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Association of ceramide risk scores with rheumatoid arthritis: a FINRISK population-based cohort study

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ABSTRACT

Objectives This study aimed to explore the association between lipid-based Cardiovascular Event Risk Tests (CERT1 and CERT2), including ceramides (Cer) and phosphatidylcholine (PC) lipid species, and rheumatoid arthritis (RA), an inflammatory disease that can increase the risk of cardiovascular diseases.

Design Prospective population-based cohort study.

Setting Primary care centres across five geographical areas in Finland.

Methods The study included 7702 individuals (selected from the FINRISK cohort) who were assessed for the prevalence and incidence of RA. At baseline, the cohort included 7518 RA-free individuals, among whom 329 developed RA during the study, and 184 had a history of RA at baseline. Serum levels of ceramides and PC were measured using mass spectrometry, and CERT scores were calculated.

Main outcome measures Prevalence and incidence of RA, CERT scores, and serum lipid levels.

Results CERT scores were associated with prevalent RA but not with incident RA in the full cohort. Adjusted ORs and 95% CI for prevalent RA were 1.24 (95% CI 1.05 to 1.46) for CERT1 and 1.42 (95% CI 1.20 to 1.68) for CERT2. Stratified analyses showed that these associations were consistent among individuals over 50 years of age and across both sexes. The Cer (d18:1/16:0)/PC (16:0/22:5) ratio was significantly associated with RA in younger individuals (OR 1.66; 95% CI (1.26 to 2.18)). Overall, the association between lipids and RA was stronger in women than men.

Conclusions The study shows a significant association between prevalent RA and bioactive lipid species used for cardiovascular risk assessment. These findings emphasise the importance of considering residual inflammatory risks, such as RA, in cardiovascular risk evaluations in clinical settings.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The use of the FINRISK cohort enhances the generalisability of the findings to the broader population.
⇒ The precise measurement of ceramides and phosphatidylcholines enabled by high-throughput mass spectrometry.
⇒ Age and sex subgroups revealed significant associations that might be masked in non-stratified analysis.
⇒ Despite the large cohort, the relatively small sample size for rheumatoid arthritis (RA) analysis might affect the robustness of some findings.
⇒ The lack of data on RA severity limits the ability to draw insights into lipid levels and disease outcome.

INTRODUCTION

Cardiovascular Event Risk Tests (CERT1 and CERT2) can predict future cardiovascular disease (CVD) events in both primary and secondary prevention.[1] CERT2 is an enhanced version of CERT1, generating a risk score ranging from 0 to 12. This score is calculated based on the concentrations and lipid-lipid ratios of specific ceramide and phospholipid species. Risk charts using the score, along with the clinical characteristics, provide a practical means of assessing both the relative and absolute risks of CVD events.[2] Understanding the underlying pathophysiological mechanisms contributing to elevated CERT2 scores is of clinical interest, as it could enable more targeted preventive actions. However, the extent to which the CERT scores may also be associated with other chronic diseases remains poorly understood.

Rheumatoid arthritis (RA) is a chronic condition with systemic inflammation. People with RA have a 30%–50% greater likelihood of cardiovascular events and face higher rates of mortality and morbidity compared with the general population.[3 4]

Ceramides and other sphingolipids are dysregulated in diseases associated with inflammatory conditions, including RA, CVD, certain cancers, inflammatory bowel disease and neurodegenerative disorders.[5–8] Ceramides, essential sphingolipids in the plasma membrane, regulate inflammation by forming ceramide-rich domains like cholesterol-rich lipid rafts. These domains facilitate the assembly of signalling complexes, interact

