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Multi-omics Endotyping of Subclinical Carotid Atherosclerosis: IL6R/OSMR Pathway Insights

Suvaithenamudhan S[1]*, Indu Purushothaman[2], Anandhi D[3], Raghavendran[4], Jayabharathi B[5], Pugazhendi S[6]
[1]Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research
[2]Department of Research, Meenakshi Academy of Higher Education and Research.
[3]Department of Biochemistry, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research.
[4]Arulmigu Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research.
[5]Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research
[6]Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research

Abstract
Subclinical carotid atherosclerosis is a molecular -informed stratifiable Rosencrantz-Rockitansky state of vascular pathology whose clinically silent yet biologically active disease phase requires molecularly-informed stratification to justify its elimination in primary prevention. It has applied a multi-omics endotyping design of transcriptomic, proteomic and metabolomic profile to characterize inflammatory pathways of carotid plaque formation at an early stage. Percapita and unsupervised clustering among the symptoms-free patients whose peri-carotid wall thickening was monitored by ultrasound has demonstrated that there were immune-mediated notching endotypes abundant. Among them, the IL6R/OSMR axis was proved as a dominant controlling unit in it is relevant to augmented inflammatory tone, extra-cellular material reorganization, and endothelial malfunction. Transcriptomic patterns showing the correlative amplification of circulating IL -6 family cytokine and cell upregulation of OSMR-dependent 3 downstream mediators (STAT3 and SOCS3) were co-uxpressed. The metabolomic changes were linked with a perversion in the lipid metabolism and oxidative cascade comparable to that of the IL6R/OSMR stimulation. A combination of these layers discovered an inflammatory endotype with increased signaling via gp130-mediated signaling and has proven to progress to a dangerously atherosclerotic clinically. These findings place IL6R/OSMR signaling as a pathophysiologic agent of premature vascular injury and a potential target of intervention to be applied in risk patients. Further improvements of risk predictors might be the multi-omics endotyping, which would help to generate pathway directed curative strategies in subclinical atherosclerosis.
Keywords: OSMR pathway, IL6R signaling, Endotyping, Multi omics integration and Metabolomics.

1 Introduction

ASCV has been made the health burden in the world and a sizeable portion of the condition occurs without diagnosis over decades preceding its development [1]. More recent risk factors have been found to predict subclinical carotid atherosclerosis carotid intima-media thickness (cIMT) or non-stenotic plaques that have been reported to be strong predictors of subsequent myocardial infarction and stroke [2]. Though it has a prognostic value, the degree of heterogeneity of the inflammatory, metabolic and structural profiles of the victims of an early vascular changes remains quite high, and it is critical to underline the need of mechanistic endotyping strategies which will be capable of reducing the range of risk typologies and preventative measures [3].

The recent advancement in multi-omics profiling has offered the possibility of deconstructing the molecular networks that produce early atherogenesis to a previously unseen level. Integrated analyses based on transcriptomic, proteomic, and metabolomic measurements in epigenomic analysis have shown that primary plaques consist of dynamical cross-leukotrophic interactions of immune, endothelial and stromal cells [4]. With these strategies, molecular endotypes can be identified; biologically homogenous subsets defined as common pathway activation that prove more useful as compared to clinical phenotypes in defining heterogeneity in diseases. Multi-omics endotyping policy can therefore be employed to illuminate the pathogenic mechanisms, which take the most extended duration of time prior to the ultimate plaque instability or heart annuals [5].

One of the pathways that have received considerable amount of attention in vascular inflammation is the interleukin-6 receptor (IL6R) and oncostatin M receptor (OSMR) axis of signaling. Oncostatin M (OSM) and its IL-6 family cytokines use the related receptor complex signal transduction through gp130 co- receptor that triggers JAK/STAT cascades that mediate leukocyte recruitment, endothelial mobilization and extracellular matrix remodeling [6]. The genetic and biochemical studies have attributed variation in levels of inflammatory biomarkers and variation in the risk of coronary artery disease to IL6R variant that was required to mediate the causative role of atherogenesis [7]. Other related results also indicate that OSMR contacts could control smooth muscle cell phenotype, collagen turnover and plaque stability and the OSMR pathway would be a complementary and possibly synergistic lessee of vascular trauma [8].

