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Impact of smoking on Cardiovascular risk and Premature Ageing: Findings from the STANISLAS Cohort

Tripti Rastogi¹; Nicolas Girerd¹; Zohra Lamiral¹; Emmanuel Bresso²; Erwan Bozec¹; Jean-Marc Boivin¹; Patrick Rossignol¹; Faiez Zannad¹; João Pedro Ferreira^{1,3}

¹Université de Lorraine, INSERM, Centre d'Investigations Cliniques Plurithématique 1433, INSERM 1116, CHRU de Nancy, FCRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France.

²Université de Lorraine, CNRS, Inria, LORIA, Nancy, France.

³Unidade de Investigação Cardiovascular-UnIC, Faculdade de Medicina Universidade do Porto, Porto, Portugal

Corresponding author: Tripti Rastogi

Centre d'Investigation Clinique Pierre Drouin -INSERM - CHRU de Nancy, Institut lorrain du cœur et des vaisseaux Louis Mathieu, Nancy, France.

Address: 4, rue du Morvan. 54500 Vandœuvre-Lès-Nancy

E-mail: t.gupta@chru-nancy.fr

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Take home message: The clinical markers of premature cardiovascular ageing seen in smokers were similar to non-smokers that were two decades older than smokers. Also, smoking was strongly associated with plasma proteins indicating inflammation, endothelial dysfunction and apoptosis.

Abstract

Background: Smoking may lead to premature ageing, but the impact on the cardiovascular system and circulating proteins needs further study. In the present study, we aim to understand the impact of smoking on heart and vessels and circulating biomarkers of multiple domains including cardiovascular damage, premature ageing and cancer-related pathways.

Methods: The STANISLAS Cohort is a longitudinal familial cohort with detailed cardiovascular examination and biomarker assessment. This study included all the participants enrolled in the fourth visit of the STANISLAS Cohort for whom information on smoking habits was available (n=1696). We assessed pulse wave velocity, intima-media thickness, echocardiographic parameters and a total of 460 proteins to study the association of circulating plasma proteins with smoking status (never vs. past vs. current smoking) while adjusting for potential confounders.

Results: Current smokers were approximately 18 years younger but had higher LVMI and similar PWV, cIMT, frequency of hypertension, diabetes and carotid plaques compared to the much older never smokers. After multivariate selection, 25 proteins were independently associated with current or past smoking. Current smoking was strongly associated with higher levels of EDIL-3, CCL11, TNFSF13B, KIT, and lower levels of IL-12B and PLTP ($p < 0.0001$) while past smoking was associated with FGF-21, CHIT1, and lower levels of CXCL10, IL1RL2 and RAGE ($p < 0.01$).

Conclusion: Current smoking is associated with signs of early onset of cardiovascular ageing and proteins biomarkers that regulate inflammation, endothelial function, metabolism, oncological processes and apoptosis.

Keywords: Smoking, circulating protein profile, premature ageing, STANISLAS cohort

Abbreviation: BMI: Body Mass Index; cIMT: Carotid Intima Media Thickness; eGFR: Estimated Glomerular Filtration Rate; GO: Gene Ontology; IVSd: septum thickness- diastolic; LVd: Left ventricular diameter, diastolic; LVEF: left ventricular ejection fraction; LVMI: Left Ventricular Mass Index; OR: Odds Ratio; PWTd: Left ventricular posterior wall thickness; PWV: Pulse Wave Velocity; SBP: Systolic Blood Pressure Office; STANISLAS: Suivi Temporaire Annuel Non-Invasif De La Santé Des Lorrains Assurés Sociaux

INTRODUCTION

Tobacco smoking is one of the most important risk factors for cardiovascular, metabolic, pulmonary, and oncological diseases, leading to more than 6 million deaths worldwide each year (1). Smoking causes life expectancy reduction, premature biological ageing, and early onset of multiple diseases (2).

Smoking can lead to an increase of vascular stiffness and carotid intima-media thickness, both of which are early markers of damage to the cardiovascular system (3, 4). On top of the damage to the cardiovascular system, smoking might induce alterations in markers related to premature ageing and oncologic diseases. Thus, assessing the impact of cigarette smoking in the cardiovascular system and in multiple circulating proteins of healthy people, might help expanding the knowledge about the early biological signature associated with smoking.

The STANISLAS (Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux) cohort is a prospective familial cohort including mostly “healthy” participants with a detailed cardiovascular and biomarker assessment, allowing to determine the impact of smoking in multiple organ systems and circulating proteins. The main aim of the present study is to assess the association of smoking status (current, past, and never) with markers of premature cardiovascular damage, and circulating protein expression that may indicate enhanced cardiovascular risk, premature ageing, and over-expression of cancer-related pathways.

PARTICIPANTS and METHODS

Study population

The details of the STANISLAS study have been published elsewhere(5, 6). Briefly, STANISLAS is a familial, longitudinal, single-centre cohort study with the objective to determine the genetic and environmental factors associated with cardiovascular disease onset, incidence and progression.

The study protocols for all examinations were reviewed and approved by the Local Ethics Committee of CPP –Est 3, France. All participants provided written informed consent to participate

in the study. The study data and materials can be fully available upon reasonable request to the authors.

Study design

The STANISLAS cohort started with 4295 participants (from 1006 families) recruited from 1993 to 1995 for the first visit (V1). Visits 2 and 3 were conducted from 1998 to 2001 and from 2003 to 2004, respectively; but detailed cardiovascular examinations were not conducted, nor circulating protein biomarkers were assessed. The visit 4 (V4) was conducted from 2011 to 2015 and included 1705 participants (from 634 families). In V4, participants underwent a detailed medical history, anthropometric measures, clinical and laboratory investigations including research-quality echocardiography, vascular examinations and assessment of circulating protein profile. For this analysis, we included all the participants included in V4 and for whom information on smoking status was available.

Standardized procedures were followed for all the investigations, blood, and urine sample collection. Blood samples (EDTA, heparin) were stored at central biobank facility between -80 °C and -196°C.

Smoking status and estimation of smoking duration

At V4, participants were interviewed for assessing present smoking status (never, past, and current), duration and intensity of smoking. Intermittent smokers who smoked at the time of interview were considered as current smokers. Current and past smokers were asked for how long have they been smoking and for how long have they smoked, respectively. In addition, past smokers were enquired for how long they have stopped smoking.

Circulating proteins

Circulating plasma proteins were analysed using the Olink Proseek® Multiplex cardiovascular (CVD) II, III, cardiometabolic (CM), organ damage (OD) and inflammatory (INF) panels. The protein assay is based on proximity extension assay (PEA) technology, where 92 Oligoneucleotide-labelled antibody probe pairs per panel (total of 92×5 ; $n=460$ proteins) are allowed to bind to their

specific target protein. Target protein bound Oligoneucleotide probe pairs hybridize giving rise to DNA amplicon with each ID- barcoding their respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark™ HD real- time PCR platform. The platform provides log₂-normalized protein expression (NPX) data. Full details are described in White paper from Olink (<https://www.olink.com/resources-support/white-papers-from-olink/>).

Statistical analysis

Baseline continuous variables are expressed as mean \pm standard deviation ($m \pm SD$) or as median and inter quartile range depending on the normality distributions, and categorical variables as frequencies (percentage). Comparison of baseline characteristics was performed using ANOVA or Kruskal–Wallis and Chi-squared or Exact Fisher test as required.

To study the association between smoking status (current, past, never smokers) and circulating proteins, multinomial logistic regression models were used (never smokers were set as the referent category). First, the potential association of each circulating protein with current and past smoking was determined while adjusting for relevant clinical confounders at V4 (sex, age, body mass index [BMI], systolic blood pressure office [SBP], diabetes, total cholesterol, microalbuminuria, estimated glomerular filtration rate [eGFR], Pulse Wave Velocity [PWV], carotid intima media thickness [cIMT], carotid plaques, left ventricular mass index [LVMI], use of antihypertensive drugs and lipid lowering drugs). We further adjusted the above mentioned model using Ankle Brachial Index (ABI) as additional clinical confounder to verify the association of smoking status with circulating proteins (*Supplemental table S4*). Second, the p-value of the association of each protein biomarker was corrected for false discovery rate (FDR set at 1%) using the Benjamini-Hochberg method. Subsequently, a multivariable stepwise forward selection model set at 5% (p-value ≤ 0.05 was considered to stay in the model and p-value > 0.05 was considered for removal from the model) was performed using all significant proteins at 1% of FDR and “forcing” the clinical variables in the model. Odds ratios and their 95% confidence intervals are reported for the proteins which had overall significant p-value of association with smoking status. Since the

circulating proteins were measured using NPX values on a \log_2 scale, the odds ratio (OR) represents the association per doubling in protein concentrations.

In addition, we assessed the association of circulating proteins with smoking status separately for the “parents” enrolled in the STANISLAS cohort to verify if the results were influenced by presence of family members (*Supplementary table S5*).

Since there was large difference in age and sex of the participants in each group, we performed a propensity score matching on age and sex on the whole population of 1696 patients. However, this method drastically reduced our sample size. We found that only 210 patients were well matched according to sex and age (70 patients in each group of never smoker, current smoker or past smoker). Thereafter, we decided to perform inverse probability weighting (IPW) analyses in order to keep the whole population in the analysis.

The propensity score (PS) representing the *a priori* likelihood of being in smoking status category (never smoker, current smoker or past smoker) was computed using multinomial logistic regression of the smoking status on characteristics for the full sample (sex, age, BMI, SBP, diabetes, total cholesterol, microalbuminuria, eGFR, PWV, cIMT, carotid plaques, LVMI, use of antihypertensive drugs and lipid lowering drugs):

An inverse probability weighting (IPW) analyses was then performed using stabilized weights (*Supplementary table S6*). The balance between the three groups (never smoker vs. current smoker, never smoker vs. past smoker and current smoker vs. past smoker) were assessed using absolute standardized mean differences. For each variable, the absolute standardized mean difference represents the absolute difference between the mean values in each two groups divided by the common SD. We performed then weighted multinomial regression models between smoking status and circulating proteins.

The two-tailed significance level was set at $p < 0.05$. All analyses were performed using SAS version 9.4.6 (SAS Institute Inc., Cary, N.C., USA).

Network Analysis

The complex network analysis was performed using the Fight Heart-Failure Graph Knowledge Box (FHF-GKB)(7), a platform representing the most available public knowledge on the human proteins, protein-GO (Gene Ontology) terms annotation and protein-pathways relationship, provided by Edgeleap company. The FHF-GKB complex network was queried in order to explore pathways and biological process that connect the biomarkers. All the GO terms from the biological process aspect associated with the biomarkers were retrieved. Then for each GO term, the number of linked biomarkers is compared to the number of annotated proteins in the whole FHF-GK Box using Fisher's exact Test. The resulting p-values are then corrected into FDR according to the Benjamini-Hochberg procedure. Only GO terms with FDR values less than 0.05 were considered for further analysis. The resulting graph is displayed in figure 2 illustrating the main connection between proteins and overrepresented biological processes.

RESULTS

Baseline characteristics

The mean age of participants was 55 years and 51% were women; 46% of the participants had never smoked, 32% had stopped smoking, and 22% were current smokers. (*Table 1 & Figure 1*).

