participate

All information was accessed in accordance with the applicable laws and ethical requirements for the study period concerned, and was compliant with the Declaration of Helsinki. The Local Research Ethics Committees of the respective recruiting centers provided ethical approval for the MARK-AGE project, which was registered retrospectively at the German Clinical Trials Register (DRKS00007713; Ethics Committee No.: 2008-075-f, Ethik-Kommission bei der Landesärztekammer Baden-Württemberg). Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Robertina Giacconi: Conceptualization, Software, Original Draft Preparation, Writing – Review & Editing; Chiara Pirazzini: Data curation, writing–review and editing, Maria Giulia Bacalini: Data curation, writing–review and editing; Paolo Garagnani: supervision, and writing–review and editing; Claudio Franceschi: Recruitment of Subjects, Formal Analysis; Miriam Capri: Recruitment of Subjects , Formal Analysis; Carlo Fortunato: Writing – Review & Editing; Gretta Veronica Badillo Pazmay: Writing – Review & Editing, Alexander Bürkle: Project Administration, Funding Acquisition, Writing – Review & Editing; María Moreno Villanueva: Methodology, Software, Writing – Review & Editing, Maurizio Cardelli: Writing – Review & Editing; Monia Cecati: Writing – Review & Editing; Laura Cianfruglia: Writing – Review & Editing; Francesco Piacenza: Data Curation, Writing – Review & Editing; Martijn E. T. Dollé: Data Curation, Formal Analysis; Eugène Jansen: Data Curation, Formal

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Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Data availability

The datasets analyzed in this study are available from the corresponding author upon reasonable request.

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Table 1. Demographic and clinical characteristics of RASIG participants

	CMV negative **n. 685**	**CMV positive** **n. 1307**	**P value**
Age (yrs)	53.6±0.43	56.7±0.31	<0.001
Males n, (%)	376 (52.7%)	611 (44.3%)	<0.001
BMI (kg/m2)	25.7±0.2	26.5±0.1	<0.001
RBC ($x10^6$/ µL)	4.76±0.43	5.08±0.35	NS
WBC ($x10^3$/µL)	5.96±0.08	6.07±0.07	NS
Neutrophils ($x10^3$/µL)	3.43±0.06	3.35±0.05	NS
Lymphocytes ($x10^3$/µL)	1.85±0.03	2.02± 0.02	<0.001
Monocytes ($x10^3$/µL)	0.48±0.01	0.49±0.01	NS
Platelets ($x10^3$/µL)	236.3±2.2	236.2±1.8	NS
Albumin (g/dL)	4.07±0.01	4.08±0.01	NS
CRP (µg/L)	1.94±0.11	2.12±0.09	NS
TC (mmol/L)	5.50±0.04	5.56±0.03	NS
HDL (mmol/L)	1.51±0.01	1.52±0.01	NS
LDL (mmol/L)	3.28±0.03	3.31±0.03	NS
TG (mmol/L)	1.21±0.03	1.29±0.02	0.062
FG (mmol/L)	5.20±0.04	5.22±0.03	NS
HbA1c (%)	6.00±0.02	5.96±0.02	NS
Creatinine (µmol/L)	73.4±0.50	74.0±0.40	NS
IHD n, (%)	19 (2.6%)	79 (5.7%)	0.002

AF n, (%)	42 (5.9%)	118 (8.6%)	0.029
Hypertension n, (%)	114 (16.0%)	355 (25.7%)	<0.001
Diabetes n, (%)	18 (3.4%)	74 (5.2%)	<0.01
Depressive symptoms n, (%)	82 (11.7%)	173 (12.7%)	NS
Osteoporosis n, (%)	44 (6.2%)	127 (8.2%)	NS
Hypothyroidism n, (%)	58 (8.1%)	110 (9.2%)	NS

Data are reported as mean ± standard error of the mean (SEM). The laboratory parameter analysis was adjusted for age, gender, country. AF= Atrial fibrillation; BMI Body Mass Index; CRP = C-reactive protein; FG = fasting glucose; HbA1c = Hemoglobin A1c; HDL = high-density lipoprotein cholesterol; IHD= Ischemic heart disease; LDL = low-density lipoprotein cholesterol; RBC = red blood cells; TC = total cholesterol; TG = triglycerides; WBC = white blood cells.

Table 2. Multivariate stepwise linear regression analysis showing the association between ELOVL2-methylation and CMV IgG levels in the RASIG population.

	β coefficient	**95% C.I.**	**P value**
ELOVL2 CpG_18.19.20.21	1.223	0.818 to 1.628	<0.0001
ELOVL2 CpG_34.35.36	-1.116	-1.574 to -0.658	<0.0001
ELOVL2 CpG_6.7	0.465	0.191 to 0.738	0.001

Adjusted for age, sex and country

Table 3. Linear regression models of CMV IgG levels with DNMT1 and DNMT3 mRNA levels.

	Unadjusted model		Model 1		Model 2	
	***β* Coefficient (95% CI)**	***p* Value**	***β* Coefficient (95% CI)**	***p* Value**	***β* Coefficient (95% CI)**	***p* Value**
DNMT1	0.345 (0.176 to 0.514)	<0.001	0.336 (0.169 to 0.504)	<0.001	0.308 (0.144 to 0.472)	<0.001
DNMT3	-0.165 (-0.304 to 0.026)	0.020	-0.168 (-0.307 to -0.03)	0.017	-.161 (-0.297 to -0.026)	0.020

Model1: adjusted for age and sex; Model 2: adjusted for age, sex and country

Table 4. Multivariate stepwise linear regression analysis showing the associations between CMV IgG levels, lipid profile and laboratory parameters in the RASIG population.