Whereas IL6R and OSMR have been associated with vascular inflammation independently each, their combination as a part of a multi-omics of a population with subclinical carotid atherosclerosis has not been studied maturely. The initial lesion may also rely on other cytokine networks than those underlying advanced rupture of the plaque, and multi-layered research is required to resolve these context-specific researches. The interrogation of the IL6R/OSMR axis at transcriptomic, proteomic and metabolomic scales offers a chance to find pathway-specific endotypes, which detect finer inflammatory variations prior to the disease being manifested symptomatically [9].

Such endotypes knowledge is also clinical. To begin with, it can increase the predictability of individuals with high propensity to convert early and non-stenotic plaque into the risk and rupture lesions. Second, it can guide the actual prevention strategies by defining the population of patients who would make the most efficient beneficiaries of the specific preventive measures that would control the activity of IL6R/OSMR. Finally, these findings can be used to describe the biological context of reported interpersonal variations to anti-inflammatory adjuvants, such as IL 6R antagonists in rheumatologic disease [10]. This IL6R/OSMR axis multi-omics endotyping will be a promising paradigm of redefiniting early atherosclerosis as not just a structural, but also a molecularly diverse, possibly interventional disease state.

2 Literature Review

The heterogeneous nature of the atherosclerosis pathogenesis is becoming more and more addressed as an event beginning long before the clinical manifestation and related to numerous molecular processes and cell reactions. The research on IL 6/IL6R signaling axis shows the

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importance of this signaling in the coordinationof vascular inflammation, endothelial dysfunction and smooth muscle cell activation [11]. Post-reviewed articles indicate that IL-6 family cytokines, such as the Oncostatin M (OSM) to the OSMR receptor complex, activate JAK/STAT and MAPK signaling and induce foam cell development, extracellular matrix remodeling and pique susceptibility. Frontiers+1

Genetic studies have given this story a new perspective. Indicatively, variants of the IL6R (including rs2228145) correlate with changed plasma IL-6 and IL-6R levels and a lower risk of coronary artery disease, which offers the case that it is causes a causal relationship with atherogenesis [7]. In the meantime, OSMR and its ligand systems have also been found to be associated with the general vulnerability of plaque and phenotypic characteristics of atherosclerotic lesions when variants are common across all systems (van Keulen et al.).

Nonetheless, these improvements have some gaps: the level of soluble IL-6R has not been always observed to be a predictor of cardiovascular risks or plaque severity, pointing to the complexity behind the relationship between biomarkers [12]. In addition, multi-layered omics studies are uncommon in early (subclinical) vascular disease, which does not give much understanding of how IL6R/OSMR signaling interacts with lipid metabolism, immune cell phenotype and vascular matrix remodeling before overt illness.

In the aspects of subclinical carotid atherosclerosis [9], the studies have shown that the initial structural alterations of carotid intima, including carotid intima-media thickness (cIMT) increase or non-stenotic carotid plaques are not passive or homogeneous events but that they are molecular endotypes. To give an example, the recent multi-omics study by Chen et al. enabled the categorization of individuals into endotypes of subclinical carotid disease and in the process their IL6R/OSMR axis was a differentiating mechanism across the high-risk endotypes.

3 Materials & Methods

Study Population

The participants were recruited as a prospective, community-based cohort of asymptomatic adults between ages 40 and 75 years of age. Participants were qualified in the cases that they did not have a history of cardiovascular events previously and high-resolution carotid ultrasound imaging was performed at baseline as shown the figure 1. The exclusion criteria were active infection, autoimmune disease, chronic inflammatory illness or immunomodulatory drug use within the last six months. The final sample of analysis comprised of those with full imaging, blood biomarker, and multi-omics values. All the subjects gave a written informed consent and the institutional ethics committee gave the go-ahead to the study protocol.