Current smokers were younger than past and never smokers; median age (pct₂₅₋₇₅): 36.5 (31.0 - 56.0) vs. 58.0 (52.0 - 62.0) vs. 55.0 (34.0 - 60.0), respectively ($p < 0.001$). In current smokers, the median duration of smoking was 16 (pct₂₅₋₇₅: 12-30) years.

Despite being ~18 years younger, current smokers had similar frequency of hypertension and diabetes, and had higher microalbuminuria, LVMi and lower HDL levels than never smokers.

Moreover, current smokers had similar cIMT (median [pct₂₅₋₇₅] 592.5 [510.9 - 669.0] vs 612.0

[525.0 714.0]), PWV (median [pct₂₅₋₇₅] 7.9 [7.1 - 8.8] vs 8.0 [7.2 - 9.2]), and carotid plaques (9.8% vs 9.1%) than never smokers.

Past and never smokers had similar age, but despite the similar age a higher proportion of past smokers had diabetes, carotid plaques and used antihypertensive and lipid lowering drugs as compared to never smokers. Furthermore, cIMT was higher in past smokers compared to never smokers. A similar pattern was observed in the past smokers who stopped smoking more than 14 years ago. (*Table 1 & Supplemental Table S1*).

In “parents” subgroup of the cohort, the median age(pct₂₅₋₇₅) of participants was 59 (57-63) years. Both, current smokers and past smokers had higher microalbuminuria, LVMi, PWV and increased presence of carotid plaques compared to never smokers. (*Supplemental Table S2*).

Association of smoking status and circulating proteins

After multivariable selection, 25 proteins were independently associated with smoking: 14 with current smoking, 6 with both current and past smoking, and 5 with past smoking alone (*Table 2*).

Particularly, compared to never smokers, current smokers expressed higher levels of EDIL-3 (EGF-like repeat and discoidin I-like domain-containing protein 3), CCL11 (Eotaxin-1), TNFSF13B (Tumour necrosis factor ligand superfamily member 13B) and KIT (Mast/stem cell growth factor receptor Kit) ($p < 0.0001$ for all after correction for potential confounders and multiple testing). On the other hand, current smokers expressed lower levels of IL-12B (Interleukin-12 subunit beta) and PLTP (Phospholipid transfer protein) ($p < 0.0001$ for both after correction for potential confounders and multiple testing). A similar pattern of association of circulating protein (EDIL-3, CCL11, TNFSF13B, IL-12B, SCF/KIT, PLTP) in current smokers was seen after adjusting for ABI (*Supplemental table S4*). Similarly, after IPW analyses, CCL11, TNFSF13B, KIT, and IL-12B remained significantly associated with current smoking status (*Supplemental table S6*).

Both current and past smoker expressed higher levels of ADM (Adrenomedullin), VEGF-D (vascular endothelial growth factor -D) and IGFBP-2 (Insulin-like growth factor binding protein-2)

and lower levels of TR (transferrin receptor) and IGFBP-1 (Insulin-like growth factor binding protein-1). However, VEGF-D did not remain significantly associated with the smoking status after adjustment for ABI (*Supplemental table 3*). After IPW analyses, VEGF-D and TR remained significantly associated current and past smoking (*Supplemental table S6*). However, EDIL-3 was significantly associated with both current and past smokers after IPW adjustments.

Past smoking was associated with higher levels of FGF-21 (Fibroblast growth factor 21), CHIT1 (Chitotriosidase-1), and lower levels of CXCL10 (C-X-C motif chemokine 10), IL1RL2 (Interleukin-1 receptor-like 2) and RAGE (Receptor for advanced glycosylation end products). After IPW analyses, CHIT1, FGF-21, RAGE and IL1RL2 remained significantly associated with past smoking status (*Supplemental table S6*).

In the “parents” subgroup of the STANISLAS cohort, a total of 17 proteins were significantly associated with smoking status. 11 proteins associated with smoking status in the “parents” subgroup were same as those identified in the whole cohort. Further, the pattern of association of circulating proteins with smoking status remained similar: Current smoker had significantly higher levels of CCL-25, KIT, VEGF-D and lower levels of IL-12 B and PLTP. Both current and past smokers had higher levels of KIM1 (Kidney injury molecule-11) and lower levels of TR and LOX-1 (Lectin-like oxidized LDL receptor1) and past smokers expressed higher levels PLTP, FGF-21 and lower levels of IL1RL2. (*Supplemental Table S5*).

DISCUSSION

This study shows that current smokers have features of premature cardiovascular ageing, similar to those of people two decades older; furthermore, current smokers express higher levels of proteins associated with inflammatory processes (e.g., IL 12B, TNFSF13B, CCL11), endothelial damage/function (e.g. EDIL-3), metabolic dysregulation (e.g. PLTP), oncologic processes (e.g., KIT, EDIL-3), and apoptosis (e.g., TNFSF13B, KIT). The different protein profile of smokers, past

and never smokers could provide an insight on the underlying protein pathways of the sub-clinical changes of premature ageing and other diseases associated with smoking.

In the STANISLAS cohort, current smokers had higher LVMI and increased presence of carotid plaques compared to non-smokers. Large observational studies have reported similar findings such as ventricular hypertrophy, subclinical atherosclerosis in smokers(8, 9). Sub-clinical cardiovascular diseases like increased LVMI, PWV and carotid plaques are independent risk markers of cardiovascular morbidity and mortality. Further, PWV is linearly associated with vascular stiffness. The reference value of PWV for age group 30-39 was found to be 6.4 (5.2–8.0) m/s while that for 50-59 was 8.1 (6.3–10.0) m/s(10). The PWV of smokers (median age 36.5 years) in our study corresponds to the PWV of 50-59 years of age group, pointing towards an accelerated vascular ageing in smokers. Additionally, low HDL suggests an adverse lipid profile among current smokers (11).

Cigarette-smoke induced oxidative stress and inflammatory immune response are the two likely mechanisms that explain premature ageing and increased incidence of chronic diseases in smokers (12). Several studies have demonstrated that smoking induces a pro-inflammatory state along with accelerated ageing called “inflamm-ageing”(13, 14). Inflamm-aging is associated with overexpression of cytokines such as the TNF superfamily and its receptors, and interleukins and leads to a decline in overall health and increases the risk of multi-morbidity (14). Among current smokers, we found a strong association between smoking and overexpression of proteins mediating inflammatory response (CCL-11 and TNFSF13B). CCL-11 is a potent chemoattractant for eosinophils associated with allergic inflammatory response, while TNFSF13B, an inflammatory cytokine, regulates B-cell and T-cell activation, recruitment and survival. Our findings are concordant with previous *in vivo* studies that demonstrated cigarette smoke induces the expression of CCL-11 and TNFSF13B in the nasal mucosa and bronco-alveolar lavage fluid, respectively(15, 16). Mechanistic studies in human cell lines have shown that increased expression of CCL-11 plays a role in vascular inflammation and the atherosclerotic process by increasing endothelial

permeability and vascular smooth muscle calcification in presence of reactive oxygen species (17, 18). On the other hand, overexpression of TNFSF13B, is associated with atherosclerotic diseases and chronic obstructive pulmonary disease (COPD)(16, 19). Moreover, TNFSF13B signalling activates NF-kB pathway and MAP/ERK kinase pathways which inhibits apoptosis and may lead to cancer progression (20). Increased expression of TNFSF13B is reported in multiple myeloma, lung and breast cancers (21). In contrast to raised TNFSF13 B and CCL-11, current smokers had reduced levels of IL-12B which is a cytokine modulator of T-cell and NK cell function. Cigarette smoke suppresses IL-12B production by activating MAP Kinase (22). Reduced levels of IL-12 predispose smokers to increased risk of bacterial infection and cancers by altering immune cell regulation (23). Moreover, in a study on the impact of lifestyle choices on ageing, it was shown that lower IL-12B predicted increase in the biological age (vs chronological age) in smokers(24).

Smokers had high expression of EDIL-3, a glycoprotein expressed by endothelial cells, which promotes neo-angiogenesis by facilitating adhesion of vascular smooth muscle cells to endothelial cells(25). EDIL-3 expression is upregulated in injured vessels walls with neo-intimal thickening (25). EDIL-3 overexpression could be a possible mechanism leading to high cIMT in smokers. Further, it is noteworthy that several studies have reported the association of EDIL-3 with pathological angiogenesis in cancer cells(26, 27). In addition, smoking upregulated KIT (receptor tyrosine kinase expression); KIT activation regulates several physiological processes. However, dysregulated KIT expression is seen in several cancers(28).

In our study, current smoking was associated with reduced levels of circulating PLTP. PLTP is a key protein in lipid metabolism and reverse cholesterol transport(29, 30). A study in normolipidemic smokers reported that smokers have 40% lower PLTP activity and had a higher concentration of pro-atherogenic lipoproteins compared to non-smokers(31). However, there are conflicting evidence regarding the PLTP activity levels and the risk of cardiovascular disease. Both, higher and lower levels of PLTP activity are associated with an increased risk of atherosclerosis(29,

30). Moreover, recent evidence suggests that PLTP may regulate inflammation and progression to COPD in lung epithelial cells in response to cigarette smoke (32).

Smoking was associated with several proteins (higher levels of EDIL-3, CCL-11, PLTP, MMP-10, TNFSF13B and lower levels of TWEAK and AXL) that are known to initiate and facilitate progression of atherosclerotic lesion. It is well-established that smoking causes atherosclerosis, a fibro fatty lesion central to cardiovascular diseases, by increasing oxidative stress, vascular inflammation and dysfunction, platelet coagulation and impaired lipid profile. CCL-11 and EDIL-3 mediate vascular inflammation - triggering vascular smooth muscle proliferation which contributes towards plaque progression. Also, CCL-11 and MMP10 cause increased calcification of vessel wall(17, 25, 33). A recent study showed that CCL-11 mediated activation of eosinophils create extracellular eosinophilic trap and enhances atherogenic platelet adhesion to vessel wall (34). Similarly, TWEAK-receptor interaction induces expression of adhesion molecule in endothelial cells and cytokine production that lead to smooth muscle cell proliferation (35). Pathological intimal thickening and calcification causes atheroma progression and plaque instability. TNFSF13B and PLTP have been associated with atherogenic potential but exact mechanisms are not yet elucidated (20, 29). In contrast, *in vitro* studies, have demonstrated that over expression of AXL inhibits calcium accumulation in vascular smooth muscle cells suggesting diminished protective effect at lower levels(36).

In addition, smokers had high levels of several other circulating proteins which have a role in regulating lung inflammation and alveolar matrix degradation leading to chronic obstructive pulmonary disease (SCGB3A2, MMP-10, CCL-11 and TNFS13B) (16, 37-39). Modified expression of SCGB3A2 in proximal and distal airway epithelium is associated with modulation of fibrosis in lungs and remodelling of airway epithelium seen in COPD (39, 40). However, most of the studies reported here were conducted on bronchoalveolar lavage fluid or in the *in vitro* cell lines and not in the blood or plasma of smokers. Profile of circulating proteins may be different from the protein expression at the local *milieu* in the lungs.

