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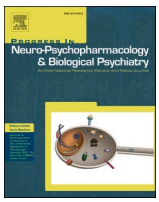

Circulating miRNAs modulating systemic low-grade inflammation and affecting neurodegeneration

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ABSTRACT

Objective and design: Inflammatory processes are an important part of the etiology of many chronic diseases across various medical domains, including neurodegeneration. Understanding their regulation on the molecular level represents a major challenge. Regulatory microRNAs (miRNAs), have been recognized for their role in post-transcriptionally modulating immune-related pathways serving as biomarkers for numerous diseases. Subjects and Methods: This study aims to investigate the association between 176 plasma-circulating miRNAs and the blood-based immune markers C-reactive protein and fibrinogen within the general population-based SHIP-TREND-0 cohort ($N = 801$) and assess their impact on neurodegeneration in linear regression and moderation analyses. Results: We provide strong evidence for miRNA-mediated regulation, particularly in relation to fibrinogen, identifying 48 significant miRNAs with a pronounced over-representation in chronic inflammatory and neurological diseases. Additional moderation analyses explored the influence of the *APOE* ε4 genotype and brain white matter neurodegeneration on the association between miRNAs and inflammation. Again, significant associations were observed for fibrinogen with special emphasize on *hsa*-miR-148a-3p, known to impact on neuroinflammation. Conclusions: Our study suggests the involvement of several plasma-circulating miRNAs in regulating immunological markers while also being linked to neurodegeneration. The strong interplay between miRNAs and inflammation holds promising potential for clinical application in many immune-related neurodegenerative diseases.

1. Introduction

Inflammatory processes are involved in the etiology of many chronic diseases, most of them closely linked to advanced age. Consequently, the term immunosenescence, defined as age-related deterioration of the function of the immune-system, has gained increasing attention in recent years and may serve as a key mechanism explaining the co-occurrence of multiple health conditions in older individuals (Chen et al., 2023b). Chronic low-grade inflammation has been identified as one major risk factor for the development of several age-related diseases. In the context of neurodegeneration, inflammatory processes in the brain, also referred to as neuroinflammation, are thought to be a main component in neurodegenerative disorders including dementia (Mohapatra et al., 2023). This systemic inflammation can arise as a consequence of genetic susceptibility, poor dietary habits, risky lifestyle behaviors, chronic diseases, or chronic stress, thereby contributing to the onset of multiple diseases. Understanding the biological mechanisms underpinning these inflammatory processes is crucial to understand the emergence of diseases and identify the molecular pathways involved. Such biological pathways can serve as targets for population-level prevention and early intervention strategies, ultimately reducing the burden of multimorbidity over the long term. C-reactive protein (CRP) is such a marker of chronic inflammatory state which is widely used in clinical routine with an application in acute inflammation, inflammation

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associated (auto-immune) disorders and acute sepsis but with low specificity to distinguish between different inflammation associated conditions (Zhou et al., 2024). Also fibrinogen is a blood-based marker for inflammation which is also discussed in neurodegenerative conditions and blood-brain-barrier dysfunction (Chandra et al., 2024; Sulimai and Lominadze, 2020).

In recent years, researchers have identified reliable biomarker candidates for various diseases on the basis of easily accessible biofluids, such as proteins, metabolites or regulatory RNAs aiming to enhance early diagnostic and understand the biological basis of diseases. This also applies to neurodegenerative disorders including Alzheimer's Dementia and other dementias (Garcia-Escobar et al., 2024; Klyucherev et al., 2022).

However, the accuracy of these biomarkers is still limited, possibly due to shared etiologies among different interrelated disorders and common biological pathways. Systemic inflammation emerges as a fundamental biological process that may cause or at least contribute to a wide range of age-related diseases. Understanding the biological mechanisms of systemic inflammation on their way to disease occurrence presents another strategy to develop effective prevention and treatment strategies.

In our recent study, we identified regulatory RNAs linked to lesions of brain white matter (van der Auwera et al., 2024). The identified targets demonstrated significant involvement in inflammatory processes and associations with circulating markers of low-grade inflammation. These micro RNAs (miRNAs) are small non-coding RNA molecules that play crucial roles in post-transcriptional regulation and gene expression and have been found to regulate various biological processes implicated in neurodegeneration and inflammation (Kaur et al., 2024; van der Auwera et al., 2024). Following up on this, we aimed to comprehensively investigate the connection between miRNAs and the low-grade inflammation markers CRP and fibrinogen which are widely used in clinical routine. All analyses are independent of specific disease outcomes in the general population *Study of Health in Pomerania* in order to identify miRNAs driving systemic inflammatory pathways in general.

The significant miRNA targets were screened for previously reported disease associations and their involvement in inflammatory processes. In the context of neuroinflammation, our objective was to examine whether an additional genetic risk for Alzheimer's Disease acts as a moderator on the biological path linking miRNAs and inflammation as it is known that the *APOE* ε4 allele may impact the blood-brain-barrier (Kirchner et al., 2023). Furthermore, the combined impact of inflammatory markers and miRNA abundance on white matter lesions (WML) were investigated, considering their role as vascular neurodegenerative correlate (van der Auwera et al., 2024).

2. Methods

2.1. SHIP sample

The investigations in the *Study of Health in Pomerania* (SHIP) were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants. The survey and study methods were approved by the institutional review boards of the University Medicine Greifswald.

SHIP is a general population project conducted in the northeast of Germany (Völzke et al., 2022). The main aim was to assess the prevalence and incidence of common diseases and lifestyle-associated risk factors in the general population. From 2008 to 2012 the SHIP-TREND-0 sample (TREND-0) was recruited including 4420 participants that underwent a standardized computer-assisted personal interview, during which they provided information on disease status, sociodemographic and lifestyle factors and also different biofluids for OMICS analyses.

2.1.1. Plasma-circulating miRNA data

Fasting blood samples in TREND-0 were taken from the cubital vein. Serum and plasma samples were stored at −80 °C in the Integrated Research Biobank of the University Medicine Greifswald and used in accordance with its regulations.

MiRNA levels were measured in a subsample of TREND-0 participants ($n = 813$) as already described previously (Ameling et al., 2015; van der Auwera et al., 2022). Circulating miRNAs were prepared from 200 µl EDTA plasma using the miRCURY™ RNA Isolation Kit –Biofluids (Qiagen, Hilden, Germany) and were measured in three batches (batch1: $n = 371$; batch2: $n = 337$; batch3: $n = 105$). For RT-qPCR based miRNA analysis the Serum/Plasma Focus microRNA PCR Panel (Qiagen, Hilden, Germany) V3.M and V4.M were used, covering 179 miRNAs. Further methodological details can be found in the supplement.