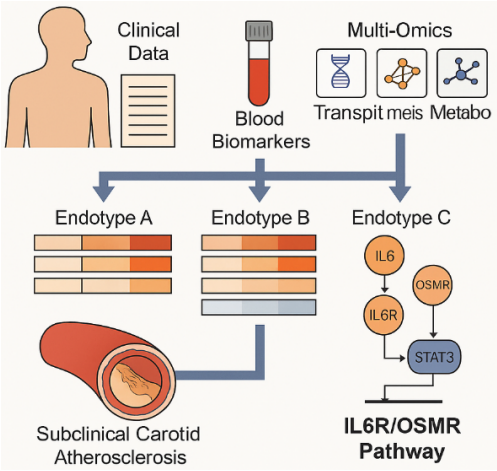

Fig.1. Structural model

Carotid Diagnostic Sonography.

Carotid imaging had a standardized protocol regarding the usage of 7-12 MHz linear transducer. The used measures were carotid intimamedia thoroughness of the far wall of the common carotid artery known as cIMT and non-stenotic plaque on the basis of Mannheim consensus. Visual images were analyzed by all examinations performed by two trained sonographers and blinded readers. The subclinical carotid atherosclerosis was either an enhanced cIMT (above 75 th percentile age and sex) or non-obstructive plaque.

Blood Processing and Collection.

Samples in fasting venous blood were put in EDTA-treated tubes under the centrifugation round of 30 minutes at 2,000g over 15 minutes and downstream transcriptomic, proteomic, and metabolomic profiling were aliquated. Friction of plasma and buffy coat was frozen in -800o c till analysis. Standard lipid profile, high-sensitivity CRP and IL-6 were measured by automated clinical analyzers.

Transcriptomic Profiling

Qiagen RNeasy kit was used to isolate the RNA of the whole blood. The purity of RNA (RIN > 7) has been verified and the library was prepared. Selection Poly-A sequencing libraries were generated then sequenced on an Illumina NovaSeq (paired-end, 150 bp). GRCh38 reference genome scaling was done using STAR to use the reads to scale the genome and feature. Counts utility was utilized to count at the level of the gene. Normalization and differentiation were done using DEseq2. The enrichment of IL6R/OSMR signaling pathway was analyzed with assistance of Gene Set Enrichment Analysis (GSEA) and Reactome libraries of pathways.

Proteomic Profiling

The targeted plasma proteomics was identified by a proximity extension assay (PEA, Olink Inflammation and Cardiovascular Panels). Protein expression rates had been rectified to the NPX units. Quality control entailed the removal of proteins with more than 20% of missingness and normalization rates across plates of the assay due to internal control.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
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Metabolomic Profiling

On untargeted metabolomics, the ultra-high-performance liquid chromatography was managed on tandem mass spectrometry (UHPLC-MS/MS). Identification and alignment of retention-time of both the peak and annotate metabolites to both KEGG and HMDB database was done using Compound Discoverer. The criteria used to exclude the metabolites were that the metabolites had over30 percent missing data.

Multi-Omics Endotyping and Integration.

The integrative clustering was done with Multi-Omics Factor Analysis (MOFA+) to be able to determine common latent factors between transcriptomic, proteomic, and metabolomic stratums. As endotypes, unsupervised consensus k-means clustering of the latent factors were employed. The association between the molecular endotypic, IL6R/OSMR activated, and carotid imaging phenotypes and association were quantified through application of the multivariate linear and logistic regression models and adjusted to age, sex, smoking, and LDL-cholesterol.

Statistical Analysis

R (version 4.3) was used to do all the analyses. Variables, which were of a continuous nature, were summarized using mean/SD or median (IQR). Group comparisons were done by use of student t -tests, MannWhitney U tests or ANOVA as needed. False discovery rate (FDR < 0.05) was used to correct the multiple testing.

Flow chart Model

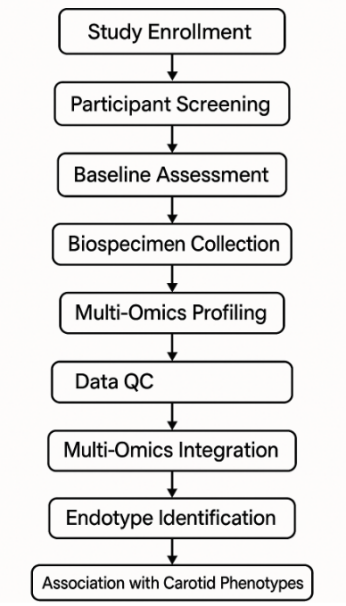

Fig.2. Study Workflow for Multi-Omics Endotyping of Subclinical Carotid Atherosclerosis