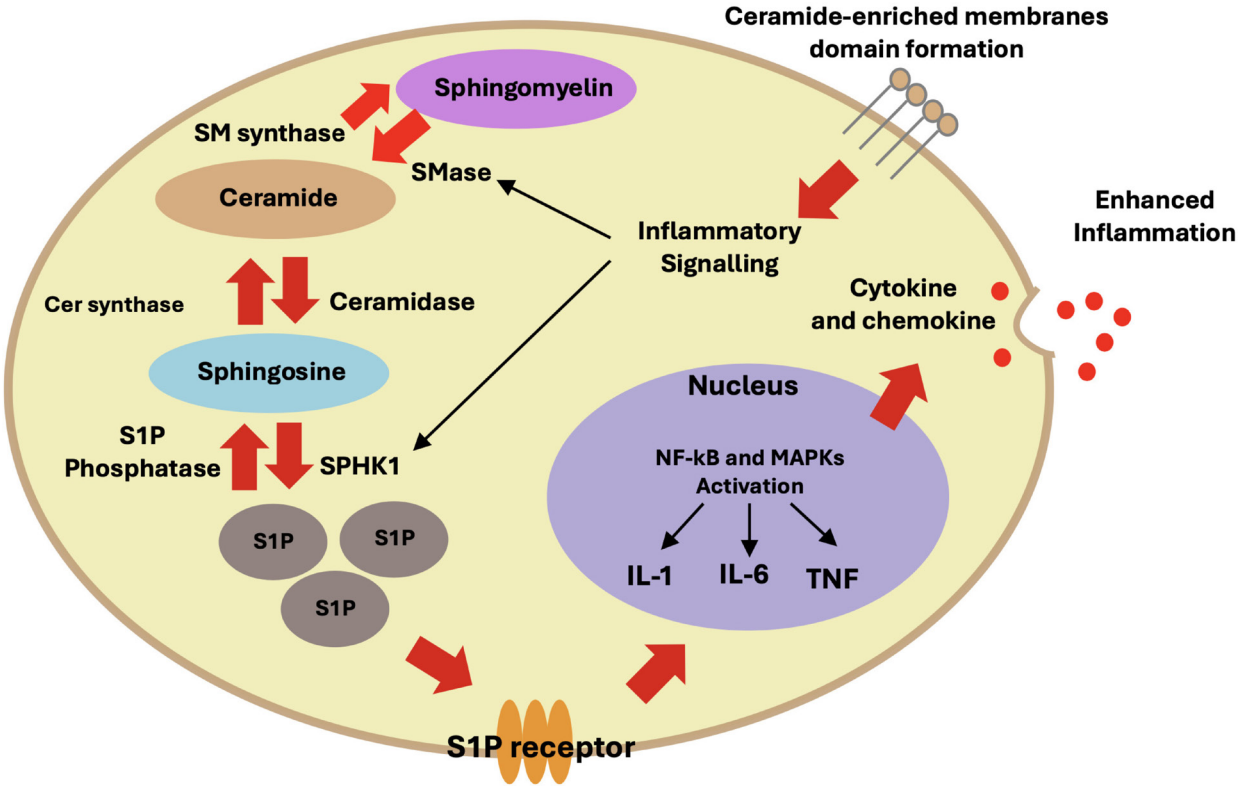

Figure 1 Biochemical pathways of sphingolipid metabolism and their role in inflammatory signalling in rheumatoid arthritis. The diagram illustrates the conversion of sphingomyelin to ceramide and subsequently to sphingosine and sphingosine-1-phosphate (S1P). Key enzymes such as sphingomyelinase (SMase), ceramidase, and sphingosine kinase 1 (SPHK1) are involved in these transformations. The interaction of S1P with its receptor initiates signalling cascades that activate nuclear factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinases (MAPKs), resulting in the production of inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF). This biochemical process highlights the critical pathways leading to enhanced inflammation, underscoring potential therapeutic targets for controlling rheumatoid arthritis.

with specific protein kinase-C isoforms and mediate inflammation-related intracellular pathways.[9–11] The formation of ceramides alone can initiate the release of pro-inflammatory cytokines like IL-1β and other inflammatory components, including tumour necrosis factor, interferon-gamma and platelet-activating factor.[12] These inflammatory factors can then boost ceramide production by turning on sphingomyelinases, which makes the overall inflammatory response stronger[13] (figure 1).

During inflammation, ceramide and sphingosine-1-phosphate (S1P) levels tend to increase, contributing to the inflammatory response. S1P is a lipid signalling molecule that influences a wide range of cellular functions[14 15] and binds to receptors involved in growth, angiogenesis, and apoptosis inhibition.[16 17] In autoimmune inflammatory rheumatic diseases, both S1P and its receptor (S1PR) are promising therapeutic targets.[16] However, certain lipid species, such as dihydroceramides and sphingosine, are considered anti-inflammatory.[18]

In earlier studies, we discovered significant associations between CERT2 lipid components and diabetes[1] and hypertension.[19] The present study explores the association between cardiovascular risk-linked ceramide and phosphatidylcholine (PC) species and RA.

MATERIALS AND METHODS

Study cohort and population representativeness

The FINRISK cohorts, initiated in 1972, comprise a series of comprehensive, population-based surveys conducted every 5 years in Finland. These surveys aim to identify factors associated with non-communicable diseases (NCDs) and their long-term consequences by systematically collecting data on CVD risk factors. The FINRISK cohorts provide invaluable insights into the epidemiology of CVD and other NCDs in the Finnish population.[20] The FINRISK study is based on random population samples obtained from the National Population Register, ensuring a good representation of the population in the study areas. Stratified random sampling was used to ensure balanced representation across age groups, genders and regions, with no exclusion criteria beyond the age range of 25–74 years.[21] Sample weights were not applied, as the random sampling approach provided adequate representation without further adjustments.

The 2002 FINRISK survey gathered information from a sample of 13 498 men and women aged 25–74 years from 5 geographical areas of Finland. The cohort comprised 6236 men (46.2%) and 7262 women (53.8%).[2 21] Details regarding the survey sample and methods have been

Table 1 Baseline characteristics of participants with prevalent (N=7702) (A) and incident (N=7511) rheumatoid arthritis (B)