In past smokers, we found that there was an increased association of smoking with diabetes, raised cIMT, presence of carotid plaques and use of lipid-lowering drugs. Correspondingly, we found a significant association with markers of cardiovascular and metabolic risk (VEGFD, IGFBP-1, IL1RL2, FGF-21). Previous studies have published similar findings where past smoking was associated with increased risk of atherosclerotic diseases compared to never smokers (41). Notably, ADM, VEGF-D, IGFBP-1, IGFBP-2 and TR were significantly associated with both current and past smoking status. The level of circulating proteins associated with inflammatory immune response became normalized in past smokers. These findings suggest that smoking-induced organ damage do not completely recede on smoking cessation. Despite the absence of active smoking in past smokers, the level of several circulating proteins having protective effect against vascular injury remained at comparable levels as of current smokers. In fact, ADM, VEGF and IGF/IGFBP may have large overlap with each other in their downstream signalling pathways in cardiac and endothelial cells(42). ADM and VEGF-D over-expression have a dual response on cardiovascular system; ADM reduces fluid shear-stress in vessel walls (eNOS pathway) (43), but its overexpression is also associated with increased risk of atherosclerotic plaque formation and calcification (44, 45). Similarly, VEGF-D protects against oxidative stress (46), but its overexpression increases the risk of cardiac fibrosis (47). Additionally, mechanistic studies in cell lines demonstrated that ADM upregulates VEGF expression in cell lines, thereby promoting angiogenesis (48). The IGF system plays a role in insulin sensitization, VSMC proliferation and endothelium mediated nitric oxide production(49). Over-expression of IGFBP-1 protects against oxidative stress induced senescence of human coronary arterial endothelial cells by activating *akt* signalling (50). In our study, smokers and past smokers had lower level of IGFBP-1 suggesting a reduced protective potential of IGFBP-1. It appears that the complex cross-talk among dysregulated protein expressions have a potential role in increased risk of cardiometabolic disease in past smokers.

Further, relatively few markers of the inflammatory response (RAGE, CXCL10) were associated with the protein profile of past smokers suggesting that smoking cessation may halt the progression towards the early onset of chronic diseases in past smokers. Similar findings have been reported previously where successful smoking cessation for over one year was associated with a significant reduction in inflammatory markers of cardiovascular diseases (51). However, lower levels of RAGE could also be due to use of statins and anti-hypertensive medication in past smokers. These drugs protect against AGE-RAGE signalling mediated harmful effects by inhibiting RAGE expression (52).

In “parents” subgroup of the cohort, a similar pattern of association was seen between circulating proteins and smoking status suggesting the results are not largely influenced by family members in the cohort. Current smoking was mostly associated with altered expressions of proteins regulating inflammatory pathway (EDIL-3, KIT, IL-12B, IL-27) whereas past smoking was associated with proteins associated with increased cardiovascular metabolic risk (FGF-21, IL1R2). Certainly, there are some changes in the protein profile in the “parents” subgroup likely due to older age of participants in this subgroup.

Few clinical trials have investigated the use of anti-inflammatory and antioxidant therapies for reducing cardiovascular risk in smokers and non-smokers and reported modest benefits (53, 54). The possible reason for the limited success of anti-inflammatory and anti-oxidant therapy could be due to the vast array of plasma proteins are altered (24, 55) and several pathways may be interlinked. However, the interaction between inflammatory markers and biomarkers of endothelial dysfunction seems to be the underlying pathological mechanisms triggered in smokers. Besides, some of the proteins may be elevated as a compensatory response to oxidative stress and organ damage. Increased expression of ADM and VEGF-D counters oxidative stress and improves vascular integrity and endothelial repair (43, 46). However, prolonged expression of these proteins may cause vascular remodelling and organ damage. These results underscore the need for a better understanding of protein pathways and assessment of novel proteins biomarkers as potential

therapeutic target in smoking-related chronic diseases. Of course, the best way to decrease the impact of smoking-related organ damage is to develop better strategies for preventing people from smoking and help those who smoke to quit as early as possible. Indeed, our study suggest that past smokers also have higher risk of organ damage, however some of that risk may be mitigated after quitting smoke.

Limitations

Some limitations should be acknowledged in the present study. First, the analysis is cross-sectional. We cannot determine the directionality of the association between circulating proteins and smoking. Second, we could not study the influence of duration and intensity of cigarette smoking and the circulating plasma protein profiles. Third, the proteins concentrations were measured as standardized NPX value at \log_2 scale and hence, no conversion to standard values is possible. Fourth, we could not study the association between passive smoking and circulating proteins because we did not have information about exposure to passive smoking. Lastly, we did not study the association between onset of COPD, overt cardiovascular disease or cancers and circulating proteins because of the very young age of smokers. Nevertheless, investigating the changes in the protein profile due to smoking will enhance the understanding of protein pathways that contribute towards the protective as well as the causative role of circulating proteins in smoking-related diseases.

Conclusion

Current smoking was associated with signs of vascular ageing such as raised PWV, cIMT and more frequent carotid plaques. Further, several protein biomarkers of premature ageing, cardiovascular disease, inflammation, and cancer-related pathways were associated with smoking. The distinct protein profile of current and past smokers provides insight into protein pathways that may be involved in the onset of smoking-related diseases and may help in the development of preventive strategies.

Conflict of interest statement

Dr. Girerd reports personal fees from AstraZeneca, personal fees from Bayer, personal fees from Boehringer, personal fees from Lilly, personal fees from Novartis, personal fees from Vifor, outside the submitted work; .Dr. ROSSIGNOL reports personal fees from Sequana medical, grants and personal fees from AstraZeneca, grants, personal fees and non-financial support from Fresenius, grants and personal fees from Novartis, personal fees from Grunenthal, non-financial support from Servier, personal fees from Stealth Peptides, grants and personal fees from Vifor Fresenius Medical Care Renal Pharma, personal fees from Idorsia, personal fees from NovoNordisk, personal fees from Ablative Solutions, personal fees and non-financial support from G3P, personal fees from Corvidia, grants and personal fees from Relypsa, a Vifor Compagny, and Vifor, personal fees from KBP, personal fees from Sanofi, grants and personal fees from Bayer, personal fees and other from CardioRenal, outside the submitted work; .Dr. Zannad reports personal fees from Janssen, personal fees from Bayer, personal fees from Amgen, personal fees from CVRx, personal fees from Boehringer, other from cardiorenal, personal fees from AstraZeneca, personal fees from Cardior, personal fees from Cereno pharmaceutical, personal fees from Applied Therapeutics, personal fees from Merck, other from CVCT, personal fees from Novartis, personal fees from NovoNordisk, personal fees from Myokardia, personal fees from Actelion, personal fees from Owkin, personal fees from Cellprothera, personal fees from Netslé HealthScience, outside the submitted work. Dr Rastogi, Dr Lamiral, Dr Bresso, Dr Boivin, Dr Bozec, Dr. Ferreira have no conflict of interest to declare.

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Author contribution

Tripti Rastogi: Writing-Original Draft, Visualization; Nicolas Girerd: Writing - Review & Editing, Supervision; Zohra Lamiral: Software, Formal analysis; Emmanuel Bresso: Software, Formal analysis, Visualization; Erwan Bozec: Investigation, Data Curation; Jean-Marc Boivin: Writing - Review & Editing; Patrick Rossignol: Writing - Review & Editing, Supervision; Faiez Zannad: Writing - Review & Editing, Supervision; João Pedro Ferreira: Conceptualization, Methodology, Writing- review & editing

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Table 1 Participants characteristics at V4 in whole population and according to smoking status

Characteristic	n	Overall	Never smoked		Current smoker		Past smoker		Over all p-value	p A vs. B	p A vs. C	p B vs. C
			n	(A)	n	(B)	n	(C)				
Clinical features												
Female gender	1696	870 (51.3 %)	787	471 (59.8 %)	366	178 (48.6 %)	543	221 (40.7 %)	< 0.0001	0.0005	< 0.0001	0.018
Age (years)	1696	55.0 (34.0 - 60.0)	787	55.0 (34.0 - 60.0)	366	36.5 (31.0 - 56.0)	543	58.0 (52.0 - 62.0)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
WC (cm)	1687	89.0 (80.0 - 99.0)	785	87.0 (78.0 - 96.0)	365	87.0 (78.0 - 96.0)	537	94.0 (84.0 - 102.0)	< 0.0001	0.87	< 0.0001	< 0.0001
BMI (Kg/m ²)	1695	25.15 (22.48 - 28.49)	787	24.62 (22.35 - 28.12)	365	24.50 (21.88 - 27.59)	543	26.33 (23.28 - 29.69)	< 0.0001	0.066	< 0.0001	< 0.0001
SBP office (mm Hg)	1696	123.7 (114.3 - 135.0)	787	123.3 (114.0 - 134.3)	366	121.7 (112.3 - 131.7)	543	126.0 (116.3 - 138.3)	< 0.0001	0.019	0.003	< 0.0001
DBP office (mm Hg)	1696	72.4 ± 8.9	787	72.1 ± 8.4	366	71.0 ± 8.7	543	74.0 ± 9.4	< 0.0001	0.045	0.0003	< 0.0001
Heart rate (bpm)	1696	63.3 (57.3 - 69.3)	787	63.7 (58.0 - 69.7)	366	62.0 (55.7 - 68.0)	543	63.3 (57.7 - 69.3)	0.001	0.001	0.56	0.007
24-h SBP (mm Hg)	1586	120.2 ± 10.3	745	119.4 ± 9.8	329	120.0 ± 10.6	512	121.5 ± 10.6	0.002	0.33	0.002	0.095
24-h DBP (mm Hg)	1586	74.3 ± 7.3	745	73.5 ± 6.9	329	74.5 ± 7.5	512	75.3 ± 7.5	< 0.0001	0.079	< 0.0001	0.078
HTA 24-h	1589	657 (41.3 %)	746	289 (38.7 %)	330	108 (32.7 %)	513	260 (50.7 %)	< 0.0001	0.059	< 0.0001	< 0.0001
HTA office	1680	544 (32.4 %)	780	238 (30.5 %)	365	83 (22.7 %)	535	223 (41.7 %)	< 0.0001	0.006	< 0.0001	< 0.0001
Treated HTA	1664	307 (18.4 %)	778	134 (17.2 %)	361	44 (12.2 %)	525	129 (24.6 %)	< 0.0001	0.029	0.002	< 0.0001
Ankle brachial index	1670	1.13 ± 0.12	768	1.12 ± 0.12	364	1.12 ± 0.12	538	1.14 ± 0.13	0.039	0.53	0.036	0.023
Diabetes	1694	70 (4.1 %)	785	24 (3.1 %)	366	8 (2.2 %)	543	38 (7.0 %)	0.0003	0.4	0.002	0.002