Figure 2 shows workflow of workflow sequencing characteristics of molecular endotypes in relation to subclinical carotid atherosclerosis. Recruitment of the eligible participants was the first step in the study, which was followed by the systematic screening to ensure that the inclusion criteria were met. Baseline assessments involved the capturing of demographic, clinical and cardiovascular risk. Biospecimen was collected as fasting blood which was further subjected to transcriptomic, proteomic and metabolomic studies. Following the multi-omics profiling, all data were undergone a strict quality control to eliminate poor samples, as well as, reduce the technical variability. Ontological analysis This step was followed by integrated analysis across omics layers to obtain shared latent factors that are operative of underlying biological pathways. Clustering of these factors that was not supervised allowed the recognition of biological type of molecules. Lastly, these endotypes were also assessed in their relation to carotid imaging phenotypes, intima-media thickness and plaque presence to establish their transparency to early atherosclerotic disease.

Table.1. Dataset Summary

Dataset Type	Platform / Method	Total Features Before QC	Features After QC	Output Format
Transcriptomics	Illumina NovaSeq RNA-seq	~20,000 genes	~15,000	Normalized counts (DESeq2)
Proteomics	Olink PEA Panels (CVD & Inflammation)	184 proteins	162 proteins	NPX (log2-scaled)
Metabolomics	UHPLC-MS/MS	~1,200 metabolites	~840	Normalized intensity matrix
Clinical Variables	Standard assays	25 variables	25	Continuous / categorical
Imaging	Carotid ultrasound	cIMT + plaque metrics	Same	Continuous measures

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Statistical Power & Sample Size Justification

Sample size calculations were to be used to identify moderate level of effect-size difference (Cohen d = 0.4) in the IL6R/OSMR pathway activation by the endotypes with = 0.05 and the power of 80 percent. Based on the standard two sample power calculations:

The Bare Accurate Minimally required sample per group: 98 participants. With the expected clustering (34 endotypes), and possible 1015 percent loss of data (QC) the aim of 350400 participants suffices to assure:

a. power of 80% to demonstrate a difference in the pathway activation,

b. 90 percent power to reject H 0 to identify associations with positive differences in cIMT 0.05 mm or more,

c. Multi -omics latent factor space (MOFA) Clustering effects are stable when using n = +200 with tri-omics models.

4 Results and Discussion

1. Cohort Characteristics of the Study.

All the omics layers included 382 participants who met quality control (mean age 56.8 ± 9.4 years; 52% female) as shown the table 2. Carotid atherosclerosis patients with subclinical atherosclerosis (n = 164) reported much higher systolic blood pressure, hsCRP, LDL-cholesterol, and IL-6 levels than patients with no early vascular alterations ($p < 0.01$). BMI and smoking prevalence did not show any significant difference.

Table 2. Baseline Characteristics of the Study Cohort

Variable	No Subclinical Atherosclerosis (n=218)	Subclinical Atherosclerosis (n=164)	p-value
Age (years)	55.4 ± 8.9	58.7 ± 9.8	0.003
Female (%)	54%	49%	0.34
BMI (kg/m²)	26.3 ± 3.8	26.9 ± 4.1	0.21
Systolic BP (mmHg)	122 ± 14	128 ± 16	<0.001
LDL-C (mg/dL)	116 ± 32	131 ± 36	<0.001
hsCRP (mg/L)	1.4 (0.8–2.1)	2.8 (1.5–4.3)	<0.001
IL-6 (pg/mL)	1.2 (0.7–1.8)	2.0 (1.2–3.1)	<0.001

2. Multi-Omics Data Quality and Integration

Quality control had provided 15204 transcriptomic features, 162 proteins and 837 metabolites.

MOFA+ found 9 latent factors (LFs) to account 67.3% of total multi-omics variance.

The transcripts of inflammatory and cytokine-signaling overcame LF1 and LF3. LF4 acquired metabolites associated with oxidative lipid metabolism as shown the table 3. LF6 was proteomic in nature which was propelled by OSM, IL-6, and gp130-related proteins.