(A)			
Variable	**People without RA (n=7518)**	**People with prevalent RA (n=184)**	**P value**
Age (year)	48.4 (37.2–58.2)	55.8 (46.6–63.6)	<0.001
Men	3556 (47.3%)	60 (32.6%)	<0.001
BMI (kg/m^2)	26.3 (23.6–29.4)	26.9 (24.0–30)	0.141
Waist circumference (cm)	88.5 (79.5–98.0)	89.5 (79.2–98.0)	0.837
Serum triglycerides (mmol/L)	1.18 (0.86–1.69)	1.14 (0.82–1.64)	0.433
Serum total cholesterol (mmol/L)	5.48 (4.84–6.21)	5.46 (4.95–6.20)	0.558
Serum HDL cholesterol (mmol/L)	1.45 (1.20–1.74)	1.49 (1.27–1.82)	0.019
Serum LDL cholesterol (mmol/L)	3.29 (2.71–3.90)	3.21 (2.72–3.80)	0.73
Serum hs-CRP (mg/L)	1.13 (0.52–2.50)	2.37 (0.77–5.67)	<0.001
Current smoking	1935 (25.7%)	32 (17.4%)	0.013
Systolic blood pressure (mm Hg)	131 (120–145)	139 (121–158)	<0.001
Diastolic blood pressure (mm Hg)	79 (71 – 86)	80 (72 – 87)	0.137
History of diabetes	434 (5.8%)	14 (7.6%)	0.374
History of lipid-lowering drug treatment	547 (7.3%)	26 (14.1%)	<0.001
History of blood pressure-lowering drug treatment	1075 (14.3%)	41 (22.3%)	0.003
(B)			
Variable	**Healthy controls (n=7182)**	**Incident RA group (n=329)**	**P value**
Age (year)	48.1 (37.0–58.1)	53.0 (41.5–61.9)	<0.001
Men	3416 (47.6%)	140 (42.6%)	0.085
BMI (Kg/m^2)	26.3 (23.6–29.4)	26.8 (23.8–30.1)	0.026
Waist circumference (cm)	88.5 (79.5–98.0)	90.0 (79.0–101.0)	0.025
Serum triglycerides (mmol/L)	1.18 (0.85–1.70)	1.19 (0.88–1.66)	0.661
Serum total cholesterol (mmol/L)	5.47 (4.83–6.21)	5.54 (4.96–6.19)	0.32
Serum HDL cholesterol (mmol/L)	1.45 (1.20–1.74)	1.44 (1.20–1.72)	0.96
Serum LDL cholesterol (mmol/L)	3.29 (2.71–3.90)	3.31 (2.79–3.85)	0.756
Serum hs-CRP (mg/dL)	1.12 (0.52–2.47)	1.41 (0.71–3.13)	<0.001
Current smoking	1841 (25.6%)	91 (27.7%)	0.449
Systolic blood pressure (mm Hg)	131 (120–145)	133 (121–149)	0.08
Diastolic blood pressure (mm Hg)	79 (71–86)	80 (73–87)	0.056
History of diabetes	402 (5.6%)	31 (9.4%)	0.005
History of lipid-lowering treatment	516 (7.2%)	30 (9.1%)	0.225
History of blood pressure-lowering treatment	998 (13.9%)	77 (23.4%)	<0.001

Data are presented as n (%) or median (IQR).
Significance value for p<0.05.
Data are presented as n (%) or median (IQR). BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein, compared using Mann-Whitney U tests and Chi-squared tests.
BMI, body mass index; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein.

previously published, highlighting the rigorous approach taken in gathering health data.[2 20] For the present prospective study, the follow-up period lasted 10 years. We defined two study approaches from the FINRISK 2002 cohort to investigate the prevalence and incidence of RA. The prevalence study included 7702 participants, comprising 7518 people without RA and 184 with a history of RA, while the incidence study approach included 7511 participants without RA at baseline, comprising 7182 people remaining free of RA and 329 who developed RA during the 10-year follow-up period.

Definition of prevalence and incident RA, and diagnosis criteria

The prevalence of RA refers to the total number of existing cases diagnosed within the population under study at the baseline examination. Incident RA refers to newly diagnosed cases of RA within the defined population who had

not been diagnosed with RA at baseline, but diagnosis of RA was confirmed during the 10-year follow-up period. The diagnoses of RA were based on data from two sources: the National Hospital Discharge Register (established in 1968) and the National Death Register. The diagnosis in the National Hospital Discharge Register is based on inpatient hospital care, with medical specialist evaluation and appropriate biomarker analyses. The diagnoses in various health registers in Finland can be ascertained using the national personal identification number assigned to all residents. In our study, both prevalence and incidence analyses were defined using the same reference group, comprising individuals without any medical history of RA. These individuals were identified through a physician's examination or by reviewing their medical history. Therefore, we can assume that there were virtually no false positive cases and very few false negative cases.

Laboratory measurements

Venous blood samples were collected, centrifuged on-site and serum was promptly frozen and transported weekly on dry ice to the Finnish Institute for Health and Welfare (THL) laboratory for analysis. Blood sampling was standardised to include a minimum fasting duration of 4 hours, with collection typically conducted between 11:00 and 19:00 hours. This standardised protocol minimises potential variation and aligns with the established procedures of the FINRISK cohort, as detailed in relevant FINRISK publications. A targeted tandem liquid chromatography-mass spectrometry method was used to measure four ceramides (Cer (d18:1/16:0), Cer (d18:1/18:0), Cer (d18:1/24:0) and Cer (d18:1/24:1)) and three PC (PC (14:0/22:6), PC (16:0/22:5) and PC (16:0/16:0)), using a validated approach from Zora Biosciences Oy.[2]

Cardiovascular Event Risk Test 1

CERT1 is a metric designed to assess ceramide levels and their respective ratios by comparing the concentrations of Cer (d18:1/16:0), Cer (d18:1/18:0) and Cer (d18:1/24:1) to Cer (d18:1/24:0). This evaluation offers insights into the specific ceramide components and their proportional relationships.[22] People with concentrations or ratios falling within the fourth quartile were assigned two score points, while those within the third quartile received one point. Participants falling within the first and second quartiles were assigned zero points. The CERT1 score was calibrated on a scale ranging from 0 to 12.[22] For an overview of how these assessments classify coronary heart disease risk among individuals, see online supplemental figure 1.