Characteristic	n	Overall	Never smoked		Current smoker		Past smoker		Over all p-value	p A vs. B	p A vs. C	p B vs. C
			n	(A)	n	(B)	n	(C)				
Laboratory Findings												
Glucose (g/L)	1689	0.88 (0.82 - 0.95)	786	0.88 (0.82 - 0.94)	363	0.86 (0.80 - 0.92)	540	0.91 (0.85 - 0.99)	< 0.0001	0.007	< 0.0001	< 0.0001
Glycated Hb (%)	1669	5.6 (5.3 - 5.8)	776	5.5 (5.3 - 5.8)	361	5.5 (5.3 - 5.8)	532	5.6 (5.4 - 5.9)	0.0002	0.72	0.0009	0.001
Total cholesterol (g/L)	1689	2.10 (1.85 - 2.40)	786	2.12 (1.87 - 2.42)	363	2.04 (1.79 - 2.28)	540	2.14 (1.87 - 2.46)	0.0001	0.0003	0.5	0.0003
Triglycerides (g/L)	1689	0.92 (0.68 - 1.26)	786	0.88 (0.64 - 1.19)	363	0.94 (0.66 - 1.34)	540	0.95 (0.74 - 1.33)	0.0005	0.065	0.0003	0.26
HDL (g/L)	1689	0.58 ± 0.14	786	0.59 ± 0.14	363	0.55 ± 0.13	540	0.58 ± 0.15	< 0.0001	< 0.0001	0.18	0.0009
LDL (g/L)	1685	1.34 ± 0.34	785	1.35 ± 0.34	362	1.29 ± 0.34	538	1.35 ± 0.35	0.006	0.008	0.94	0.008
Microalbuminuria (mg/L)	1684	6.0 (3.5 - 9.9)	782	5.4 (3.1 - 9.0)	362	7.2 (4.4 - 11.9)	540	6.0 (3.8 - 10.3)	< 0.0001	< 0.0001	0.004	0.006
eGFR (mL/min/1.73m ²)	1689	96.5 (86.9 - 107.6)	786	96.1 (86.4 - 107.3)	363	104.7 (95.2 - 115.3)	540	93.3 (84.3 - 99.9)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PWV (m/s)	1662	8.1 (7.3 - 9.3)	768	8.0 (7.2 - 9.2)	361	7.9 (7.1 - 8.8)	533	8.6 (7.6 - 9.9)	< 0.0001	0.009	< 0.0001	< 0.0001
cIMT	1658	621.0 (532.0 - 723.0)	770	612.0 (525.0 - 714.0)	358	592.5 (510.9 - 669.0)	530	671.5 (572.0 - 781.0)	< 0.0001	0.004	< 0.0001	< 0.0001
cIMT>900	1658	99 (6.0 %)	770	38 (4.9 %)	358	12 (3.4 %)	530	49 (9.2 %)	0.0005	0.23	0.003	0.002
Carotid plaques	1660	210 (12.7 %)	772	70 (9.1 %)	358	35 (9.8 %)	530	105 (19.8 %)	< 0.0001	0.7	< 0.0001	< 0.0001
LVEF (%)	1582	65.3 (61.0 - 69.3)	732	65.6 (61.7 - 69.4)	341	64.4 (60.1 - 68.5)	509	65.4 (60.6 - 69.9)	0.014	0.008	0.42	0.082
IVSd 2D (mm)	1675	0.95 ± 0.18	777	0.93 ± 0.18	364	0.92 ± 0.17	534	0.99 ± 0.18	< 0.0001	0.66	< 0.0001	< 0.0001
LVd 2D (cm)	1673	4.55 ± 0.52	777	4.48 ± 0.50	364	4.64 ± 0.50	532	4.60 ± 0.55	< 0.0001	< 0.0001	0.0006	0.14

Characteristic	n	Overall	Never smoked		Current smoker		Past smoker		Over all p-value	p A vs. B	p A vs. C	p B vs. C
			n	(A)	n	(B)	n	(C)				
PWTd 2D (mm)	1673	0.88 ± 0.17	777	0.86 ± 0.17	364	0.87 ± 0.17	532	0.91 ± 0.18	< 0.0001	0.36	< 0.0001	0.001
LVMi (g/m ²)	1672	74.0 (62.6 - 87.4)	777	71.7 (60.5 - 84.3)	363	74.6 (64.1 - 86.6)	532	76.7 (66.3 - 91.5)	< 0.0001	0.018	< 0.0001	0.014
Medications												
Antihypertensive drugs	1696	339 (20.0 %)	787	143 (18.2 %)	366	49 (13.4 %)	7543	147 (27.1 %)	< 0.0001	0.042	0.0002	< 0.0001
Lipid lowering drugs	1696	259 (15.3 %)	787	94 (11.9 %)	366	40 (10.9 %)	543	125 (23.0 %)	< 0.0001	0.62	< 0.0001	< 0.0001

IVSd: septum thickness- diastolic; LVd: Left ventricular diameter, diastolic; PWTd: Left ventricular posterior wall thickness; LVEF: left ventricular ejection fraction; PWV: Pulse Wave Velocity

Table 2: Final selection of biomarkers at V4 associated to smoking status (multivariable multinomial model obtained from forward selection)

Variable	Olink Name	Status	OR (95% CI)	p-value
Age per increment of 10 yrs		Current smoker	0.477 (0.345 - 0.660)	<.0001
		Past smoker	1.092 (0.901 - 1.323)	0.37
Female sex		Current smoker	0.377 (0.207 - 0.689)	0.002
		Past smoker	0.554 (0.375 - 0.817)	0.003
BMI per increment of 10 Kg/m ²		Current smoker	0.802 (0.425 - 1.515)	0.50
		Past smoker	0.779 (0.514 - 1.181)	0.24
SBP office measure per increment of 10 mm Hg		Current smoker	0.928 (0.778 - 1.107)	0.41
		Past smoker	0.827 (0.743 - 0.919)	0.0004
Diabetes		Current smoker	0.728 (0.204 - 2.605)	0.63
		Past smoker	1.960 (0.984 - 3.902)	0.055
Total cholesterol (g/L)		Current smoker	1.177 (0.643 - 2.153)	0.60

Variable	Olink Name	Status	OR (95% CI)	p-value
Microalbuminuria per increment of 100 mg/L		Past smoker	1.288 (0.888 - 1.868)	0.18
		Current smoker	1.159 (0.956 - 1.406)	0.13
		Past smoker	0.831 (0.584 - 1.183)	0.30
eGFR per increment of 10 mL/min/1.73m ²		Current smoker	1.432 (1.166 - 1.758)	0.0006
		Past smoker	1.071 (0.946 - 1.214)	0.28
Pulse Wave Velocity per increment of 10 m/s		Current smoker	0.732 (0.131 - 4.087)	0.72
		Past smoker	1.676 (0.657 - 4.279)	0.28
cIMT per increment of 100		Current smoker	1.196 (0.997 - 1.435)	0.054
		Past smoker	1.119 (1.007 - 1.244)	0.036
Carotid plaques		Current smoker	0.976 (0.475 - 2.005)	0.95
		Past smoker	1.773 (1.196 - 2.630)	0.004
LVMi per increment of 10 g/m ²		Current smoker	1.165 (1.028 - 1.321)	0.017
		Past smoker	1.036 (0.958 - 1.119)	0.37
Antihypertensive drugs		Current smoker	1.091 (0.559 - 2.126)	0.80
		Past smoker	0.827 (0.574 - 1.190)	0.31
Lipid lowering agents		Current smoker	1.658 (0.817 - 3.364)	0.16
		Past smoker	1.551 (1.042 - 2.309)	0.031
SCGB3A2	Secretoglobin family 3A member 2	Current smoker	1.542 (1.203 - 1.976)	0.0006
		Past smoker	0.791 (0.650 - 0.963)	0.020
EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	Current smoker	2.132 (1.614 - 2.815)	<.0001
		Past smoker	1.251 (0.987 - 1.584)	0.064
IL-12B	Interleukin-12 subunit beta	Current smoker	0.563 (0.434 - 0.731)	<.0001
		Past smoker	1.128 (0.959 - 1.328)	0.15
DPP6	Dipeptidyl aminopeptidase-like protein 6	Current smoker	0.706 (0.546 - 0.913)	0.008
		Past smoker	0.863 (0.732 - 1.018)	0.080
ADM	ADM	Current smoker	2.021 (1.493 - 2.736)	<.0001
		Past smoker	1.247 (1.032 - 1.506)	0.022
VEGFD	Vascular endothelial growth factor D	Current smoker	1.348 (1.041 - 1.746)	0.023
		Past smoker	1.223 (1.051 - 1.423)	0.009

Variable	Olink Name	Status	OR (95% CI)	p-value
MMP-10	Matrix metalloproteinase-10	Current smoker	1.454 (1.161 - 1.820)	0.001
		Past smoker	1.073 (0.927 - 1.241)	0.35
CCL11	Eotaxin-1	Current smoker	2.113 (1.596 - 2.797)	<.0001
		Past smoker	1.025 (0.862 - 1.220)	0.78
FGF-21 (CVD II panel)	Fibroblast growth factor 21	Current smoker	1.106 (0.867 - 1.412)	0.42
		Past smoker	1.286 (1.091 - 1.515)	0.003
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	Current smoker	1.739 (1.341 - 2.255)	<.0001
		Past smoker	1.086 (0.919 - 1.283)	0.33
TWEAK	Tumor necrosis factor	Current smoker	0.606 (0.457 - 0.803)	0.0005
		Past smoker	0.998 (0.837 - 1.191)	0.98
CHIT1	Chitotriosidase-1	Current smoker	1.200 (0.950 - 1.516)	0.13
		Past smoker	1.253 (1.088 - 1.443)	0.002
AXL	Tyrosine-protein kinase receptor UFO	Current smoker	0.707 (0.542 - 0.922)	0.011
		Past smoker	0.933 (0.788 - 1.105)	0.42
CXCL10	C-X-C motif chemokine 10	Current smoker	0.797 (0.607 - 1.047)	0.10
		Past smoker	0.835 (0.714 - 0.978)	0.025
IL1RL2	Interleukin-1 receptor-like 2	Current smoker	0.974 (0.751 - 1.263)	0.84
		Past smoker	0.689 (0.584 - 0.813)	<.0001
EGFL7	Epidermal growth factor-like protein 7	Current smoker	0.661 (0.510 - 0.857)	0.002
		Past smoker	0.894 (0.766 - 1.043)	0.15
TR	Transferrin receptor protein 1	Current smoker	0.677 (0.545 - 0.840)	0.0004
		Past smoker	0.848 (0.737 - 0.976)	0.021
PLTP	Phospholipid transfer protein	Current smoker	0.532 (0.403 - 0.703)	<.0001
		Past smoker	1.090 (0.920 - 1.293)	0.32
ST2	ST2 protein	Current smoker	0.646 (0.509 - 0.819)	0.0003
		Past smoker	0.920 (0.785 - 1.077)	0.30
RAGE	Receptor for advanced glycosylation end products	Current smoker	0.975 (0.748 - 1.272)	0.85
		Past smoker	0.815 (0.693 - 0.959)	0.014
IGFBP-2	Insulin-like growth factor-binding protein 2	Current smoker	1.701 (1.255 - 2.305)	0.0006
		Past smoker	1.235 (1.018 - 1.499)	0.032

Variable	Olink Name	Status	OR (95% CI)	p-value
IGFBP-1	Insulin-like growth factor-binding protein 1	Current smoker	0.595 (0.452 - 0.783)	0.0002
		Past smoker	0.693 (0.576 - 0.834)	0.0001
PAPPA	Pappalysin-1	Current smoker	0.724 (0.566 - 0.925)	0.010
		Past smoker	0.984 (0.840 - 1.154)	0.84
RASSF2	Ras association domain-containing protein 2	Current smoker	1.415 (1.124 - 1.781)	0.003
		Past smoker	1.079 (0.914 - 1.273)	0.37
KIT	Mast/stem cell growth factor receptor Kit	Current smoker	1.727 (1.323 - 2.254)	<.0001
		Past smoker	0.959 (0.809 - 1.137)	0.63

Figure Legends

Figure 1: Study flow chart

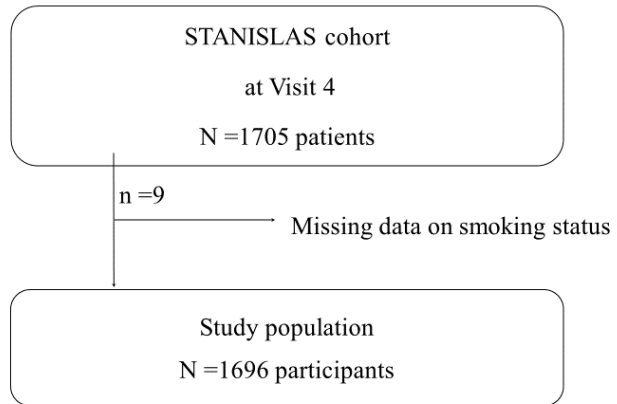


Figure 2: Network analysis of circulating proteins (in red) linked to GO terms (in blue) to explore the protein- pathways involved in current smokers.