	β coefficent	**95% C.I.**	**P value**
VLDL-Cholesterol	0.573	0.179 to 0.967	<0.01
HDL1-Cholesterol	0.949	0.443 to 1.456	<0.0001
BMI	0.045	0.024 to 0.066	<0.0001
Lymphocyte count	0.452	0.200 to 0.704	<0.0001
VLDL-Triglycerides	-1.067	-1.456 to -0.657	<0.0001
Total Triglycerides	2.040	-1.225 to 2.855	<0.0001
LDL2-Triglycerides	-0.539	-0.800 to -0.277	<0.0001
α-2macroglobulin	0.469	0.110 to 0.828	0.010
HDL-Cholesterol	-1.406	-2.470 to -0.341	0.010
CCI	0.127	0.017 to 0.237	0.024

Adjusted for age, sex and country
VLDL: Very low-density lipoprotein; LDL:Low-density lipoprotein; HDL: high-density lipoprotein; CCI: Charlson Comorbidity Index

Table 5. Linear regression model of mean ELOVL2 methylation with lymphocyte subsets in RASIG population

	β coefficient	95% C.I.	P value
CD16+ CD56+ NK cells	-0.005	-0.020 to 0.009	NS
CD19 + B-cells	-0.001	-0.013 to 0.012	NS
CD3 + T-cells	-0.020	-0.117 to 0.077	NS
CD4 + T-cells	-0.075	-0.111 to -0.038	<0.0001
CD8 + T-cells	0.065	0.044 to 0.086	<0.0001

Model: adjusted for age, sex and country

Figure 1. Baseline DNA integrity, DNA repair capacity according to percentiles of CMV IgG levels

Baseline DNA integrity and DNA repair was measured in PBMCs from RASIG participants using the automated FADU assay. **Fig.1 (A)** Increased CMV IgG levels was associated with lower intact DNA and thus higher baseline DNA damage (Linear regression, β = -0.063; p = 0.012 adjusted for age, sex and country).

Fig. 1 (B) DNA repair after 3.5 Gy X-irradiation according to percentiles of CMV IgG levels

Increased CMV IgG levels showed a decreased DNA repair capacity (Linear regression, β = -0.058; p = 0.020, adjusted for age, sex and country).

*p<0.05

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Figure

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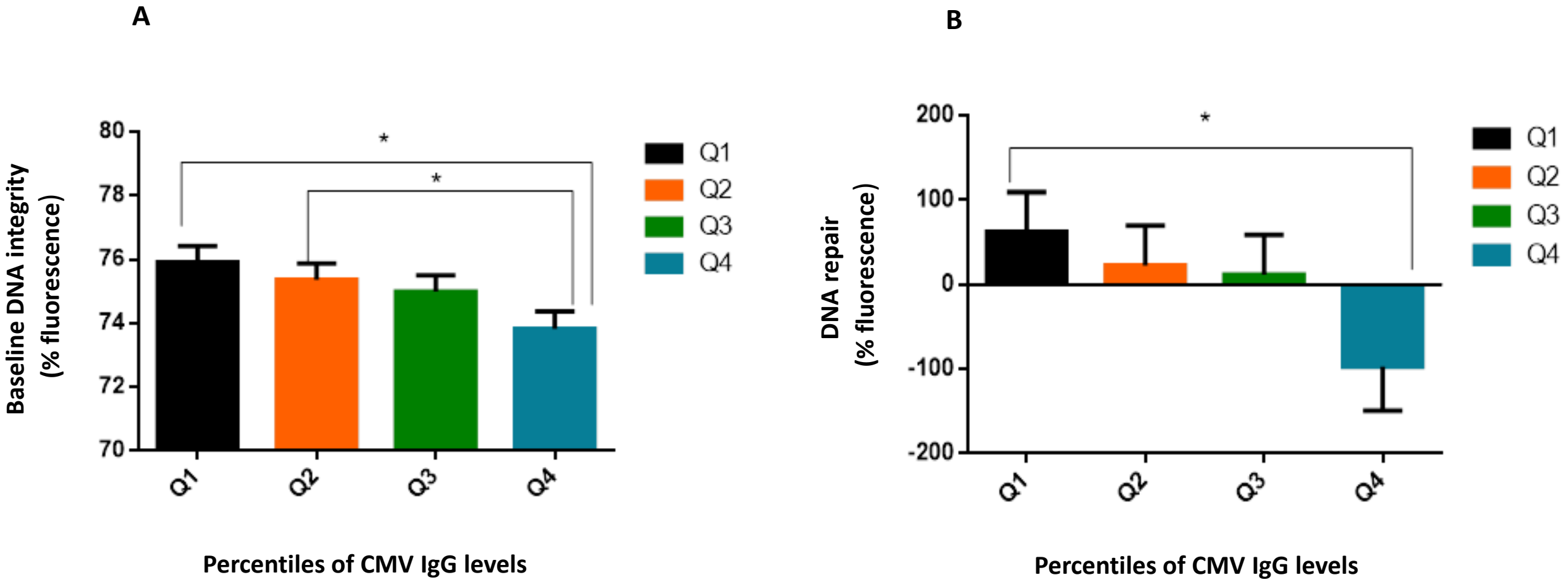

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Association of Cytomegalovirus Serostatus with ELOVL2 Methylation: Implications for Lipid Metabolism, Inflammation, DNA Damage, and Repair Capacity in the MARK-AGE Study Population

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