Cardiovascular Event Risk Test 2

To enhance CERT1 and integrate the predictive capabilities of PCs for heart disease,[2 23] an upgraded version known as CERT2 was introduced. In CERT2, one ceramide/ceramide ratio (Cer (d18:1/24:1)/(d18:1/24:0)) was included, along with two ceramide/PC ratios (Cer (d18:1/16:0) / PC (16:0/22:5) and Cer (d18:1/18:0)/(PC 14:0/22:6)), in addition to a single PC (PC 16:0/16:0). The scoring system for CERT2 ranges from 0 to 12 points, based on the accumulated points obtained by the individual, as shown in online supplemental figure 1.

Statistical analyses

We characterised the study population using medians in IQRs and counts while comparing the prevalent and incident RA groups using Mann-Whitney U tests and Chi-squared tests. The normality of the distribution of variables was assessed visually, and due to some of them being non-normally distributed, we used non-parametric methods for all of them. We also employed logistic regression models to explore association with prevalent RA and Cox regression models to examine the incidence of RA, with age as the time scale. All the regression model estimates were calculated per SD for CERT scores or per log-transformed SD. Using the locally estimated scatterplot smoothing method, we assessed the risk of prevalent RA, producing risk plots for selected variables, as shown in online supplemental figure 2. We stratified the analysis by age group (under 50 years old and 50 years or older). We also conducted sex-specific analyses to explore the association between the CERT scores and ceramide components with prevalent RA.

Adjustments were made to account for variables associated with both the exposure (CERT scores and their components) and the outcome (the prevalence or incidence of RA). For the prevalence models, these covariates included log-transformed age, sex, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, body mass index (BMI), current smoking, former smoking and high-sensitivity C reactive protein (hs-CRP). For the incidence models, the adjustments also include history of diabetes, total cholesterol and apolipoprotein B. Sex was also included as a stratifying variable when possible.

All analyses were performed by using R software V.4.3.1.[24] We used the survival package[25] for Cox regression models. All p values below 0.05 were considered significant.

RESULTS

Baseline characteristics

Table 1 presents the selected characteristics of the study population included in the evaluation of prevalent RA (n=7702) and incident RA (n=7511). Participants with prevalent RA were more often female, older, had higher hs-CRP and systolic blood pressure (SBP), were more frequently on lipid-lowering and BP-lowering drug treatments, and were non-smokers compared with those without RA (table 1A). Participants who developed RA during follow-up were more frequently on BP-lowering drug treatment compared with those who remained RA-free; they also had slightly elevated hs-CRP and were older than those remaining free of RA (table 1B).

Association of CERT scores and their components with the prevalence and incidence of RA

CERT1 showed significant associations in both unadjusted and adjusted models with prevalent RA (OR 1.47, 95% CI 1.28 to 1.70; OR 1.24, 95% CI 1.05 to 1.46, respectively), but not with incident RA. CERT2 had significant associations with both prevalent and incident RA in the unadjusted analysis (OR 1.63, 95% CI 1.40 to 1.89, and HR 1.13, 95% CI 1.01 to 1.26, respectively). After adjusting for confounding factors, CERT2 remained significantly associated with prevalent RA (OR 1.42, 95% CI 1.20 to 1.68), while the association with incident RA became non-significant (HR 1.08, 95% CI 0.96 to 1.23).

Among the CERT lipid components, the Cer (18:1/16:0)/ PC (16:0/22:5) ratio showed significant associations with RA prevalence, both before and after adjustment, with ORs of 1.50 (95% CI 1.30 to 1.73) and 1.41 (95% CI 1.21 to 1.65), respectively. Similarly, Cer (d18:1/18:0) showed significant associations with RA prevalence both before and after adjustment, with ORs of 1.43 (95% CI 1.24 to 1.66) and 1.29 (95% CI 1.07 to 1.57), respectively. CRP was significantly associated with both RA prevalence and incidence in unadjusted and adjusted models. For prevalence, the ORs were 1.74 (95% CI 1.52 to 2.00) and 1.75 (95% CI 1.51 to 2.03), respectively. For incidence, the HRs were 1.19 (95% CI 1.07 to 1.33) and 1.16 (95% CI 1.03 to 1.31), respectively. For detailed statistics including additional variables, see table 2.

Association between CERT scores and their components in the prevalence and incidence of RA stratified by age

Prevalence analysis

Figure 2 shows the associations between CERT scores and their components with prevalent RA stratified by age. In participants aged under 50 years of age, the most significant associations were observed between PC (16:0/22:5) and prevalent RA, as well as between the Cer (d18:1/16:0) to PC (16:0/22:5) ratio and prevalent RA. In people aged 50 years or older, both CERT2 and CERT1 scores were significantly associated with prevalent RA. These associations were mainly driven by Cer (d18:1/16:0) and Cer (d18:1/18:0).