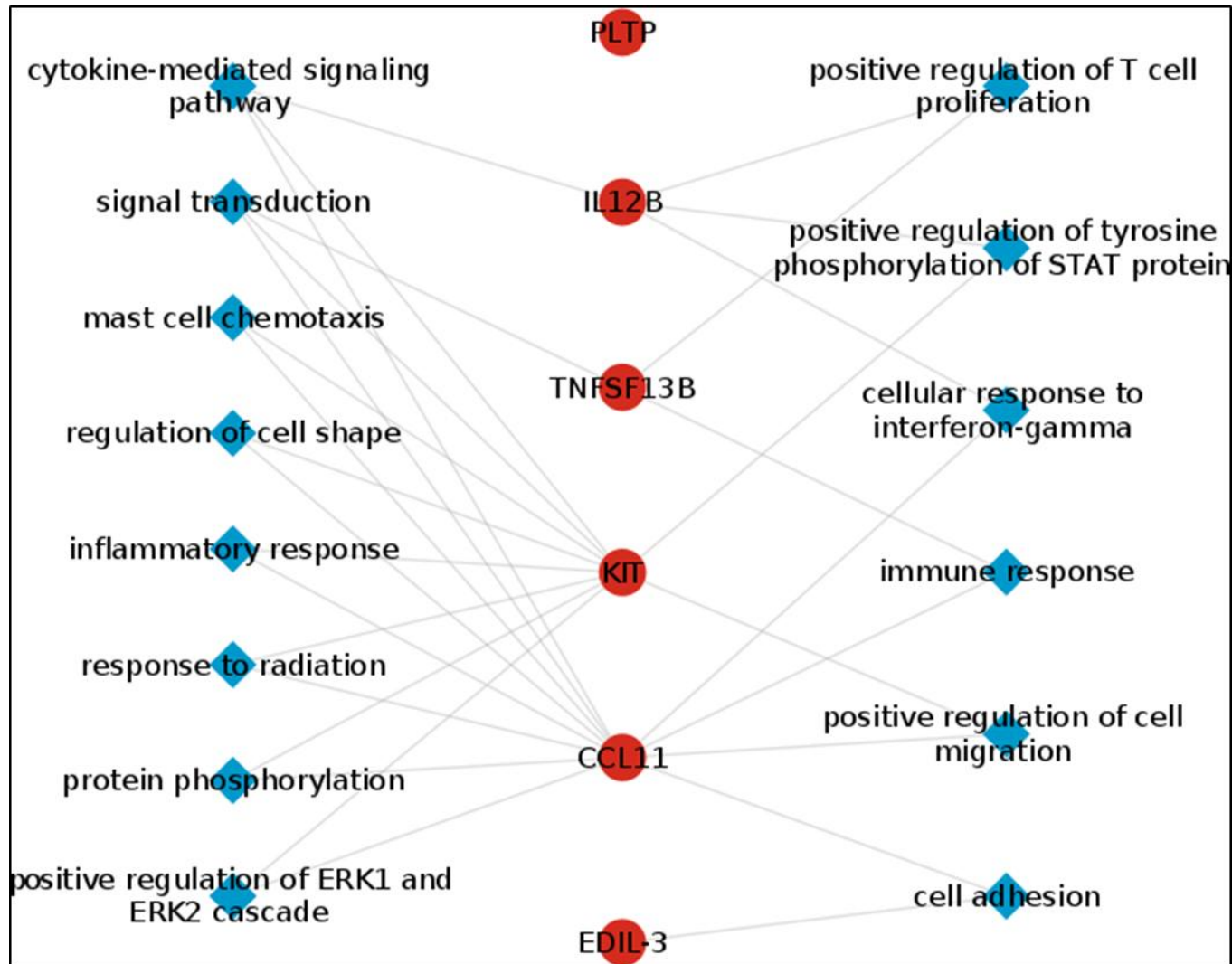
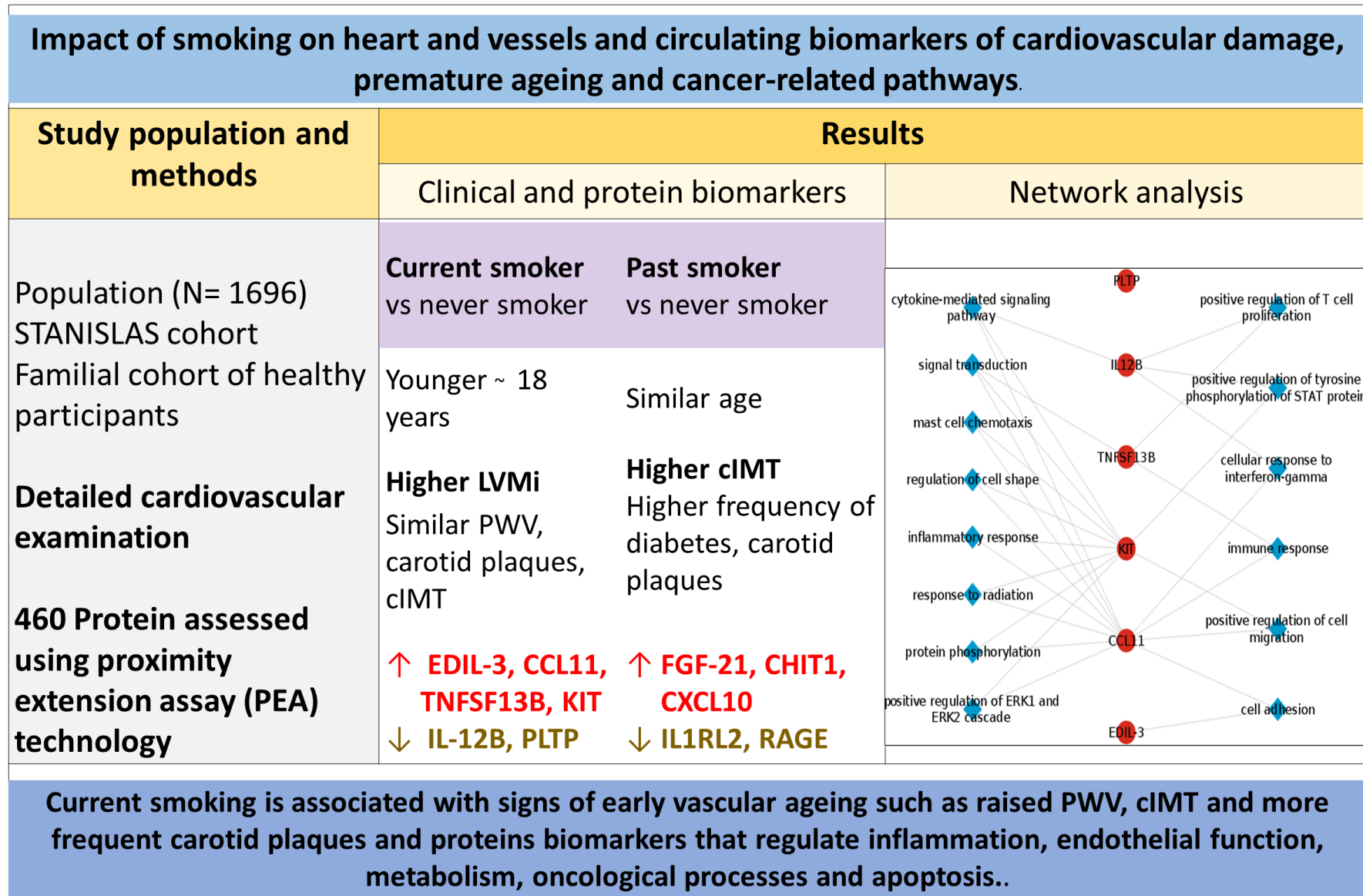


Figure: Graphical Abstract



Supplementary Material

Table S1: Participants characteristics at V4 in whole population and according to tobacco use and smoking exposure duration within Past smoker group

Characteristic	n	Overall	n	Never smoked	n	Current smoker	n	Past smoker	n	Past smoker	p- value
						Median (16)		< median (14 years)		≥ median (14 years)	
Female gender	1694	868 (51.2 %)	787	471 (59.8 %)	366	178 (48.6 %)	270	132 (48.9 %)	271	87 (32.1 %)	< 0.0001
Age (years)	1694	55.0 (34.0 - 60.0)	787	55.0 (34.0 - 60.0)	366	36.5 (31.0 - 56.0)	270	57.0 (36.0 - 60.0)	271	60.0 (56.0 - 62.0)	< 0.0001
WC (cm)	1685	89.0 (80.0 - 99.0)	785	87.0 (78.0 - 96.0)	365	87.0 (78.0 - 96.0)	268	91.0 (81.0 - 99.0)	267	97.0 (87.0 - 106.0)	< 0.0001
BMI (Kg/m ²)	1693	25.15 (22.48 - 28.49)	787	24.62 (22.35 - 28.12)	365	24.50 (21.88 - 27.59)	270	25.68 (22.79 - 28.49)	271	27.14 (24.21 - 30.26)	< 0.0001
SBP office (mm Hg)	1694	123.7 (114.3 - 135.0)	787	123.3 (114.0 - 134.3)	366	121.7 (112.3 - 131.7)	270	123.3 (115.0 - 135.0)	271	129.0 (118.3 - 140.7)	< 0.0001
DBP office (mm Hg)	1694	72.4 ± 8.9	787	72.1 ± 8.4	366	71.0 ± 8.7	270	73.0 ± 9.0	271	75.0 ± 9.6	< 0.0001
Heart rate (bpm)	1694	63.3 (57.3 - 69.3)	787	63.7 (58.0 - 69.7)	366	62.0 (55.7 - 68.0)	270	63.3 (58.0 - 69.0)	271	63.7 (57.7 - 69.7)	0.004
24-h SBP (mm Hg)	1584	120.2 ± 10.3	745	119.4 ± 9.8	329	120.0 ± 10.6	250	120.4 ± 10.8	260	122.5 ± 10.4	0.0005
24-h DBP (mm Hg)	1584	74.3 ± 7.3	745	73.5 ± 6.9	329	74.5 ± 7.5	250	74.8 ± 7.6	260	75.8 ± 7.5	< 0.0001
HTA 24-h	1587	657 (41.4 %)	746	289 (38.7 %)	330	108 (32.7 %)	253	115 (45.5 %)	258	145 (56.2 %)	< 0.0001
HTA office	1678	544 (32.4 %)	780	238 (30.5 %)	365	83 (22.7 %)	269	92 (34.2 %)	264	131 (49.6 %)	< 0.0001
Treated HTA	1662	307 (18.5 %)	778	134 (17.2 %)	361	44 (12.2 %)	266	49 (18.4 %)	257	80 (31.1 %)	< 0.0001
Ankle brachial index	1668	1.13 ± 0.12	768	1.12 ± 0.12	364	1.12 ± 0.12	268	1.14 ± 0.12	268	1.15 ± 0.14	0.063
Diabetes	1692	70 (4.1 %)	785	24 (3.1 %)	366	8 (2.2 %)	270	10 (3.7 %)	271	28 (10.3 %)	< 0.0001
Glucose (g/L)	1687	0.88 (0.82 - 0.95)	786	0.88 (0.82 - 0.94)	363	0.86 (0.80 - 0.92)	268	0.89 (0.83 - 0.96)	270	0.94 (0.87 - 1.03)	< 0.0001
Glycated Hb (%)	1668	5.6 (5.3 - 5.8)	776	5.5 (5.3 - 5.8)	361	5.5 (5.3 - 5.8)	265	5.6 (5.3 - 5.8)	266	5.7 (5.4 - 6.0)	< 0.0001
Total cholesterol (g/L)	1687	2.10 (1.85 - 2.40)	786	2.12 (1.87 - 2.42)	363	2.04 (1.79 - 2.28)	268	2.17 (1.86 - 2.46)	270	2.13 (1.90 - 2.44)	0.0005