For each batch, a miRNA was selected for further analysis if Ct (cycle threshold) ≤ 37 was detected in at least 40 % of the samples. In order to consider the influence of technical parameters, the Ct-values of synthetic spiked-in miRNAs — monitoring the efficiency of miRNA extraction (UniSp2 and the difference between Ct-values of UniSp4 and UniSp2) — the interplate calibrator (UniSp3), as well as the storage time of the biosamples were regressed out of the data. The resulting residuals were used as independent variables in later models to detect associations between miRNAs and phenotypes of interest. A batch was included in the analysis for a specific miRNA if at least 80 subjects contained a valid measurement of the respective miRNA (complete list of valid miRNAs is given in the supplement).

2.1.2. Circulating inflammatory markers

High-sensitivity C-reactive protein (hs-CRP) concentrations were determined in serum by nephelometry on the Dimension VISTA (Siemens Healthcare Diagnostics, Eschborn, Germany). Plasma fibrinogen concentrations were measured using Clauss method assessed by coagulation analyzers (BCS-XP; Siemens Healthcare Diagnostics, Germany).

2.1.3. Brain imaging data

In addition, TREND-0 participants were asked for a whole-body MRI assessment. After exclusion of subjects who refused participation or fulfilled MRI exclusion criteria (e.g., cardiac pacemaker) 2047 subjects underwent the MRI scanning procedure (see supplement) (Hosten et al., 2021). White matter lesions (WML) were segmented by the lesion growth algorithm (Schmidt et al., 2012) as implemented in the LST toolbox version 3.0.0 (www.statistical-modelling.de/lst.html) for SPM (Statistical Parametric Mapping, https://www.fil.ion.ucl.ac.uk/spm/) using both the T1-weighted and the FLAIR MRI sequences. We set the initial threshold kappa to 0.25 and used a threshold of 0.5 to generate binary lesion maps based on the obtained probability maps to be able to extract the total lesion volume and the number of lesions present. For analyses, the total volume of all lesions was used as outcome.

2.1.4. APOE genotype

Genome-wide SNP information was taken from the genetic data in TREND-0 (see supplement). The *APOE* genotypes were determined based on the two SNPs rs429358 (C; T) and rs7412 (T; C) from the resulting imputation (imputation quality >0.8; Hardy–Weinberg-Equilibrium, $p > 0.05$) (Pitchika et al., 2022). As we used the data from the genome-wide SNP chip instead of strand-specific genotyped SNPs for the determination of *APOE* status, two ambiguous SNP combinations occurred where *APOE* ε2/ε4 and ε1/ε3 could not be distinguished from each other (http://www.snpedia.com/index.php/APOE; accessed on June 27th 2022). Those participants in TREND-0 were excluded from the *APOE* analyses ($n = 27$ in the genetic batch containing 986 individuals). Subjects were defined as *APOE* ε4 carriers if they had at least one ε4 allele.

2.2. Statistical analysis

Subject characteristics of the non-missing final study sample were

assessed by mean and standard deviation for metric variables and by numbers and percentages for categorical data. Significant differences between males and females were tested via *t*-test for metric variables and Chi^2-test for categorical data. Different regression analyses were performed to assess the associations between the inflammatory markers CRP and fibrinogen and plasma-circulating miRNAs in the general population. Linear regression models with the inflammatory markers as outcome and the miRNA residuals as predictors of interest were calculated adjusted for age (non-linear), sex, BMI (Body mass index), HCT (hematocrit), platelets, smoking status, and miRNA batch. These covariates were chosen either based on the experience from our own data (Ameling et al., 2015) or based on the literature (Dahiya et al., 2015; Kaur et al., 2019; Pritchard et al., 2012). CRP-levels were log-transformed prior to analysis.

CRP ~ miRNA + age + sex + BMI + smoking + HCT + PLT + batch.

FIB ~ miRNA + age + sex + BMI + smoking + HCT + PLT + batch.

In subsequent analyses, the moderating effect of the *APOE* ε4 genotype was tested by including an interaction term between miRNA and genetic factor into the models.

CRP ~ miRNA×APOE + age + sex + BMI + smoking + HCT + PLT + batch.

FIB ~ miRNA×APOE + age + sex + BMI + smoking + HCT + PLT + batch.

To test the moderating effect of miRNA abundance and low-grade inflammation on neurodegeneration, a miRNA × inflammation interaction term was added to the model with total WML volume (log-transformed) as outcome. These analyses were additionally adjusted for intracranial volume (ICV) and *APOE* ε4 genotype.

WML ~ miRNA×CRP + APOE + ICV + age + sex + BMI + smoking + HCT + PLT + batch.

WML ~ miRNA×FIB + APOE + ICV + age + sex + BMI + smoking + HCT + PLT + batch.

For the significant results normal distribution of residuals was verified manually. For each analysis on the miRNA set multiple testing correction was done by Benjamini-Hochberg correction. All analyses were performed using R version 4.3.0.

2.2.1. *In-silico analysis*

Tissue specific expression: GTExPortal (https://www.gtexportal.org/home/), miRNA TissueAtlas (https://ccb-web.cs.uni-saarland.de/tissueatlas2) (Keller et al., 2022), and CNS microRNA Profiles database for mouse (Pomper et al., 2020) were used to compare the expression of miRNAs and genes in brain tissues of human and mouse samples and thus justify the impact of circulating miRNAs on brain related processes and diseases.

Over-representation in diseases: We used the over-representation analysis implemented in the miRNA Enrichment Analysis and Annotation Tool (miEAA 2.0) (Aparicio-Puerta et al., 2023) (https://ccb-compute2.cs.uni-saarland.de/mieaa2/) to search for significant over-representation of miRNA sets in disease outcomes. MiEAA incorporates data from large miRNA, tissue, and pathway databases, such as Gene Ontology, miRTarBase 8.0, TissueAtlas, MNDR v2.0, TAM 2.0, HMDD v3.0 and miRWalk2.0. To identify the primary source of a specific miRNA in blood, we used data from the miR-Blood database (https://mir-blood.com/) (Jehn et al., 2024) which is a small RNA expression atlas for the eleven major components of human peripheral blood.

3. Results

3.1. Sample characteristic

The study sample with non-missing information on both inflammatory markers, miRNA measurement and basic covariates included 772 subjects. Of them, 52 % were females, with a mean age of 49 years. Significant differences ($p < 0.001$) between males and females were observed for smoking status, HCT, PLT, both inflammatory markers, total WML volume and miRNA batch where males showed a higher prevalence for smoking, higher BMI, higher HCT values and a larger WML volume and females revealed higher levels of PLT, CRP and fibrinogen and were over-represented in miRNA batch 3 (see Table 1). The final sample for CRP analyses was 781 and for fibrinogen 801.

3.2. Identification of miRNAs associated with circulating markers for low-grade inflammation

In the final analyses 176 plasma-circulating miRNAs were tested for an association between miRNA abundance and blood markers for systemic inflammation. In the CRP analysis 36 miRNAs revealed a nominal significant effect with three of them surviving multiple testing correction, *hsa*-miR-92a-3p, *hsa*-miR-16-5p, and *hsa*-miR-451a; all with lower abundance in case of higher CRP levels (see Table 2, supplementary Table S1, Fig. 1A). According to data from the miRNA expression databases, especially *hsa*-miR-9a-3p and *hsa*-miR-16-5p are expressed in the human brain and both miRNAs are highly expressed in mouse brain immune cells.