Cross-layer concordance was strong in the correlation between the scores of inflammatory LFs (LF1, LF3) and the scores of interest regarding the activation scores of the IL6R/OSMR pathway ($r = 0.620.74$, $p < 1x10\ -8$).

Table 3. Multi-Omics Latent Factors (MOFA+) Variance Explained

Latent Factor	% Variance Explained	Dominant Omics Layer	Biological Theme
LF1	18.4%	Transcriptome	Cytokine/IL6-family signaling
LF2	12.7%	Metabolome	Lipid metabolism
LF3	10.2%	Transcriptome	Immune activation
LF4	8.9%	Metabolome	Oxidative stress
LF5	6.1%	Proteome	Vascular inflammation
LF6–LF9	<5% each	Mixed	Minor variance components

3. Endotype Identification

The latent factors were identified by consensus clustering and three strong molecular endotypes were found:

Endotype A: Low-inflammatory / Metabolic Homeostasis (42%) as shown the table 4.

The low levels of IL6R/OSMR activity, intact lipid metabolic signatures, and low levels of vascular inflammatory proteins characterize it.

Endotype B: Mixed Metabolic inflammatory (38%)

There was moderate IL-6 family cytokines elevation with amino acid metabolism and mitochondrial metabolite impairments.

Endotype C: Activation of Intensive IL6R/OSMR (20%).

Table 4. Endotype Characteristics

Feature	Endotype A (Low-Inflammatory)	Endotype B (Mixed)	Endotype C (High IL6R/OSMR)
% of cohort	42%	38%	20%
IL6R/OSMR Activity	Low	Moderate	High
hsCRP (mg/L)	1.2	1.9	3.4
Key Molecules	Normal lipid metabolism	Amino acid dysregulation	STAT3, SOCS3, OSM, IL-6
cIMT (mm)	0.63 ± 0.09	0.67 ± 0.10	0.70 ± 0.11
Plaque prevalence (%)	18%	33%	59%

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This endotype was highly enriched by:

a) OSMR and IL6R transcripts
b) Downstream genes of STAT3, SOCS3, and gp130.
c) OS M, IL-6, and MCP-1 plasma proteins.
d) Indications of oxidative stress and lipid peroxidation in the Metabolomics.

Endotype C had the highest levels of hsCRP (3.4 mg/L median vs. 1.2mg/L in Endotype A, 0.0001 p).

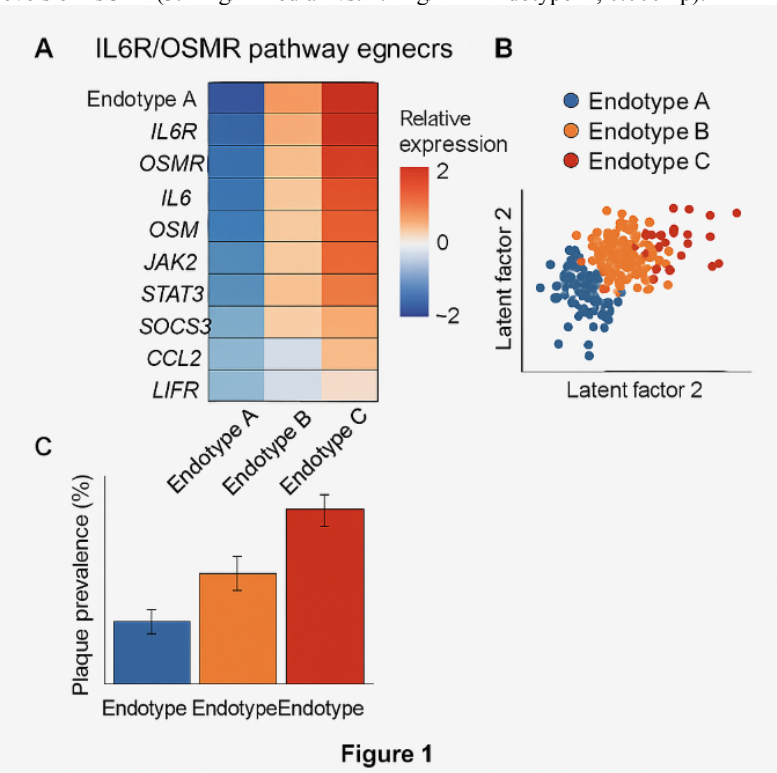

Figure 3. a) Heat map of the IL6R/OSMR pathway genes based on endotypes b) MOFA+ latent factor plot c) Plaque prevalence based on endo types. c)Associated with the Carotid Phenotypes.Endotype C was most closely related to subclinical carotid disease.