Characteristic	n	Overall	n	Never smoked	n	Current smoker	n	Past smoker	n	Past smoker	p- value
						Median (16)		< median (14 years)		≥ median (14 years)	
Triglycerides (g/L)	1687	0.92 (0.68 - 1.27)	786	0.88 (0.64 - 1.19)	363	0.94 (0.66 - 1.34)	268	0.91 (0.68 - 1.25)	270	1.01 (0.77 - 1.42)	< 0.0001
HDL (g/L)	1687	0.58 ± 0.14	786	0.59 ± 0.14	363	0.55 ± 0.13	268	0.59 ± 0.14	270	0.58 ± 0.15	< 0.0001
LDL (g/L)	1683	1.34 ± 0.34	785	1.35 ± 0.34	362	1.29 ± 0.34	268	1.36 ± 0.35	268	1.34 ± 0.34	0.015
Microalbuminuria (mg/L)	1682	6.0 (3.5 - 9.9)	782	5.4 (3.1 - 9.0)	362	7.2 (4.4 - 11.9)	267	5.7 (3.5 - 10.3)	271	6.3 (4.1 - 10.4)	< 0.0001
eGFR (mL/min/1.73m ²)	1687	96.5 (87.0 - 107.6)	786	96.1 (86.4 - 107.3)	363	104.7 (95.2 - 115.3)	268	94.9 (85.8 - 102.5)	270	92.6 (82.9 - 97.6)	< 0.0001
PWV (m/s)	1660	8.1 (7.3 - 9.3)	768	8.0 (7.2 - 9.2)	361	7.9 (7.1 - 8.8)	263	8.3 (7.4 - 9.5)	268	8.8 (7.8 - 10.1)	< 0.0001
cIMT (µm)	1656	621.0 (532.0 - 723.0)	770	612.0 (525.0 - 714.0)	358	592.5 (510.9 - 669.0)	263	638.0 (534.0 - 748.0)	265	698.0 (615.0 - 801.0)	< 0.0001
cIMT>900 µm	1656	99 (6.0 %)	770	38 (4.9 %)	358	12 (3.4 %)	263	19 (7.2 %)	265	30 (11.3 %)	0.0003
Carotid Plaques	1658	210 (12.7 %)	772	70 (9.1 %)	358	35 (9.8 %)	263	34 (12.9 %)	265	71 (26.8 %)	< 0.0001
LVEF (%)	1580	65.3 (61.0 - 69.3)	732	65.6 (61.7 - 69.4)	341	64.4 (60.1 - 68.5)	257	65.2 (61.0 - 69.4)	250	65.4 (60.6 - 70.1)	0.034
IVSd 2D (mm)	1673	0.95 ± 0.18	777	0.93 ± 0.18	364	0.92 ± 0.17	269	0.95 ± 0.17	263	1.04 ± 0.19	< 0.0001
LVd 2D (cm)	1671	4.55 ± 0.52	777	4.48 ± 0.50	364	4.64 ± 0.50	269	4.57 ± 0.54	261	4.62 ± 0.55	< 0.0001
PWTd 2D (mm)	1671	0.88 ± 0.17	777	0.86 ± 0.17	364	0.87 ± 0.17	269	0.88 ± 0.16	261	0.95 ± 0.18	< 0.0001
LVMi (g/m ²)	1670	73.9 (62.6 - 87.4)	777	71.7 (60.5 - 84.3)	363	74.6 (64.1 - 86.6)	269	73.5 (63.3 - 87.5)	261	80.7 (69.5 - 94.4)	< 0.0001
Antihypertensive drugs	1694	339 (20.0 %)	787	143 (18.2 %)	366	49 (13.4 %)	270	53 (19.6 %)	271	94 (34.7 %)	< 0.0001
Lipid lowering agents	1694	259 (15.3 %)	787	94 (11.9 %)	366	40 (10.9 %)	270	45 (16.7 %)	271	80 (29.5 %)	< 0.0001

IVSd: septum thickness- diastolic; LVd: Left ventricular diameter, diastolic; PWTd: Left ventricular posterior wall thickness; LVEF: left ventricular ejection fraction; PWV: Pulse Wave Velocity; WC : waist circumference

Table S2: Participants characteristics of Parents at V4 in whole population and according to smoking exposure.

Characteristic	Overall	Never smoked (A)	Current smoker (B)	Past smoker (C)	Overall	<i>p</i>	<i>p</i>	<i>p</i>
	(N=995)	(N=457)	(N=118)	(N=420)	P	A vs. B	A vs. C	B vs. C
Female gender	498 (50.0 %)	288 (63.0 %)	51 (42.9 %)	159 (37.9 %)	< 0.0001	< 0.0001	< 0.0001	0.32
Age (years)	59.0 (57.0 - 63.0)	59.0 (57.0 - 63.0)	59.0 (56.0 - 61.0)	60.0 (57.0 - 62.0)	0.043	0.018	0.77	0.02
WC (cm)	93.0 (84.0 - 101.0)	91.0 (82.0 - 99.0)	93.0 (84.0 - 100.0)	96.0 (86.0 - 103.5)	< 0.0001	0.098	< 0.0001	0.15
IMC (Kg/m2)	26.34 (23.62 - 29.44)	25.82 (23.48 - 29.21)	25.88 (23.51 - 28.84)	26.85 (23.88 - 30.05)	0.048	0.63	0.036	0.053
SBP office (mm Hg)	128.3 (118.2 - 140.0)	128.3 (118.0 - 139.3)	128.3 (117.0 - 142.3)	128.7 (118.3 - 140.0)	0.94	0.78	0.78	0.95
DBP office (mm Hg)	74.4 ± 9.0	74.0 ± 8.6	74.6 ± 9.4	74.8 ± 9.4	0.52	0.63	0.26	0.78
Heart rate (bpm)	62.7 (57.0 - 68.5)	63.3 (57.7 - 69.0)	59.0 (54.3 - 65.7)	62.7 (57.2 - 69.0)	0.0003	< 0.0001	0.57	0.0005
24-h SBP (mm Hg)	121.6 ± 10.9	120.6 ± 10.4	123.8 ± 12.4	122.1 ± 10.9	0.029	0.019	0.059	0.25
24-h DBP (mm Hg)	75.0 ± 7.6	74.1 ± 7.3	77.2 ± 8.3	75.5 ± 7.7	0.0002	0.0004	0.002	0.13
Glucose (g/L)	0.92 (0.85 - 0.99)	0.91 (0.85 - 0.97)	0.91 (0.85 - 0.98)	0.93 (0.87 - 1.01)	0.0007	0.97	0.0003	0.016
Total cholesterol (g/L)	2.22 (1.99 - 2.49)	2.26 (2.02 - 2.49)	2.16 (1.93 - 2.48)	2.21 (1.95 - 2.48)	0.097	0.12	0.056	0.78
Triglycerides (g/L)	0.99 (0.74 - 1.34)	0.98 (0.73 - 1.32)	1.01 (0.71 - 1.34)	1.00 (0.76 - 1.36)	0.41	0.62	0.19	0.7
HDL (g/L)	0.60 ± 0.15	0.61 ± 0.14	0.58 ± 0.13	0.59 ± 0.15	0.093	0.081	0.072	0.63
LDL (g/L)	1.41 ± 0.34	1.43 ± 0.33	1.40 ± 0.35	1.38 ± 0.35	0.094	0.37	0.03	0.65
Microalbuminuria (mg/L)	5.8 (3.4 - 9.8)	5.3 (3.0 - 8.8)	7.2 (4.2 - 12.2)	5.9 (3.7 - 10.3)	0.0003	0.0004	0.004	0.089
Glycated Hb(%)	5.7 (5.5 - 5.9)	5.7 (5.5 - 5.9)	5.8 (5.5 - 6.0)	5.7 (5.5 - 5.9)	0.1	0.056	0.74	0.038
eGFR (mL/min/1.73m2)	90.4 (80.4 - 96.9)	89.6 (78.9 - 96.3)	93.9 (84.5 - 99.0)	90.6 (81.0 - 96.9)	0.018	0.005	0.46	0.017
Pulse Wave Velocity (m/s)	8.9 (7.9 - 10.1)	8.7 (7.9 - 9.9)	8.9 (8.1 - 10.0)	9.0 (8.0 - 10.3)	0.064	0.27	0.021	0.66
cIMT (µm)	692.00 (616.50 - 787.50)	685.00 (612.00 - 774.00)	674.00 (619.00 - 766.00)	705.00 (623.00 - 804.00)	0.058	0.79	0.02	0.2
cIMT>900	93 (9.5 %)	38 (8.5 %)	9 (7.7 %)	46 (11.2 %)	0.33	0.79	0.17	0.27
Carotid plaques	203 (21.0 %)	69 (15.5 %)	29 (25.4 %)	105 (25.7 %)	0.0005	0.013	0.0002	0.96