With regard to fibrinogen 58 miRNAs were nominal significant of which 48 remained significant after multiple testing correction with different directions of effect (Table 2, Supplementary Table S2, Fig. 1B; even 16 miRNAs still significant after more conservative Bonferroni correction for multiple testing). The lowest *p*-value was observed for *hsa*-miR-451a ($p = 3.5 \times 10^{-8}$) which was also among the significant miRNAs for CRP levels with the same effect direction. This is not surprising given the high correlation of $r = 0.44$ between CRP and fibrinogen levels in our sample. In fact, all significant results for CRP vanished if additionally adjusted for fibrinogen levels indicating that the main effects on these miRNAs was attributed to fibrinogen. A correlation matrix of the nominal significant miRNAs for fibrinogen is given in the supplementary material (Supplementary Fig. S1) showing a high correlation among several miRNAs. Data from the miR-Blood database

Table 1
Sample characteristic of the non-missing final study sample with non-missing data for age, sex, miRNA measurement, BMI, HCT, PLT, smoking, CRP, and fibrinogen ($n = 772$).

Variable	Males (n = 370)	Females (n = 402)	Comparison
Age (in years)	49.3 (14.5), 49.5	49.1 (13.4), 50.0	$T = 0.13$, $p = 0.89$
Smoking			$Chi^2 = 22.7$, $p < 0.001$
Never	114 (30.8 %)	187 (46.5 %)	
Former	169 (45.7 %)	126 (31.3 %)	
Current	87 (23.5 %)	89 (22.1 %)	
BMI (kg/m^2)	27.7 (3.6), 27.7	27.3 (5.1), 26.3	$T = 1.28$, $p = 0.20$
HCT	0.44 (0.03), 0.44	0.40 (0.03), 0.40	$T = 18.3$, $p < 0.001$
PLT (Gpt/l)	215 (45), 210	243 (50), 238	$T = -8.19$, $p < 0.001$
FIB (g/l)	2.8 (0.67), 2.7	3.1 (0.70), 3.2	$T = -6.96$, $p < 0.001$
CRP (mg/l)	1.7 (2.54), 0.96	2.7 (4.41), 1.32	$T = -3.80$, $p < 0.001$
APOE ε4 carrier	90 (24.3 %)	91 (22.6 %)	$Chi^2 = 0.08$, $p = 0.77$
total WML volume in ml*	0.71 (2.63), 0.06	0.39 (1.23), 0.07	$T = 2.06$, $p = 0.04$
miRNA batch			$Chi^2 = 18.4$, $p < 0.001$
1	178 (48.1 %)	176 (43.8 %)	
2	166 (44.9 %)	157 (39.1 %)	
3	26 (7.0 %)	69 (17.1 %)	

BMI: Body Mass Index, HCT: hematocrit, PLT: platelets, FIB: fibrinogen, CRP: C-reactive protein, WML: white matter lesions. *MRI data not available for all subjects in the CRP and fibrinogen analyses. For metric variables mean (standard deviation) and median are given; for categorical variables numbers and percentage.

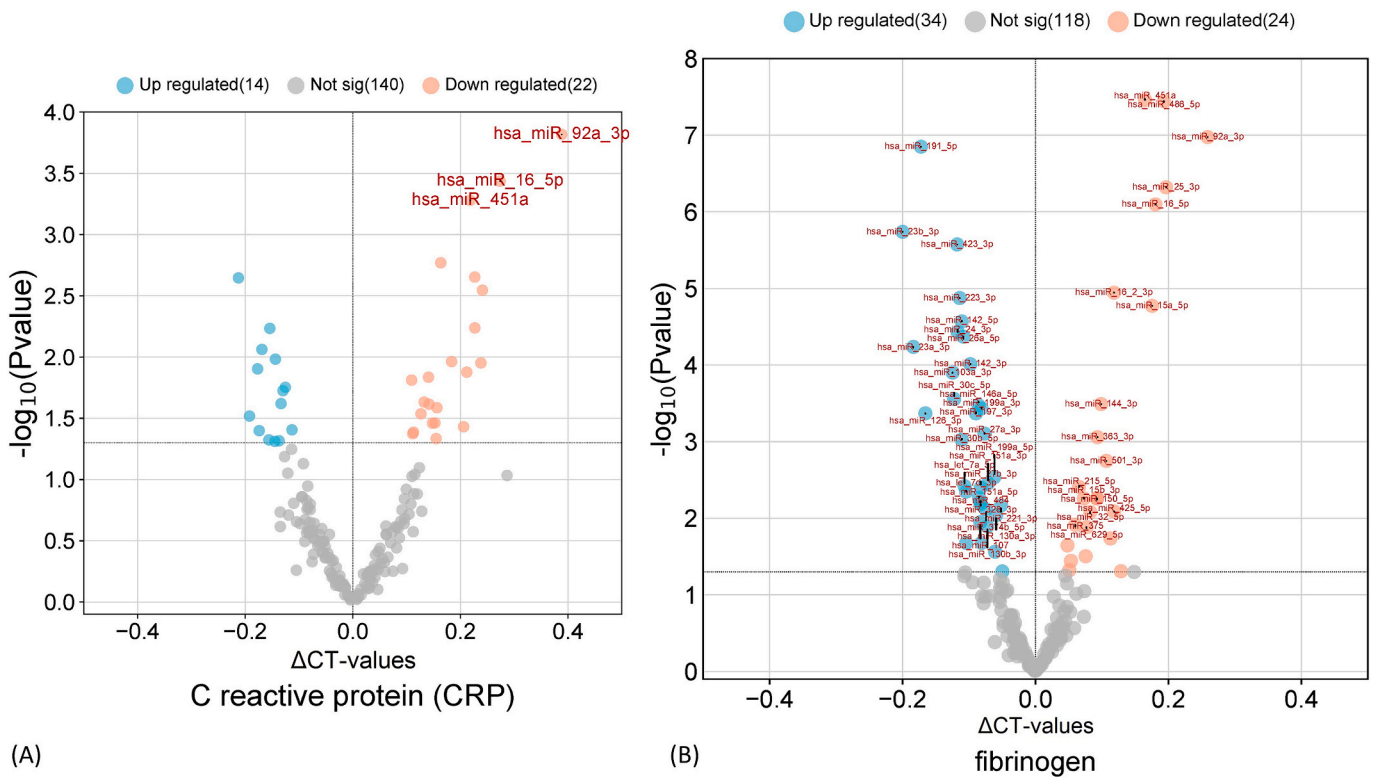

Fig. 1. Volcano-like plot for the association results between plasma-circulating miRNAs and inflammatory markers. A) CRP levels with three miRNAs surviving multiple testing correction. B) fibrinogen levels with 48 miRNAs surviving multiple testing correction. The horizontal line indicates nominal significance. MiRNAs surviving multiple testing correction are displayed in red (up-regulated, left side; down-regulated, right side) depending on their direction of effect. Higher ΔCT-values as estimates from the linear regression indicate lower abundance and lower ΔCT-values indicate higher abundance. Created with SRplot (Tang et al., 2023). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed that the primary cellular source of most of the significant miRNAs were either erythrocytes or thrombocytes. However, additional adjustment for age×sex interaction, white blood cell count, red blood cell count or albumin (marker for liver function as CRP and fibrinogen are primarily synthesized in the liver) did not change the significance of the results.