Incidence analysis

Online supplemental figure 3 illustrates the associations between CERT scores and their components with incident RA stratified by age. In participants aged 50 years or under, neither CERT scores nor their components showed significant associations with incident RA in the adjusted models. However, for individuals aged 50 years or older, a weak association was observed with CERT2 (HR 1.13, 95% CI 0.93 1.36), although it did not reach statistical significance. None of the Cer species or PC ratios showed significant associations with incident RA in either age group.

Association between CERT scores and their components with prevalent and incident RA by sex subgroups

Prevalence analysis

Figure 3 shows associations between CERT scores and their components with RA prevalence by sex. In men (N=3616), significant associations with prevalent RA were observed for CERT2 in both unadjusted (OR 1.42, 95% CI 1.10 to 1.85) and adjusted (OR 1.35, 95% CI 1.02 to 1.78) analyses. The Cer (d18:1/16:0)/ PC (16:0/22:5) ratio showed significant association in both unadjusted (OR 1.38, 95% CI 1.07 to 1.77) and adjusted (OR 1.40, 95% CI 1.08 to 1.82) analyses.

In women (n=4086), both CERT1 and CERT2 showed significant associations in both unadjusted (CERT2: OR 1.81, 95% CI 1.50 to 2.17; CERT1: OR 1.61, 95% CI 1.36 to 1.92) and adjusted analyses (CERT2: OR 1.72, 95% CI 1.42 to 2.10; CERT1: OR 1.49, 95% CI 1.23 to 1.81). Cer components Cer (d18:1/16:0) and Cer (d18:1/18:0) showed significant associations with prevalent RA in both unadjusted and adjusted analyses, and the Cer (d18:1/16:0)/ PC (16:0/22:5) ratio was significantly associated with prevalent RA before and after adjustments (ORs of 1.62, 95% CI 1.35 to 1.93 and 1.66, 95% CI 1.37 to 2.00), respectively.

Incidence analysis

Online supplemental figure 4 presents the associations between CERT scores and their components with incident RA, in gender subgroups. In men (N=3556) and women (N=3955), CERT2 showed a stronger association in women (univariable HR 1.21; 95% CI 1.04 to 1.40, adjusted HR 1.16; 95% CI 0.99 to 1.36) compared with men (adjusted HR 0.99; 95% CI 0.82 to 1.20), though the association was not statistically significant after adjustments. CERT1 and individual Cers (eg, Cer (d18:1/16:0)) showed numerically slightly higher HRs in women, but the confidence intervals included 1, indicating a lack of statistical significance. PC and Cer ratios exhibited no significant sex-based variation.

DISCUSSION

While previous studies have confirmed the efficacy of the CERT scores in predicting CVD risk, their association with other diseases, including chronic inflammatory conditions such as RA, remains less well understood. For the first time, our study revealed significant associations between the distinct CERT score components and RA-associated inflammation. An earlier study showed that people with RA had a 50% higher risk of CVD death compared with the general population, similar to what is seen, for instance, in people with type 2 diabetes mellitus.[26] CVD is influenced by inflammation, and people with RA are more likely to develop CVD because of the inflammatory nature of RA.[27]

Based on our observations, CERT score components, alongside hs-CRP, a well-known marker of inflammation, demonstrated a strong association with the presence of

Table 2 Univariable and multivariable regression for CERT scores and their components in the study on the (A) prevalent RA (N=7702) and (B) incident RA (N=7511)