LVEF (%)	66.3 (61.9 - 70.1)	66.7 (63.0 - 70.0)	66.7 (62.5 - 70.2)	65.8 (60.6 - 70.2)	0.057	0.94	0.022	0.15
IVSd 2D (mm)	1.00 ± 0.18	0.98 ± 0.18	0.99 ± 0.17	1.03 ± 0.18	0.0002	0.28	< 0.0001	0.11
LVd 2D (cm)	4.52 ± 0.54	4.43 ± 0.50	4.60 ± 0.53	4.58 ± 0.56	0.0001	0.002	0.0002	0.59
PWTd 2D (mm)	0.92 ± 0.17	0.90 ± 0.16	0.92 ± 0.16	0.94 ± 0.17	0.037	0.25	0.011	0.62
LVMi (g/m ²)	77.9 (66.9 - 91.7)	76.2 (64.3 - 90.4)	78.5 (68.6 - 91.9)	80.9 (68.9 - 94.2)	0.001	0.061	0.0005	0.55
High blood pressure office	255 (25.6 %)	113 (24.7 %)	33 (27.7 %)	109 (26.0 %)	0.77	0.5	0.68	0.7
High blood pressure 24-h	324 (33.8 %)	133 (30.2 %)	47 (40.5 %)	144 (35.7 %)	0.058	0.034	0.085	0.35
Treated HTA	289 (29.9 %)	127 (28.3 %)	35 (30.7 %)	127 (31.6 %)	0.57	0.61	0.29	0.86
Diabetes	63 (6.3 %)	23 (5.1 %)	3 (2.5 %)	37 (8.8 %)	0.017	0.24	0.028	0.021
Antihypertensive drugs	320 (32.1 %)	135 (29.5 %)	40 (33.6 %)	145 (34.5 %)	0.27	0.39	0.11	0.85
Hypercholesterolemia treatment	256 (25.7 %)	92 (20.1 %)	39 (32.8 %)	125 (29.8 %)	0.0007	0.003	0.001	0.53

Table S3a: Baseline Characteristics before inverse probability of treatment weighting

	Never smoked	Current smoker	Past smoker						
	(A)	(B)	(C)	SD	P-value	SD	P-value	SD	P-value
Characteristics	m ± SD / n(%)	m ± SD /n(%)	m ± SD /n(%)	A vs. B	A vs. B	A vs. C	A vs. C	B vs. C	B vs. C
Female gender	438 (60%)	166 (49%)	207 (41%)	22.40%	0.0007	37.50%	< 0.0001	14.80%	0.036
Age (years)	48 ± 15	41 ± 13	54 ± 12	49.20%	< 0.0001	40.40%	< 0.0001	100.80%	< 0.0001
BMI (Kg/m ²)	25.6 ± 4.6	25.0 ± 4.5	26.8 ± 4.9	11.90%	0.08	24.80%	< 0.0001	36.30%	< 0.0001
SBP office measure (mm Hg)	125 ± 14	123 ± 15	128 ± 16	14.20%	0.031	18.80%	0.001	31.50%	< 0.0001
Total cholesterol (g/L)	2.13 ± 0.39	2.05 ± 0.38	2.17 ± 0.42	21.60%	0.001	8.10%	0.16	28.70%	< 0.0001
Microalbuminuria (mg/L)	13.17 ± 69.87	20.12 ± 135.86	11.62 ± 25.84	7.30%	< 0.0001	2.70%	0.015	9.60%	0.003
eGFR (mL/min/1.73m ²)	96.1 ± 15.1	104.0 ± 14.7	92.6 ± 14.1	52.90%	< 0.0001	23.80%	< 0.0001	80.00%	< 0.0001
Pulse Wave Velocity (m/s)	8.4 ± 1.7	8.1 ± 1.5	9.0 ± 1.9	19.30%	0.019	32.70%	< 0.0001	51.50%	< 0.0001
cIMT	628.9 ± 146.1	604.0 ± 131.2	683.1 ± 154.9	17.60%	0.004	36.20%	< 0.0001	54.30%	< 0.0001

Carotid plaques	67 (9%)	34 (10%)	102 (20%)	2.80%	0.66	32.20%	< 0.0001	29.40%	< 0.0001
LVMi (g/m ²)	73.8 ± 19.0	76.3 ± 18.4	79.4 ± 19.7	13.60%	0.009	29.40%	< 0.0001	16.20%	0.064
Diabetes	23 (3%)	8 (2%)	35 (7%)	4.90%	0.47	17.70%	0.002	22.20%	0.003
Antihypertensive drugs	130 (18%)	46 (14%)	127 (25%)	11.70%	0.08	18.70%	0.001	30.40%	< 0.0001
Hypercholesterolemia treatment	85 (12%)	38 (11%)	114 (23%)	1.40%	0.83	30.00%	< 0.0001	31.40%	< 0.0001

Table S3b: Baseline Characteristics after stabilized inverse probability of treatment weighting

	Never smoked	Current smoker	Past smoker						
	(A)	(B)	(C)	SD	P-value	SD	P-value	SD	P-value
Characteristic	m ± SD / n(%)	m ± SD / n(%)	m ± SD / n(%)	A vs. B	A vs. B	A vs. C	A vs. C	B vs. C	B vs. C
Female gender	380 (52%)	169 (51%)	274 (54%)	2.80%	0.68	4.30%	0.46	7.00%	0.32
Age (years)	48 ± 15	48 ± 13	49 ± 14	3.30%	0.62	2.10%	0.72	5.70%	0.42
BMI (Kg/m ²)	25.8 ± 4.6	25.6 ± 4.5	25.7 ± 4.8	4.30%	0.52	0.50%	0.94	3.70%	0.6
SBP office measure (mm Hg)	125 ± 14	125 ± 16	125 ± 16	0.70%	0.92	1.10%	0.84	1.70%	0.81
Total cholesterol (g/L)	2.13 ± 0.39	2.11 ± 0.38	2.13 ± 0.41	4.30%	0.52	0.90%	0.87	5.10%	0.47
Microalbuminuria (mg/L)	13.94 ± 73.54	15.19 ± 83.47	10.76 ± 26.73	1.60%	0.81	5.40%	0.38	7.80%	0.33
eGFR (mL/min/1.73m ²)	96.6 ± 15.4	96.9 ± 15.2	96.7 ± 15.3	1.70%	0.79	0.20%	0.97	1.60%	0.82
Pulse Wave Velocity (m/s)	8.5 ± 1.8	8.4 ± 1.6	8.5 ± 1.7	3.70%	0.58	0.50%	0.93	4.30%	0.54
cIMT	639.5 ± 156.8	633.0 ± 132.9	641.2 ± 152.1	4.30%	0.51	1.10%	0.85	5.60%	0.42
Carotid Plaques	90 (12%)	41 (12%)	63 (13%)	0.50%	0.94	0.60%	0.92	1.10%	0.88
LVMi (g/m ²)	76.1 ± 20.0	75.4 ± 18.0	75.8 ± 19.1	3.70%	0.58	1.30%	0.83	2.50%	0.73

Diabetes	27 (4%)	11 (3%)	20 (4%)	1.60%	0.81	1.30%	0.82	2.90%	0.69
Antihypertensive drugs	138 (19%)	68 (21%)	94 (19%)	4.00%	0.55	0.80%	0.9	4.70%	0.5
Hypercholesterolemia treatment	105 (14%)	52 (16%)	72 (14%)	3.70%	0.57	0.20%	0.97	3.90%	0.58

Table S4: Final selection of biomarkers at V4 associated to smoking status (multivariable multinomial model obtained from forward selection) in the whole cohort after adding ankle brachial index in adjustment variables

Variable	Olink Name	Status	OR (95% CI)	p-value	Overall p-value
Age per increment of 10 yrs		Current smoker	0.448 (0.321 - 0.625)	<.0001	<.0001
		Past smoker	1.091 (0.898 - 1.326)	0.38	
Female gender		Current smoker	0.437 (0.238 - 0.804)	0.008	0.001
		Past smoker	0.534 (0.363 - 0.785)	0.001	
BMI per increment of 10 Kg/m ²		Current smoker	0.787 (0.411 - 1.509)	0.47	0.53
		Past smoker	0.798 (0.523 - 1.216)	0.29	
SBP office measure per increment of 10 mm Hg		Current smoker	0.904 (0.753 - 1.086)	0.28	0.003
		Past smoker	0.827 (0.741 - 0.922)	0.0006	
Diabetes		Current smoker	0.501 (0.135 - 1.856)	0.30	0.089
		Past smoker	1.707 (0.852 - 3.420)	0.13	
Total cholesterol (g/L)		Current smoker	1.443 (0.772 - 2.697)	0.25	0.31
		Past smoker	1.286 (0.880 - 1.881)	0.19	
Microalbuminuria per increment of 100 mg/L		Current smoker	1.190 (0.990 - 1.430)	0.064	0.083
		Past smoker	0.850 (0.599 - 1.207)	0.36	
eGFR per increment of 10 mL/min/1.73m ²		Current smoker	1.455 (1.168 - 1.812)	0.0008	0.003
		Past smoker	1.093 (0.962 - 1.242)	0.17	
Pulse Wave Velocity per increment of 10 m/s		Current smoker	0.452 (0.079 - 2.597)	0.37	0.20
		Past smoker	1.830 (0.710 - 4.715)	0.21	
EIM per increment of 100		Current smoker	1.188 (0.989 - 1.427)	0.065	0.038
		Past smoker	1.131 (1.017 - 1.258)	0.023	

Carotid plaques		Current smoker	1.108 (0.538 - 2.285)	0.78	0.008
		Past smoker	1.852 (1.241 - 2.765)	0.003	
LVMi per increment of 10 g/m ²		Current smoker	1.110 (0.975 - 1.264)	0.12	0.28
		Past smoker	1.029 (0.950 - 1.115)	0.48	
Antihypertensive drugs		Current smoker	1.027 (0.517 - 2.043)	0.94	0.29
		Past smoker	0.756 (0.522 - 1.096)	0.14	
Lipid lowering agents		Current smoker	1.489 (0.713 - 3.109)	0.29	0.17
		Past smoker	1.457 (0.968 - 2.193)	0.071	
ABI		Current smoker	1.424 (0.229 - 8.835)	0.70	0.92
		Past smoker	1.171 (0.385 - 3.559)	0.78	
PLTP	Phospholipid transfer protein	Current smoker	0.740 (0.573 - 0.955)	0.021	0.011
		Past smoker	1.088 (0.939 - 1.261)	0.26	
ADM	ADM	Current smoker	1.620 (1.173 - 2.238)	0.003	0.005
		Past smoker	1.257 (1.028 - 1.536)	0.026	
IL1RL2	Interleukin-1 receptor-like 2	Current smoker	0.984 (0.753 - 1.285)	0.90	<.0001
		Past smoker	0.684 (0.577 - 0.810)	<.0001	
LOX-1	Lectin-like oxidized LDL receptor 1	Current smoker	0.643 (0.452 - 0.915)	0.014	0.001
		Past smoker	0.680 (0.543 - 0.852)	0.0008	
SCF (CVD II panel)	Stem cell factor	Current smoker	0.697 (0.544 - 0.893)	0.004	0.017
		Past smoker	0.913 (0.776 - 1.075)	0.27	
FGF-21 (CVD II panel)	Fibroblast growth factor 21	Current smoker	0.995 (0.758 - 1.305)	0.97	0.009
		Past smoker	1.299 (1.090 - 1.547)	0.003	
PRSS8	Prostasin	Current smoker	1.666 (1.234 - 2.249)	0.0009	0.004
		Past smoker	1.125 (0.936 - 1.353)	0.21	
MARCO	Macrophage receptor MARCO	Current smoker	0.628 (0.479 - 0.822)	0.0007	0.002
		Past smoker	1.006 (0.852 - 1.189)	0.94	
MMP12	Matrix metalloproteinase-12	Current smoker	1.459 (1.116 - 1.909)	0.006	0.021
		Past smoker	1.099 (0.934 - 1.293)	0.26	
TNFRSF13B	Tumor necrosis factor receptor superfamily member 13B	Current smoker	1.437 (1.095 - 1.885)	0.009	0.031
		Past smoker	1.092 (0.926 - 1.288)	0.29	
BLM hydrolase	Bleomycin hydrolase	Current smoker	0.669 (0.518 - 0.865)	0.002	0.009
		Past smoker	0.907 (0.763 - 1.078)	0.27	