3.3. In-silico analysis of target miRNAs

Mapping the significant miRNAs from the direct association analyses to their genomic positions revealed two clusters of significant miRNAs for fibrinogen on chromosome 9 and 17 (Fig. S2).

We also searched for over-represented diseases among the 48 significant miRNAs from the direct association analyses applying the miRNA over-representation (miEAA) tool. Results revealed a long list of over-represented diseases mainly autoimmune diseases, different cancer types, neurological, and neurodegenerative diseases (Fig. 2, Supplementary Table S3). On the level of enriched KEGG pathways results were comparable with many diseases associated as well as regulatory pathways being the most significant (supplementary Fig. S4, Supplementary Table S3). As list of the miRNA~target gene pairs used by miEAA and based on the information of miRTarBase 9.0 is given in the supplement (supplementary Table S6). The miRTargetscan database revealed 255 genes targeted by at least two of the 48 miRNAs (see Table S5) which were enriched in immune system, signal transduction and gene expression pathways (Fig. S4). There was also significant enrichment of interactions among these 255 genes and known AD genes and miRNAs according to the AlzCode database (http://www.alzcode.xyz/; $p = 0.0001$) (Lin et al., 2022).

3.4. Interaction with APOE ε4 genotype

As the significant miRNAs for the circulating inflammatory markers exhibited a strong association with neurodegenerative diseases, we tested for a moderating effect of the *APOE* ε4 genotype to reveal inflammation-associated miRNAs with a specific impact in AD risk allele carriers. In these analyses, only for fibrinogen a significant *APOE*×miRNA association was observed for *hsa*-miR-148a-3p (see Fig. 3A). For *APOE* ε4 carriers higher *hsa*-miRNA-148a-3p abundance was associated with higher fibrinogen levels, whereas for none-*APOE* ε4 carriers there was no effect. *Hsa*-miR-148a is located on chromosome 7 and exhibits low expression within the human brain, except the pituitary gland, and shows mainly expression in astrocytes in mouse brainstem and spinal cord (see Fig. S5) (Pomper et al., 2020). According to the *Japanese miRNA-eQTL database* (https://jamir-eqtl.org/) several eQTLs of *hsa*-miR-148a-3p have been identified that were associated with mild cognitive impairment or AD (see Fig. S6).

3.5. Moderating effects of miRNAs and inflammation markers on brain white matter lesions

Moderation analysis between miRNAs and inflammatory markers on WMLs revealed 13 significant interactions after multiple testing correction but only for the interaction with fibrinogen (Table 2, Fig. S7). Significant direct effects of the inflammatory markers on total WML volume were not observed (all $p > 0.27$). Again, including an interaction term between age×sex had no effect on the results. Comparing this set of miRNAs with those from the direct effects on fibrinogen markers, three miRNAs were significantly associated in both analyses (*hsa*-miR-140-3p, *hsa*-miR-27b-3p, *hsa*-miR-27a-3p) meaning that despite their strong direct effect on plasma fibrinogen levels, they also exhibited a strong

Table 2
Significant miRNAs for direct associations with CRP and fibrinogen as well as for miRNA × fibrinogen interaction on WML.