(A)	Unadjusted		Adjusted*	
Variable	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
CERT1	1.47 (1.28 to 1.70)	<0.001	1.24 (1.05 to 1.46)	0.01
CERT2	1.63 (1.40 to 1.89)	<0.001	1.42 (1.20 to 1.68)	<0.001
Cer (d18:1/16:0)	1.32 (1.15 to 1.53)	<0.001	1.28 (1.06 to 1.54)	0.009
Cer (d18:1/18:0)	1.43 (1.24 to 1.66)	<0.001	1.29 (1.07 to 1.57)	0.008
Cer (d18:1/24:0)	1.06 (0.91 to 1.23)	0.452	1.12 (0.92 to 1.36)	0.25
Cer (d18:1/24:1)	1.25 (1.08 to 1.45)	0.003	1.23 (1.02 to 1.49)	0.033
PC (14:0/22:6)	1.09 (0.94 to 1.26)	0.275	0.91 (0.78 to 1.08)	0.294
PC (16:0/16:0)	1.25 (1.09 to 1.44)	0.002	1.02 (0.85 to 1.23)	0.804
PC (16:0/22:5)	0.86 (0.75 to 1.00)	0.05	0.81 (0.68 to 0.95)	0.01
Cer (d18:1/16:0)/Cer (d18:1/24:0) ratio	1.28 (1.11 to 1.47)	<0.001	1.09 (0.94 to 1.27)	0.234
Cer (d18:1/18:0)/Cer (d18:1/24:0) ratio	1.47 (1.27 to 1.70)	<0.001	1.17 (0.99 to 1.38)	0.063
Cer (d18:1/24:1)/Cer (d18:1/24:0) ratio	1.28 (1.10 to 1.48)	0.001	1.09 (0.94 to 1.27)	0.252
Cer (d18:1/16:0)/PC (16:0/22:5) ratio	1.50 (1.30 to 1.73)	<0.001	1.41 (1.21 to 1.65)	<0.001
Cer (d18:1/18:0)/PC (14:0/22:6) ratio	1.18 (1.02 to 1.37)	0.023	1.22 (1.04 to 1.43)	0.017
Cer (d18:1/18:0)/Cer (d18:1/16:0) ratio	1.28 (1.11 to 1.49)	<0.001	1.11 (0.94 to 1.32)	0.228
LDL	1.00 (0.86 to 1.16)	1	0.94 (0.80 to 1.10)	0.423
CRP	1.74 (1.52 to 2.00)	<0.001	1.75 (1.51 to 2.03)	<0.001
(B)	**Unadjusted**		**Adjusted†**	
Variable	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CERT1	1.01 (0.90 to 1.12)	0.922	0.94 (0.83 to 1.07)	0.34
CERT2	1.13 (1.01 to 1.26)	0.033	1.08 (0.96 to 1.23)	0.194
Cer (d18:1/16:0)	0.99 (0.88 to 1.11)	0.835	0.96 (0.83 to 1.11)	0.598
Cer (d18:1/18:0)	1.04 (0.93 to 1.16)	0.521	0.97 (0.84 to 1.12)	0.663
Cer (d18:1/24:0)	0.98 (0.87 to 1.10)	0.716	1.00 (0.86 to 1.16)	0.993
Cer (d18:1/24:1)	1.05 (0.93–1.17)	0.449	1.06 (0.91 to 1.23)	0.457
PC (14:0/22:6)	0.96 (0.85 to 1.07)	0.43	0.95 (0.83 to 1.09)	0.46
PC (16:0/16:0)	1.06 (0.95 to 1.19)	0.306	1.08 (0.93 to 1.26)	0.299
PC (16:0/22:5)	0.93 (0.83 to 1.04)	0.185	0.90 (0.78 to 1.04)	0.146
Cer (d18:1/16:0)/Cer (d18:1/24:0) ratio	1.01 (0.91 to 1.13)	0.809	0.97 (0.87 to 1.09)	0.652
Cer (d18:1/18:0)/Cer (d18:1/24:0) ratio	1.06 (0.95 to 1.19)	0.286	0.97 (0.86 to 1.10)	0.664
Cer (d18:1/24:1)/Cer (d18:1/24:0) ratio	1.09 (0.98 to 1.22)	0.127	1.05 (0.94 to 1.18)	0.417
Cer (d18:1/16:0) /PC (16:0/22:5) ratio	1.06 (0.95 to 1.18)	0.292	1.05 (0.93 to 1.18)	0.449
Cer (d18:1/18:0)/PC (14:0/22:6) ratio	1.06 (0.95 to 1.18)	0.29	1.02 (0.90 to 1.15)	0.743
Cer (d18:1/18:0)/Cer (d18:1/16:0) ratio	1.06 (0.95 to 1.19)	0.29	0.99 (0.88 to 1.12)	0.889
LDL	0.95 (0.85 to 1.07)	0.413	0.98 (0.74 to 1.29)	0.877
CRP	1.19 (1.07 to 1.33)	<0.002	1.16 (1.03 to 1.31)	0.018

The table outlines the OR and HR along with their corresponding 95% CI and p values. The estimates are per SD (CERT scores) or per log SD.
*Adjusted for log-transformed age, sex, HDL cholesterol, LDL cholesterol, BMI, current smoking, former smoking, high-sensitivity CRP.
†Adjusted for HDL cholesterol, LDL cholesterol, BMI, current smoking, former smoking, high-sensitivity CRP, history of diabetes, total cholesterol, apolipoprotein B. Sex was included as a stratifying variable.
BMI, body mass index; CERT, Cardiovascular Event Risk Tests; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RA, rheumatoid arthritis.

