

The merged matrix was standardized and processed. (A-B) The figure shows two-dimensional PCA cluster plots before and after correcting for inter-batch differences removed for GSE21545 and GSE41571. (C-D) The figure shows two-dimensional PCA cluster plots before and after correcting for inter-batch differences removed for GSE28829 and GSE43292. (E-F) The figure shows box plots before and after normalization for GSE21545 and GSE41571. (G-H) The figure shows box plots before and after normalization for GSE28829 and GSE43292.



Top 30 KEGG pathways ranked by GSVA score differences between non-DEMGs cluster vs. DEMGs

cluster.



UMAP plot visualizing 10 myeloid subtypes identified from scRNA-seq in AS vs. normal groups.



The MR analysis indicated a significant causal association between the eQTL of FLT3LG and AS. (A) Dot plot shows a significant causal association between the eQTL of FLT3LG and AS. (B-C) Forest plot of MR effect size FLT3LG on AS in different datasets, respectively. (D) Forest plot shows results of forward and reverse MR Analysis in AS. (E) Left: Scatter plot shows the relationship between the significance of eQTLs for the FLT3LG gene and the significance of GWAS. Each dot represents a single nucleotide polymorphism (SNP). The color coding (referenced in the legend as r²) indicates the degree of linkage disequilibrium (LD) between the SNPs, with warmer colors like red indicating higher LD. Right: Regional association plot in on a specific region of chromosome 12 (chr12), where the FLT3LG gene is located. It shows the -log10 p-values of the association between SNPs in this region and the gene expression levels (eQTLs) and their association with AS.

Supplementary Table 1

Data source	Group	Туре	date	patient	sample	platforms	manufacturer
Carotid artery plaque	3AS:3non-AS	scRNA	10/2020	3	6	GPL18573	Illumina NextSeq 500 (Homo sapiens)
Carotid artery plaque	1AS:2non-AS	scRNA	08/2020	3	3	GPL24676	Illumina NovaSeq 6000 (Homo sapiens)
Carotid artery plaque	32AS:32non-AS	bulk	04/2013	32	64	GPL6244	Affymetrix Human Gene 1.0 ST Array
Carotid artery plaque	13EAS:16AAS	bulk	04/2011	29	29	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
Carotid artery plaque	126AS (carotid plaque):97AS (PBMC)	bulk	03/2012	223	126	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
Carotid artery plaque	16EAS:27AAS	bulk	12/2020	43	43	GPL6104	Illumina humanRef-8 v2.0 expression beadchip
Carotid artery plaque	6EAS:5AAS	bulk	12/2012	11	11	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array
	Data source Carotid artery plaque Carotid artery plaque Carotid artery plaque Carotid artery plaque Carotid artery plaque Carotid artery plaque	Data source Group Carotid artery plaque 3AS:3non-AS Carotid artery plaque 1AS:2non-AS Carotid artery plaque 3ZAS:32non-AS Carotid artery plaque 13EAS:16AAS Carotid artery plaque 12EAS:16AAS Carotid artery plaque 15EAS:27AAS Carotid artery plaque 6EAS:57AAS	Data source Group Type Carotid artery plaque 3AS:3non-AS scRNA Carotid artery plaque 1AS:2non-AS scRNA Carotid artery plaque 32AS:32non-AS bulk Carotid artery plaque 13EAS:16AAS bulk Carotid artery plaque 16AS(carotid plaque):97AS (PBMC) bulk Carotid artery plaque 16EAS:27AAS bulk	Data source Group Type ater Carotid artery plaque 3AS: 3non-AS scRNA 10/2020 Carotid artery plaque 1AS: 2non-AS scRNA 08/2020 Carotid artery plaque 32AS: 32non-AS bulk 04/2013 Carotid artery plaque 13EAS: 16AAS bulk 04/2011 Carotid artery plaque 126AS (carotid plaque): 97AS (PBMC) bulk 03/2012 Carotid artery plaque 16EAS: 27AAS bulk 12/2020 Carotid artery plaque 6EAS: 5AAS bulk 12/2012	Data sourceGroupTypedatepatientCarotid artery plaque3AS:3non-ASscRNA0/20203Carotid artery plaque1AS:2non-ASscRNA08/20203Carotid artery plaque23AS:32non-ASbulk04/201332Carotid artery plaque13EAS:16AASbulk04/201129Carotid artery plaque16EAS:cortid plaque):97AS (PBMC)bulk03/2012223Carotid artery plaque6EAS:27AASbulk12/202043	Data sourceGroupTypedatepatientsampleCarotid artery plaque3AS:3non-ASscRNA10/202036Carotid artery plaque1AS:2non-ASscRNA08/202033Carotid artery plaque12AS:32non-ASbulk04/20133264Carotid artery plaque12AS:16AASbulk04/20112929Carotid artery plaque16AS(carotid plaque):97AS (PBMC)bulk03/2012232126Carotid artery plaque16EAS:27AASbulk12/20124343Carotid artery plaque6EAS:5AASbulk12/20121111	Data sourceGroupTypedatepatientsampleplatformsCarotid artery plaque3AS:3non-ASscRNA10/202036GPL18573Carotid artery plaque1AS:2non-ASscRNA08/202033GPL24676Carotid artery plaque12AS:32non-ASbulk04/20133264GPL6244Carotid artery plaque12AS:16AASbulk04/20112929GPL570Carotid artery plaque16EAS:cr27AASbulk03/201223126GPL6104Carotid artery plaque6EAS:27AASbulk12/20124343GPL5104

EAS, early a the rosclerotic plaque; AAS, advanced a the rosclerotic plaque

The source of the datasets.

Supplementary Table 2

Items	Identification of Hub Genes in AS Based on Bioinfomatics Analysis					
	Our Findings	Cell Paper (PMID:36855107)	Cell Paper (PMID:36061826)			
Years	2024	2023	2022			
Atherosclerotic plaque subtypes with prognostic significance	cluster3 AS plaques	-	-			
A subtype of macrophage with prognostic significance	$\textbf{SPP1}^{+}/\textbf{VCAN}^{+} \text{ macrophages, IL1B}^{+} \text{ macrophages, FLT3LG}^{+} \text{ macrophages}$	SPP1 ⁺ macrophages	-			
Single cell analysis strategies	Monocle2, PySCENIC, scMetabolism, CellChart, BayesPrism	Monocle2, SCENIC, scMetabolism, NicheNet, CIBERSORTx	-			
Mendelian Randomization (MR) analysis	use		-			
Machine learning algorithms	10 algorithms		3 algorithms			
Bulk datasets use	GSE21545, GSE41571, GSE163154, GSE28829, GSE43292	GSE21545, GSE100927 GSE163154, GSE43292	GSE28829, GSE43292, GSE100927			
Verification	Mice Atherosclerotic plaque: qPCR, WB, mIF		Mice Atherosclerotic plaque: WB, mIF			

qPCR, quantitative real-time polymerase chain reaction; WB, western blot; mIF, multiplex immunofluorescence

Several findings in this study are significantly novel in comparing to what is published.