miRNA	Estimate	*p*-value	Nis	p_{BH}	Harboring gene	Highest contribution*
Significant miRNAs associated with CRP						
hsa-miR-92a-3p	Negative	1.5×10^{-4}	778	2.7×10^{-2}	Chr13, *MIR17HG*	plasma
hsa-miR-16-5p	Negative	3.7×10^{-4}	776	3.1×10^{-2}	Chr13, *DLEU2*	Plasma, erythrocytes
hsa-miR-451a	Negative	5.3×10^{-4}	778	3.1×10^{-2}	Chr17, *FAM222B*	Plasma, erythrocytes
Significant miRNAs associated with fibrinogen						
hsa-miR-451a	Negative	3.5×10^{-8}	798	3.2×10^{-6}	Chr17, *FAM222B*	Plasma, erythrocytes
hsa-miR-486-5p	Negative	3.6×10^{-8}	798	3.2×10^{-6}	Chr8, *ANK1*	plasma
hsa-miR-92a-3p	Negative	1.1×10^{-7}	799	6.2×10^{-6}	Chr13, *MIR17HG*	plasma
hsa-miR-191-5p	Positive	1.4×10^{-7}	798	6.2×10^{-6}	Chr3, *DALRD3*	Plasma, erythrocytes
hsa-miR-25-3p	Negative	4.8×10^{-7}	799	1.7×10^{-5}	Chr7, *MCM7*	Plasma, erythrocytes
hsa-miR-16-5p	Negative	8.0×10^{-7}	797	2.4×10^{-5}	Chr13, *DLEU2*	Plasma, erythrocytes
hsa-miR-23b-3p	Positive	1.8×10^{-6}	796	4.6×10^{-5}	Chr9, *AOPEP*	thrombocytes
hsa-miR-423-3p	Positive	2.7×10^{-6}	755	5.9×10^{-5}	Chr17, *NSRP1*	Plasma, erythrocytes
hsa-miR-16-2-3p	Negative	1.1×10^{-5}	776	2.2×10^{-4}	Chr3, *SMC4*	Plasma, erythrocytes
hsa-miR-223-3p	Positive	1.3×10^{-5}	800	2.4×10^{-4}	ChrX, *MIR223HG*	neutrophils
hsa-miR-15a-5p	Negative	1.7×10^{-5}	798	2.7×10^{-4}	Chr13, *DLEU2*	erythrocytes
hsa-miR-142-5p	Positive	2.7×10^{-5}	792	4.0×10^{-4}	Chr17, *TSPOAP1*	erythrocytes
hsa-miR-24-3p	Positive	3.7×10^{-5}	801	5.0×10^{-4}	Chr9, *AOPEP*	Plasma, thrombocytes
hsa-miR-26a-5p	Positive	4.3×10^{-5}	793	5.4×10^{-4}	Chr3, *CTDSPL*	erythrocytes
hsa-miR-23a-3p	Positive	5.8×10^{-5}	794	6.8×10^{-4}	Chr19, *MIR23AHG*	Plasma, thrombocytes
hsa-miR-142-3p	Positive	9.8×10^{-5}	800	1.1×10^{-3}	Chr17, *TSPOAP1*	erythrocytes
hsa-miR-103a-3p	Positive	1.3×10^{-4}	797	1.3×10^{-3}	Chr5, *PANK3*	Plasma, erythrocytes
hsa-miR-30c-5p	Positive	2.8×10^{-4}	792	2.7×10^{-3}	Chr1, *NFYC*	erythrocytes
hsa-miR-144-3p	Negative	3.2×10^{-4}	797	3.0×10^{-3}	Chr17, *ERAL1*	erythrocytes
hsa-miR-146a-5p	Positive	3.4×10^{-4}	795	3.0×10^{-3}	Chr5, *MIR3142HG*	thrombocytes
hsa-miR-199a-3p	Positive	3.7×10^{-4}	782	3.1×10^{-3}	Chr19, *DNM2*	thrombocytes
hsa-miR-197-3p	Positive	4.3×10^{-4}	741	3.3×10^{-3}	Chr1, *GNAI3*	plasma
hsa-miR-126-3p	Positive	4.3×10^{-4}	796	3.3×10^{-3}	Chr9, *EGFL7*	erythrocytes
hsa-miR-27a-3p	Positive	7.9×10^{-4}	771	5.8×10^{-3}	Chr19, *MIR23AHG*	various
hsa-miR-363-3p	Negative	8.7×10^{-4}	778	6.2×10^{-3}	ChrX, *MIG106AHG*	Plasma, erythrocytes
hsa-miR-30b-5p	Positive	9.4×10^{-4}	795	6.4×10^{-3}	Chr8, *ZFAT*	erythrocytes
hsa-miR-501-3p	Negative	1.8×10^{-3}	422	1.2×10^{-2}	ChrX, *CLCN5*	plasma
hsa-miR-199a-5p	Positive	2.9×10^{-3}	653	1.8×10^{-2}	Chr19, *DNM2*	thrombocytes
hsa-miR-151a-3p	Positive	3.5×10^{-3}	725	2.1×10^{-2}	Chr8, *PTK2*	Plasma, erythrocytes
hsa-let-7a-5p	Positive	3.8×10^{-3}	797	2.2×10^{-2}	Chr9, *MIRLET7A*	erythrocytes
hsa-miR-215-5p	Negative	3.9×10^{-3}	737	2.2×10^{-2}	Chr1, *IARS2*	Plasma, erythrocytes
hsa-miR-27b-3p	Positive	4.0×10^{-3}	795	2.2×10^{-2}	Chr9, *AOPEP*	thrombocytes
hsa-let-7d-3p	Positive	4.5×10^{-3}	791	2.4×10^{-2}	Chr9, *MIRLET7A1HG*	plasma
hsa-miR-151a-5p	Positive	5.4×10^{-3}	787	2.6×10^{-2}	Chr8, *PTK2*	erythrocytes
hsa-miR-140-3p	Negative	5.5×10^{-3}	798	2.6×10^{-2}	Chr16, *WWP2*	plasma
hsa-miR-15b-3p	Negative	5.5×10^{-3}	772	2.6×10^{-2}	Chr3, *SMC4*	erythrocytes
hsa-miR-150-5p	Negative	5.5×10^{-3}	798	2.6×10^{-2}	Chr19	CD4 T-cells
hsa-miR-484	Positive	6.2×10^{-3}	789	2.9×10^{-2}	Chr16, *NDE1*	plasma
hsa-miR-328-3p	Positive	6.6×10^{-3}	573	3.0×10^{-2}	Chr16, *ELMO3*	plasma
hsa-miR-221-3p	Positive	6.8×10^{-3}	796	3.0×10^{-2}	ChrX, *MIR222HG*	Plasma, thrombocytes
hsa-miR-374b-5p	Positive	7.6×10^{-3}	585	3.3×10^{-2}	ChrX, *FTX*	erythrocytes
hsa-miR-425-5p	Negative	8.2×10^{-3}	793	3.4×10^{-2}	Chr3, *DALRD3*	plasma
hsa-miR-32-5p	Negative	8.3×10^{-3}	790	3.4×10^{-2}	Chr9, *TMEM245*	plasma
hsa-miR-130a-3p	Positive	9.1×10^{-3}	790	3.6×10^{-2}	Chr11, *MIR130AHG*	erythrocytes
hsa-miR-107	Positive	1.1×10^{-2}	787	4.4×10^{-2}	Chr10, *PANK1*	thrombocytes
hsa-miR-375	Negative	1.2×10^{-2}	535	4.7×10^{-2}	Chr2, *CFAP65*	plasma
hsa-miR-130b-3p	Positive	1.3×10^{-2}	608	4.8×10^{-2}	Chr22, unknown locus	erythrocytes
hsa-miR-629-5p	Negative	1.3×10^{-2}	447	4.8×10^{-2}	Chr15, *TLE3*	Plasma, erythrocytes
Significant miRNAs for fibrinogen × miRNA interaction on total white matter lesion volume						
hsa-miR-148a-3p	Negative	2.0×10^{-6}	629	3.5×10^{-4}	Chr7, unknown locus	Plasma, erythrocytes
hsa-miR-144-5p	Positive	1.4×10^{-5}	601	1.2×10^{-3}	Chr17, *ERAL1*	erythrocytes
hsa-miR-1260a	Negative	8.4×10^{-5}	266	4.8×10^{-3}	Chr14, *NGB*	plasma
hsa-miR-140-3p	Negative	2.0×10^{-4}	638	6.8×10^{-3}	Chr16, *WWP2*	plasma
hsa-miR-27b-3p	Negative	1.6×10^{-4}	636	6.8×10^{-3}	Chr9, *AOPEP*	thrombocytes
hsa-miR-424-5p	Negative	2.4×10^{-4}	631	6.8×10^{-3}	ChrX	erythrocytes
hsa-miR-125b-5p	Negative	6.7×10^{-4}	623	1.7×10^{-2}	Chr11, *MIR100HG*	plasma
hsa-miR-128-5p	Negative	1.7×10^{-3}	306	2.8×10^{-2}	Chr2, *R3HDM1*	erythrocytes
hsa-miR-143-3p	Positive	2.1×10^{-3}	504	2.8×10^{-2}	Chr5, *CARMN*	Plasma, neutrophils
hsa-miR-19b-3p	Negative	2.0×10^{-3}	637	2.8×10^{-2}	Chr13, *MIR17HG*	Plasma, erythrocytes
hsa-miR-27a-3p	Negative	1.4×10^{-3}	612	2.8×10^{-2}	Hr19, *MIR23AHG*	various
hsa-miR-320c	Negative	1.9×10^{-3}	311	2.8×10^{-2}	Chr18, *ABHD3*	plasma
hsa-miR-362-3p	Positive	1.7×10^{-3}	165	2.8×10^{-2}	Chr1, *IARS2*	erythrocytes

p_{BH}: Benjamini-Hochberg corrected *p*-values. The number of individuals with non-missing data for CRP was 781 and for fibrinogen 801. *based on data from the miRBlood database (Jehn et al., 2024), the blood component with the highest contribution to whole-blood is listed.