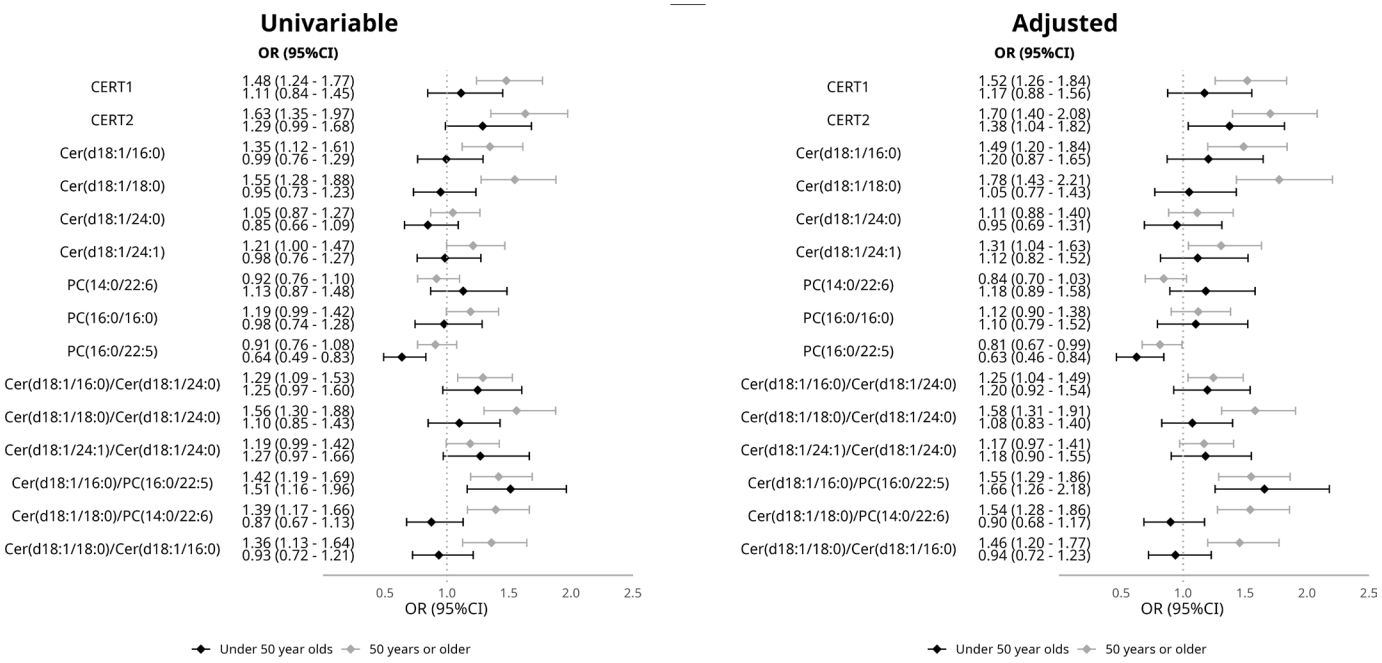

Figure 2 A forest plot displays the results of univariable and multivariable logistic regression analyses for CERT scores and their components in the study of RA prevalence, segmented by age group (under 50 years old and 50 years or older). ORs and their 95% CIs are depicted. Estimates are presented per log SD or per SD of CERT scores. The multivariable models are adjusted for covariates including age, serum high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. CERT, Cardiovascular Event Risk Tests; RA, rheumatoid arthritis.

RA among participants in the study. Alteration of ceramides and PCs with hs-CRP can highlight the importance of these lipids in regulating inflammation processes.[28]

The present study showed significant associations between specific Cers and prevalent RA. A prior study also found that various sphingolipids, including

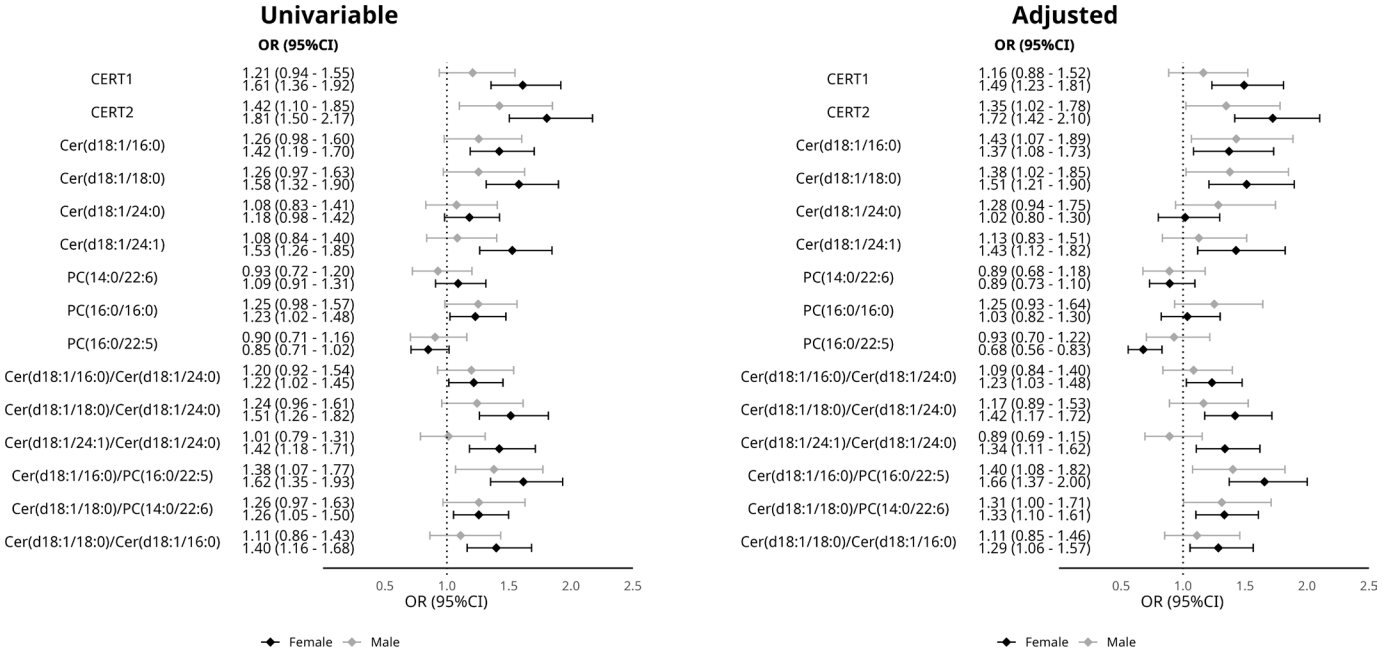

Figure 3 A forest plot displays the results of univariable and multivariable logistic regression analyses for CERT scores and their components in the study of RA prevalence among men and women. ORs and their 95% CIs are depicted. Estimates are presented per log SD or per SD of CERT scores. The multivariable models are adjusted for covariates including age, serum high-density lipoprotein cholesterol and low-density lipoprotein cholesterol.