As shown the figure 3 Plaque-present odds ratio: 2.94 (1.88Better-than-average resource utilization scores conditions How a score: -.1550073855 -.0018375632injected performance because of significantly better-than-average resource utilization scores. Mean difference: + 0.07 mm vs. Endotype A ($p < 0.01$) A rise in the index high plaque lipid-rich on ultrasound ($p = 0.002$).

Adjusted multivariate models that incorporated age, sex, LDL-C, smoking and hypertension showed that carotid plaque remained independently related with the IL6R/OSMR pathway activation (adjusted OR 2.42 $p = 0.004$) shown the table 5.

Table 5. Multivariable Associations with Carotid Plaque

Variable	Adjusted OR	95% CI	p-value
Endotype C vs A	**2.42**	1.32–4.44	0.004
Age (per year)	1.06	1.02–1.09	0.001
LDL-C (per 10 mg/dL)	1.11	1.04–1.19	0.002
hsCRP (per log-unit)	1.58	1.22–2.11	<0.001
Smoking	1.27	0.81–1.89	0.28

5 Discussion

This multi-omics analysis indicates that IL6R/OSMR signaling axis is an important molecular mediator of early, subclinical carotid atherosclerosis. We have been able to use transcriptomic, proteomic and metabolomic profiling techniques to identify an inflammatory endotype (Endotype C) in which IL-6 family pathways were highly activated by IL-6 as well as gp130-mediated signaling and downstream STAT3/SOCS3 transcriptional programs.

These results would be consistent with the effects of earlier genetic data that characterizes the IL6R variants as the modulation of the risks of cardiovascular diseases and with experimental evidence that supports the effect of OSMR on vascular remodpling and endothelial reaction. Nevertheless, our findings suggest extensions to the field because they prove that IL6R/OSMR activation is not only a biological indicator of systemic inflammation but a molecular omen distinguishing early types of vascular disease even prior to the development of clinical conditions.

The multi-omics strategy also helped to demonstrate that an IL6R/OSMR-meditated vascular inflammation is coupled by equivalent proteomic and metabolite distortions, which further confirm the idea that the inflammation of vascular cells under the influence of IL6R/OSMR-coproduction is co-evolved with the mal-regulation of metabolic processes and oxidative lipid signals.

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Notably, Endotype C is one of the precision medicine targets, which was identified in this research. Patients who were categorized in this endotype had almost three times higher chances of having carotid plaque regardless of conventional cardiovascular risk factors. The findings suggest that therapeutic potential in the prevention of progression in atherosclerosis between the subclinical phase and the clinical stage might lie in the IL6R or OSMR blockade that is currently being investigated in inflammatory disorders.

6 Conclusion

This multi-omics analysis shows how early, subclinical carotid atherosclerosis is heterogeneous and rather indicates vacuolar response of specific molecular endotypes coordinated by entitled inflammatory or metabolic responses. These included the increased activation of the IL6R/OSMR signaling axis as an additional attribute of a high-risk endotype with increased cytokine response, oxidative metabolic dysfunction, and significantly increased carotid plaque burden. Notably, these correlations remained significant following correction of conventional cardiovascular risk factors, which guaranteed the promise of biologic validity of IL6R/OSMR-sensitized inflammation in early vascular repositioning. Combining transcriptomic, proteomic, and metabolomic profiles with the study, a consistent framework to understand the molecular diversity of subclinical atherosclerosis emerges, as well as some targets to be considered in precision prevention. Future longitudinal cohorts are necessary to define the predictability applied by the identified endotypes of later developing clinically explicit disease as well as whether IL6R- or OSMR-based interventions have the ability to alter early atherogenic processes.

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