effect on WMLs moderated by fibrinogen. None of these miRNAs was associated with CRP levels and nine miRNAs (*hsa*-miR-148a-3p, *hsa*-miR-144-5p, *hsa*-miR-1260a, *hsa*-miR-424-5p, *hsa*-miR-125b-5p, *hsa*-miR-128-5p, *hsa*-miR-143-3p, *hsa*-miR-19b-3p, *hsa*-miR-320c, *hsa*-miR-362-3p) attained only significance in the moderation analysis. The top miRNA of this analysis was the same as for the interaction with *APOE* ε4 status, *hsa*-miR-148a-3p. Here, the association between fibrinogen levels and total WML volume was dependent on the level of *hsa*-miR-148a-3p in the sense that in case of high fibrinogen levels additional high levels of the miRNA led to a smaller total lesion volume whereas low amount of the miRNA increased the total lesion volume. This effect was more evident in the non *APOE* ε4 allele carriers ($p = 0.0017$, Fig. S8). Stratified for APOE ε4 carrier status, significant interactions were primarily observed in non-carriers. Neither fibrinogen levels nor *hsa*-miR-148a-3p showed a direct significant effect on WML.

4. Discussion

This is the first study to investigate the pattern of plasma-circulating miRNAs associated with the low-grade inflammatory markers CRP and fibrinogen in the general population irrespective of a specific disease or clinical setting. The study identified a large number of miRNAs involved in the regulation of blood-based inflammatory markers. These miRNAs have been associated previously with immunological and inflammation-associated diseases from different medical areas including autoimmune diseases, cancer, neurological, and neurodegenerative as well as cardiovascular diseases; all of which are known to exhibit a strong inflammatory component. In addition, analyses identified miRNAs specifically impacting neurodegeneration while moderated by inflammatory markers drawing a line from miRNA regulated peripheral inflammation to neurodegenerative processes.

In our analyses we identified 48 plasma-circulating miRNAs associated with plasma fibrinogen with three of them also associated with serum CRP in our general population sample. Most of the miRNAs have been found to primarily originate from erythrocytes or thrombocytes but all significant results were independent from these blood cell components. Regarding previous results, there are only few studies investigating the association between CRP and miRNA abundance, and most of them were performed in disease population and with a limited set of preselected miRNAs tested. A couple of studies were performed in obese children (Bayoumy et al., 2023; Lauria et al., 2022; Lischka et al., 2005) and included not more than 200 subjects. Unfortunately, we were not able to replicate these findings regarding CRP, partly due to missing miRNA information in TREND-0. Other studies were performed in small groups of adult patients but again replication was unsuccessful (Li et al., 2022; Xu et al., 2022). With regard to fibrinogen there are no previous studies investigating their associations with miRNAs. However, analyses in the Rotterdam Study on the association between plasma-circulating miRNAs and blood-based immune markers indicated a strong miRNA signal for the neutrophil-to-lymphocyte ratio, the platelet-to-lymphocyte ratio, and the systemic immune-inflammation index (Leonard et al., 2023). From their list of proposed main target miRNAs only *hsa*-miR-150-5p was available in the TREND-0 cohort and revealed a significant association with fibrinogen. The by far stronger results for fibrinogen may indicate a stronger miRNA regulation or may reflect that both, fibrinogen and miRNAs, were measured in plasma. Analysing the biological impact of the set of 48 significant miRNAs results showed a strong over-representation with regard to inflammatory diseases and pathways. The three miRNAs associated with both inflammatory markers, CRP and fibrinogen, have previously been identified as biomarkers for various inflammation-associated diseases. The miRNA *hsa*-miR-451a was found as potential biomarker for psychotic-like experiences (Tomita et al., 2023), diabetic chronic kidney disease (Zapała et al., 2023), acute ischemic stroke (Aldous et al., 2022), anaphylaxis (Francuzik et al., 2022), autism (Frye et al., 2021) or rheumatoid arthritis (Prajzlerová et al., 2021). For *hsa*-miR-92a-3p associations with ischemic stroke (Zhang et al., 2023a), anxiety with comorbid substance use disorder (Chen et al., 2021), amyotrophic lateral sclerosis (Joilin et al., 2020), Parkinson's Disease (Taguchi and Wang, 2018), or lupus (Kim et al., 2016) have been reported. Most associations have been reported for *hsa*-miR-16-5p, such as psoriasis (Alatas et al., 2020), amyotrophic lateral sclerosis (Joilin et al., 2020), Alzheimer's Dementia (Herrera-Espejo et al., 2019), multiple sclerosis (Yuan et al., 2023), diverse cancer types (Ghafouri-Fard et al., 2022), or ischemic stroke (Jiang et al., 2022). This strengthens the assumption that one miRNA alone is not very specific as biomarker for a single disease but is associated with the complex underlying immune related biological processes which are shared by all of the diseases and their associated comorbidity patterns.

Both inflammatory markers have previously been associated with neurodegeneration (Zhang et al., 2023b) and especially fibrinogen is known as a potent neurotoxin in the brain also interacting with amyloid β (Kim et al., 2021; Wen and Zhang, 2023) and impairing the blood-brain-barrier (Pyun et al., 2020). To get a better impression on the effect of miRNA associated inflammation on neurodegeneration, we performed two different types of moderation analyses: 1. the association between miRNAs and inflammatory markers moderated by *APOE* ε4 status as major genetic marker associated with AD. Previous results have shown an involvement of the *APOE* ε4 allele in blood-brain-barrier dysfunction which could promote the influx of neurotoxins and inflammatory components into the brain (Kirchner et al., 2023). Moreover, *APOE* ε4 is known to drive chronic inflammation and oxidative stress (Duro et al., 2022). Analyses in TREND-0 revealed one miRNA with significant association on fibrinogen moderated by *APOE* ε4 status. A positive association between levels of *hsa*-miR-148a-3p and fibrinogen was only observed in *APOE* ε4 carriers with a dose-response relationship depending on the number of *APOE* ε4 alleles (Fig. 3A). Many studies suggest a downregulation of *hsa*-miR-148a-3p in patients with cognitive impairment and AD (Chen et al., 2023a; Dag et al., 2023; Islam et al., 2021; Satoh et al., 2015; Zhang et al., 2021). And it was also shown that upregulation of miR-148a-3p might have neuroprotective effects in AD or stroke (Huang et al., 2021; Zeng et al., 2022; Zhang et al., 2021; Zhong et al., 2023). Contrary, higher inflammation levels were found to be positively associated with this miRNA (Akbaba et al., 2021; Chen et al., 2023a) but not in AD patients. Our results indicate that in the group *APOE* ε4 carriers with additionally low levels of *hsa*-miR-148a-3p, which have been associated with higher AD risk and lower pro-inflammatory levels, also lower fibrinogen levels could be observed (Fig. 3B). This might sound contra intuitive as systemic chronic inflammation is thought to be a major driver of neurodegeneration. But various studies have observed an inverse association between the inflammatory marker CRP and the *APOE* ε4 allele across all ages and before the onset of any AD pathology (Wang et al., 2022b). As the subjects from TREND-0 are apparently healthy and the inflammatory markers CRP and fibrinogen are highly correlated with each other, this observation might also apply to our cohort (see Fig. 3B). According to high levels of miR-148a-3p observed in mouse astrocytes, studies in humans suggest that astrocyte derived *hsa*-miR-148a-3p has a protective effect on neuroinflammation, neurological function and inhibits hippocampal pyroptosis (Qian et al., 2024; Zhong et al., 2023). 2. In a second analysis the association between inflammatory markers and brain WML moderated by miRNAs was tested. 13 miRNAs revealed a moderating effect of the association between fibrinogen and WMLs with again *hsa*-miR-148a-3p showing the strongest association especially in non *APOE* ε4 carriers. For those ten miRNAs exclusively emerging in the moderation analysis, strong implications for neurodegenerative and neurological disorders as well as inflammation have previously been observed (Chen et al., 2022; Harati et al., 2022; Keller et al., 2014; Li et al., 2020; Li et al., 2023; Lin et al., 2019; Raheja et al., 2018; Ren et al., 2023; Satoh et al., 2015; Sun et al., 2020; Sundquist et al., 2021; Visconte et al., 2023; Wang et al., 2022a; Wu et al., 2019; Yang et al., 2022). Especially, *hsa*-miR-143-3p was found to be associated with different AD markers