sphingomyelin (SM), SM (d18:0/18:2), SM (d18:1/18:1) and Cer (d18:1/16:0) were significantly increased in people with RA. Also, associations between Cer levels and RA severity, including correlations with disease activity scores, were noted.[5] Our study confirms the previously described association between Cer (d18:1/16:0) and RA.

Our research revealed a significant association between Cer (d18:1/24:1) and the prevalence of RA. A recent study on synovial fluid samples, employing lipidomic analysis, also showed elevated ceramide levels in people with RA. Particularly, Cer (d18:1/24:1) was found to be 3.5 times higher compared with people without RA. These findings highlight disruptions in lipid metabolism in RA and underscore the potential of Cer (d18:1/24:1) as a predictive marker for RA.[29]

In our sex-stratified analysis, only CERT2 showed a significant association with RA in men, while both CERT1 and CERT2 showed significant associations in women. Notably, many Cers and their ratios showed a significant association in women but not in men. Women with RA often experience more severe disease activity and disability outcomes compared with men, while the extent of joint destruction is comparable between both sexes.[30] Different Cer and PC patterns between the sexes in our study may be associated with hormonal changes during important life stages. Hormone replacement therapy and menopause are considered important factors in initiating or worsening autoimmune processes, potentially also contributing to the development of RA in women.[31] However, our study did not include data on the severity of RA.

CERT scores and their several components were associated with the prevalence of RA. Our analyses revealed a significant association between CERT2 and prevalent RA, indicating that CERT2's lipid components reflect the inflammatory profile characteristic of RA. This suggests that CERT2 could be valuable for identifying people with CVD who may also carry an added inflammatory risk from conditions such as RA. However, incident RA was not associated with CERT scores or their components. This suggests that alterations in Cer and PC levels occur closer to the clinical onset of RA. This implies that these lipids may not be effective for predicting the future onset of RA in the long term. The onset age of RA typically occurs in people aged 30–50 years,[32] and our findings suggest that Cers may play a more limited role in the development and progression of RA in those aged 50 years or under. However, significant associations were observed with CERT2, Cer (d18:1/16:0)/PC (16:0/22:5) and PC (16:0/22:5) in this age group. In contrast, Cer species such as Cer (d18:1/16:0), Cer (d18:1/18:0) and CERT1 and CERT2 appear to be more closely linked with prevalent RA in people aged 50 years or older.

Strengths and limitations

Our study has several strengths. The large, population-based FINRISK cohort enhances the generalisability of our findings, allowing us to draw meaningful conclusions about the Finnish population. The detailed lipid analysis, using high-throughput mass spectrometry provided accurate measurements of specific Cers and PCs, offered valuable insights into lipid metabolism in RA. Additionally, the analyses stratified by age and sex uncovered significant associations that might be overlooked in a non-stratified approach, highlighting sex-specific and age-specific factors. However, our study also has limitations. The relatively small sample size of the RA cases and unmeasured potential confounding factors—such as diet, medication use and other lifestyle factors—could introduce residual confounding, influencing the observed associations. Additionally, we lacked information on the treatment status of people with RA, which may have impacted their lipid profiles. To address these challenges, larger sample sizes and further research are needed to address the variability in metabolism of Cers and PCs among different ethnicities or genetic backgrounds to clarify the specificity of Cers, PCs and CERT1 and 2 for RA. As this is an observational study, we cannot infer causality from the association between CERT2 and RA. While CERT2 components are linked with RA-related inflammation, it remains unclear whether these associations reflect a direct or indirect effect. Longitudinal studies and controlled trials will be necessary to determine causative mechanisms underlying these findings.

In conclusion, this study demonstrates that CERT2 and its distinct lipid components are associated with prevalent RA. These findings are important for interpreting CERT2 test results and for developing prevention protocols tailored for people with CVD or high risk of CVD who may also have residual inflammatory risks, such as those associated with RA.

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Competing interests RL is a shareholder of Zora Biosciences Oy, which holds related patents. We assert that this has not influenced the conduct or outcomes of our research, and all findings reported here are the result of objective analysis. All other authors have no competing interests to declare.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and the FINRISK 2002 Study was approved by the Epi-demiological and Public Health Ethics Committee of the Helsinki Hospital District on 19 December 2001 (Dnro 558/E3/2001). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. This study used the FINRISK 2002 dataset, which includes clinical and demographic data collected from participants. The dataset is not publicly available due to privacy and confidentiality restrictions but can be accessed on reasonable request to the THL Biobank.

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