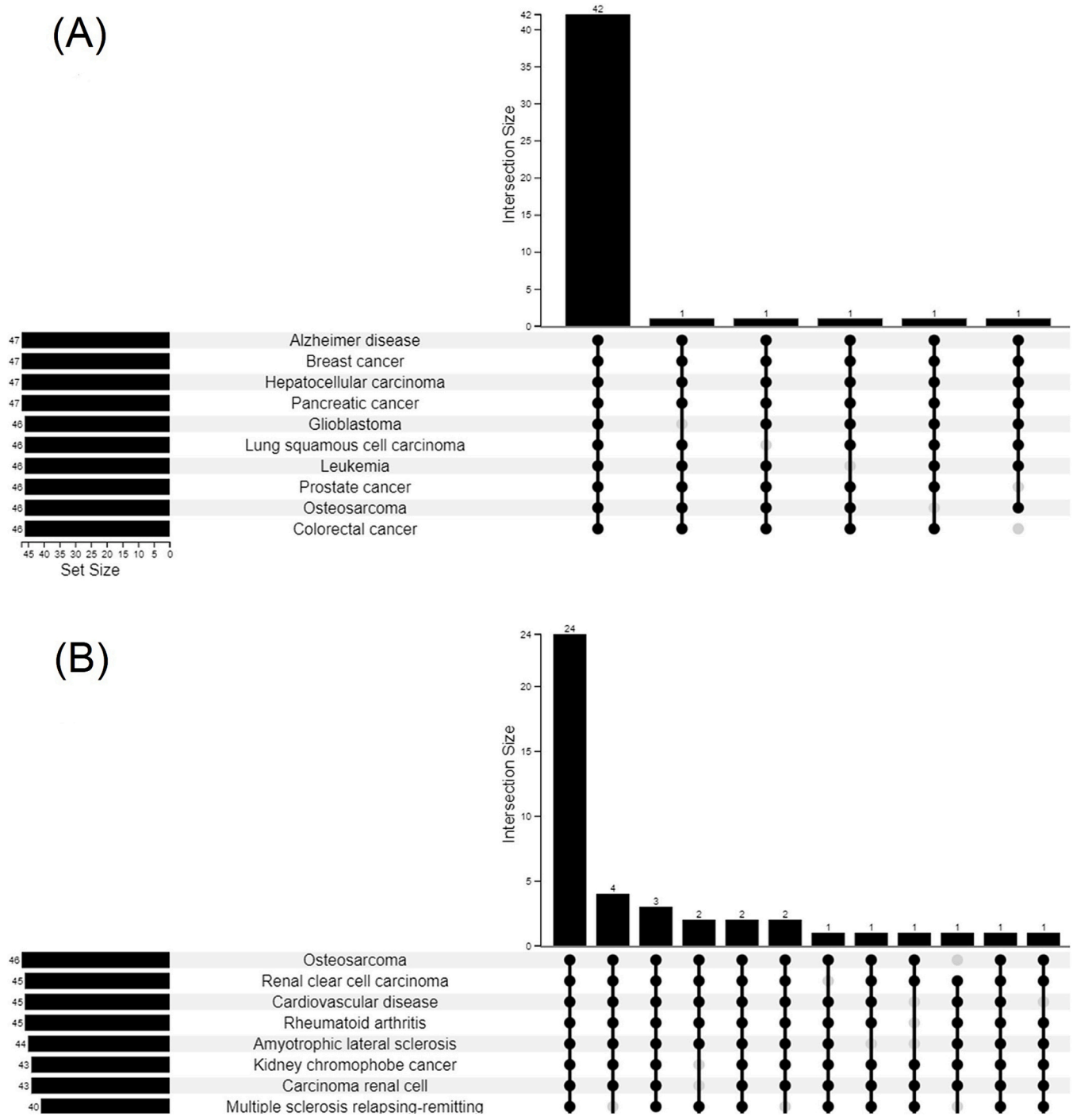

Fig. 2. Upset-plot of the results for the over-represented diseases based on the 48 significant target miRNAs (miEAA tool). A) Diseases sorted by the number of disease-associated miRNAs. B) Diseases sorted by over-representation *p*-value. In both cases autoimmune diseases, cancer, cardiovascular and neuropsychiatric diseases are among the top ones. The numbers besides the horizontal bars next to the disease names represent the total number of miRNAs in miEAA associated with the specific disease. The numbers on top of the vertical bars reflect the number of miRNAs within the testset. The black dots indicate that a miRNA set from the significant miRNAs was associated with the specific disease.

such as tau phosphorylation (Wang et al., 2022a), P-tau/Aβ42 ratio (Jia et al., 2021) or with dementia in general (Sun et al., 2020; Yang et al., 2022). Also, several of these miRNAs have been linked to blood-brain-barrier integrity or amyloid β induced neurotoxicity (Bazrgar et al., 2022; Harati et al., 2022; Li et al., 2020; Lin et al., 2019). As fibrinogen and CRP were not directly associated with WML in the TREND-0 population, the moderation by miRNAs might be an important path how inflammation affects neurodegeneration.

Taken together, our analyses show that systemic chronic inflammation is closely linked to circulating miRNA abundance in terms of regulation and moderation. The broad disease association pattern of the target miRNAs strengthens the hypothesis of an underlying common mechanism shared by all these disorders, namely chronic inflammation. Understanding this mechanism and how it promotes specific diseases might help to identify their biological causes and possible targets for intervention. A very strong signal was especially found for fibrinogen which was also measured in plasma. This is in line with findings from the population-based Rotterdam Study also observing a strong

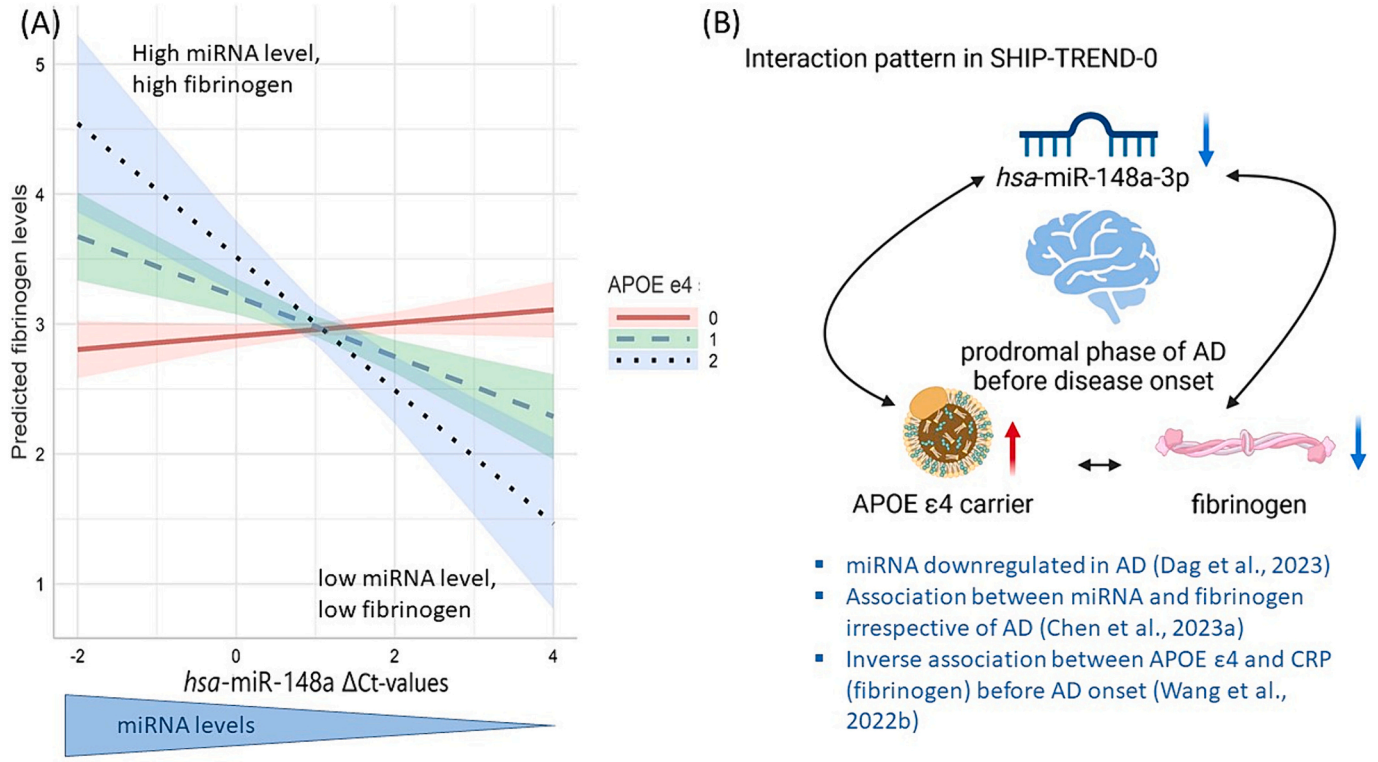

Fig. 3. A) Interaction plot for the interaction between plasma-circulating *hsa*-miR-148a-3p and *APOE* ε4 allele carrier status (here separated as 0 = non-allele carriers, 1 = one ε4 allele, 2 = ε4/ε4 carriers) on fibrinogen levels ($n = 757$, $p = 3.5 \times 10^{-4}$). Higher estimates indicate higher ΔCt-values and thus lower miRNA abundance. This is indicated by the blue triangle. B) Visualization of association pattern observed in TREND-0. Arrows indicate higher (red) or lower (blue) level of respective analyte with APOE ε4 carrier status. Supporting literature findings are coloured in blue. This pattern applies to the prodromal phase of AD before disease onset. Association patterns in AD patients may differ. Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inflammatory signal associated with miRNAs. In addition, we identified a neurodegeneration associated miRNA signal involving *APOE* ε4 carrier status and white matter lesion volume. Especially the interplay between *hsa*-miR-148a-3p, fibrinogen levels and *APOE* ε4 allele on white matter lesions could be the focus of further investigations. Implicated mechanisms of this miRNA signal were among others blood-brain-barrier permeability, amyloid β induced neurotoxicity or neuroinflammation.

Limitations of our study might be technical as our miRNA data was measured in three different batches, our data only included a limited set of miRNAs due to a fixed miRNAs PCR panel, or different biofluids for the measurement of inflammation biomarkers. However, a major limitation might be the lack of an independent replication sample not able to confirm the proposed hypotheses as well as the lack of additional mechanistic results. Drawing mechanistic conclusions out of this kind of epidemiological data is difficult as often extreme groups are missing and reveals rather unspecific associations. Further molecular research on that topic is required for example working with disease groups or exploring more specific inflammatory markers, such as interleukins, or growth factors which will be available in the SHIP-TREND study soon.

5. Conclusion

Nevertheless, we were able to prove that the levels of inflammatory markers are closely linked to changes in miRNA abundance which has a broad impact on the emergence of inflammation associated diseases and also on neurodegeneration. Further studies are needed to identify miRNA-associated molecular mechanisms linking chronic inflammation with specific diseases and especially peripheral inflammation with neurodegeneration.

Ethics statement

The investigations in the *Study of Health in Pomerania* (SHIP) were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants. The survey and study methods were approved by the institutional review boards of the University Medicine Greifswald.

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CRediT authorship contribution statement

Sandra Van der Auwera: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Sabine Ameling:** Writing – review & editing, Methodology, Data curation. **Katharina Wittfeld:** Writing – review & editing, Resources, Data curation. **Robin Bülow:** Writing – review & editing, Resources, Data curation. **Matthias Nauck:** Writing – review & editing, Resources, Funding acquisition. **Henry Völzke:** Writing – review & editing, Resources, Funding acquisition. **Uwe**

Völker: Writing – review & editing, Resources, Funding acquisition. **Hans J. Grabe:** Writing – review & editing, Resources, Project administration, Conceptualization.

Declaration of competing interest

HJG has received travel grants and speakers honoraria from Neuraxpharm, Servier, Indorsia and Janssen Cilag. All other authors declare that they have nothing to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2024.111130.

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