TR	Transferrin receptor protein 1	Current smoker	0.715 (0.573 - 0.891)	0.003	0.006
		Past smoker	0.866 (0.751 - 0.998)	0.047	
AZU1	Azurocidin	Current smoker	1.371 (0.982 - 1.914)	0.064	0.004
		Past smoker	1.415 (1.145 - 1.750)	0.001	
IGFBP-1	Insulin-like growth factor-binding protein 1	Current smoker	0.602 (0.451 - 0.804)	0.0006	0.0001
		Past smoker	0.721 (0.597 - 0.870)	0.0007	
CHIT1	Chitotriosidase-1	Current smoker	1.141 (0.902 - 1.443)	0.27	0.012
		Past smoker	1.245 (1.077 - 1.438)	0.003	
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	Current smoker	1.833 (1.404 - 2.393)	<.0001	<.0001
		Past smoker	1.096 (0.922 - 1.301)	0.30	
ST2	ST2 protein	Current smoker	0.581 (0.455 - 0.740)	<.0001	<.0001
		Past smoker	0.937 (0.799 - 1.098)	0.42	
SCGB3A2	Secretoglobin family 3A member 2	Current smoker	1.662 (1.278 - 2.160)	0.0001	<.0001
		Past smoker	0.830 (0.679 - 1.014)	0.069	
CTSZ	Cathepsin Z	Current smoker	0.833 (0.644 - 1.078)	0.16	0.027
		Past smoker	0.804 (0.683 - 0.947)	0.009	
IGFBP-2	Insulin-like growth factor-binding protein 2	Current smoker	1.749 (1.282 - 2.386)	0.0004	0.001
		Past smoker	1.245 (1.018 - 1.524)	0.033	
CCL11	Eotaxin-1	Current smoker	1.718 (1.277 - 2.311)	0.0003	0.001
		Past smoker	1.023 (0.852 - 1.228)	0.81	
TNFSF14	Tumor necrosis factor ligand superfamily member 14	Current smoker	1.180 (0.876 - 1.589)	0.28	0.031
		Past smoker	1.294 (1.067 - 1.569)	0.009	
IL-12B	Interleukin-12 subunit beta	Current smoker	0.512 (0.391 - 0.670)	<.0001	<.0001
		Past smoker	1.059 (0.894 - 1.254)	0.51	
MMP-10	Matrix metalloproteinase-10	Current smoker	1.354 (1.071 - 1.713)	0.011	0.040
		Past smoker	1.056 (0.908 - 1.229)	0.48	
CXCL10	C-X-C motif chemokine 10	Current smoker	0.778 (0.582 - 1.039)	0.089	0.022
		Past smoker	0.808 (0.685 - 0.953)	0.012	
CCL25	C-C motif chemokine 25	Current smoker	1.340 (1.055 - 1.702)	0.017	0.038
		Past smoker	0.986 (0.848 - 1.146)	0.85	
TWEAK	Tumor necrosis factor	Current smoker	0.605 (0.459 - 0.798)	0.0004	0.002
		Past smoker	0.914 (0.764 - 1.094)	0.33	

EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	Current smoker	2.232 (1.683 - 2.962)	<.0001	<.0001
		Past smoker	1.243 (0.980 - 1.578)	0.073	
PLXDC1	Plexin domain-containing protein 1	Current smoker	0.721 (0.567 - 0.917)	0.008	0.028
		Past smoker	0.965 (0.837 - 1.112)	0.62	
NCF2	Neutrophil cytosol factor 2	Current smoker	1.547 (1.059 - 2.260)	0.024	0.042
		Past smoker	0.956 (0.752 - 1.216)	0.71	
EGFL7	Epidermal growth factor-like protein 7	Current smoker	0.630 (0.483 - 0.822)	0.0007	0.002
		Past smoker	0.879 (0.751 - 1.030)	0.11	

Table S5: Final selection of biomarkers at V4 associated to smoking status in “Parents” subgroup (multivariable multinomial model obtained from forward selection)

Variable	Olink Name	Status	OR (95% CI)	P-value	Overall P-value
Age per increment of 10 yrs		Current smoker	0.436 (0.193 - 0.988)	0.047	0.009
		Past smoker	0.560 (0.373 - 0.839)	0.005	
Female gender		Current smoker	0.397 (0.176 - 0.896)	0.026	0.002
		Past smoker	0.473 (0.305 - 0.733)	0.0008	
BMI per increment of 10 Kg/m ²		Current smoker	0.863 (0.406 - 1.835)	0.7	0.87
		Past smoker	0.907 (0.599 - 1.374)	0.65	
SBP office measure per increment of 10 mm Hg		Current smoker	1.010 (0.807 - 1.263)	0.93	0.013
		Past smoker	0.845 (0.750 - 0.951)	0.005	
Diabetes		Current smoker	0.106 (0.015 - 0.748)	0.024	0.03

		Past smoker	1.339 (0.662 - 2.707)	0.42	
Total cholesterol (g/L)		Current smoker	0.786 (0.341 - 1.812)	0.57	0.53
		Past smoker	1.188 (0.750 - 1.882)	0.46	
Microalbuminuria per increment of 100 mg/L		Current smoker	1.552 (0.955 - 2.522)	0.076	0.096
		Past smoker	0.878 (0.632 - 1.222)	0.44	
eGFR per increment of 10 mL/min/1.73m ²		Current smoker	1.314 (0.985 - 1.752)	0.063	0.17
		Past smoker	1.065 (0.921 - 1.233)	0.39	
Pulse Wave Velocity per increment of 10 m/s		Current smoker	0.549 (0.070 - 4.332)	0.57	0.18
		Past smoker	2.227 (0.793 - 6.253)	0.13	
cIMT per increment of 100		Current smoker	1.107 (0.882 - 1.389)	0.38	0.27
		Past smoker	1.098 (0.976 - 1.236)	0.12	
Carotid plaques		Current smoker	1.085 (0.521 - 2.261)	0.83	0.006
		Past smoker	1.868 (1.254 - 2.782)	0.002	
LVMi per increment of 10 g/m ²		Current smoker	1.092 (0.935 - 1.275)	0.27	0.5
		Past smoker	1.032 (0.945 - 1.127)	0.48	
Antihypertensive drugs		Current smoker	1.123 (0.550 - 2.293)	0.75	0.37
		Past smoker	0.789 (0.538 - 1.157)	0.22	
Lipid lowering agents		Current smoker	1.796 (0.866 - 3.724)	0.12	0.024
		Past smoker	1.757 (1.156 - 2.670)	0.008	
SCGB3A2	Secretoglobin family 3A member 2	Current smoker	1.859 (1.313 - 2.631)	0.0005	0.0002
		Past smoker	0.890 (0.720 - 1.100)	0.28	
EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	Current smoker	1.813 (1.383 - 2.377)	<.0001	<.0001
		Past smoker	1.114 (0.898 - 1.382)	0.32	
IL1RL2	Interleukin-1 receptor-like 2	Current smoker	0.949 (0.680 - 1.322)	0.76	<.0001

		Past smoker	0.650 (0.540 - 0.783)	<.0001	
IL-12B	Interleukin-12 subunit beta	Current smoker	0.502 (0.358 - 0.703)	<.0001	0.0001
		Past smoker	1.009 (0.848 - 1.202)	0.92	
DPP6	Dipeptidyl aminopeptidase-like protein 6	Current smoker	0.514 (0.365 - 0.722)	0.0001	0.0005
		Past smoker	0.835 (0.689 - 1.011)	0.064	
KIM1 (OD panel)	Kidney injury molecule 1	Current smoker	1.732 (1.227 - 2.444)	0.002	0.003
		Past smoker	1.241 (1.037 - 1.485)	0.018	
VEGFD	Vascular endothelial growth factor D	Current smoker	1.592 (1.140 - 2.223)	0.006	0.016
		Past smoker	1.157 (0.975 - 1.373)	0.095	
ST2	ST2 protein	Current smoker	0.587 (0.421 - 0.820)	0.002	0.007
		Past smoker	0.898 (0.754 - 1.071)	0.23	
TR	Transferrin receptor protein 1	Current smoker	0.704 (0.503 - 0.986)	0.041	0.04
		Past smoker	0.838 (0.707 - 0.994)	0.043	
PRTN3	Myeloblastin	Current smoker	1.692 (1.191 - 2.406)	0.003	0.0004
		Past smoker	1.487 (1.203 - 1.838)	0.0002	
FGF-21 (CVD II panel)	Fibroblast growth factor 21	Current smoker	0.978 (0.689 - 1.387)	0.9	0.013
		Past smoker	1.301 (1.079 - 1.570)	0.006	
PLTP	Phospholipid transfer protein	Current smoker	0.510 (0.348 - 0.749)	0.0006	<.0001
		Past smoker	1.256 (1.025 - 1.539)	0.028	
KIT	Mast/stem cell growth factor receptor Kit	Current smoker	1.937 (1.347 - 2.787)	0.0004	0.0002
		Past smoker	0.912 (0.749 - 1.111)	0.36	
CCL25	C-C motif chemokine 25	Current smoker	1.479 (1.078 - 2.029)	0.015	0.016
		Past smoker	0.935 (0.793 - 1.103)	0.43	
LOX-1	Lectin-like oxidized LDL receptor 1	Current smoker	0.671 (0.465 - 0.970)	0.034	0.014

PRSS8	Prostasin	Past smoker	0.766 (0.627 - 0.937)	0.009	0.02
		Current smoker	1.710 (1.168 - 2.503)	0.006	
IL-27	Interleukin-27	Past smoker	1.125 (0.925 - 1.370)	0.24	0.046
		Current smoker	0.715 (0.522 - 0.980)	0.037	
		Past smoker	0.844 (0.712 - 1.002)	0.052	

Table S6: Final selection of biomarkers at V4 associated to smoking status (multivariable multinomial model obtained from forward selection) using ITPW analyses

Variable	Olink Name	Status	OR (95% CI)	p-value
AZU1	Azurocidin	Current smoker	1.419 (1.118 - 1.800)	0.004
		Past smoker	1.314 (1.103 - 1.566)	0.002
CCL11	Eotaxin-1	Current smoker	2.084 (1.617 - 2.686)	<.0001
		Past smoker	1.051 (0.889 - 1.242)	0.56
CCL25	C-C motif chemokine 25	Current smoker	1.327 (1.076 - 1.636)	0.008
		Past smoker	0.975 (0.850 - 1.117)	0.71
CHIT1	Chitotriosidase-1	Current smoker	1.125 (0.924 - 1.369)	0.24
		Past smoker	1.249 (1.095 - 1.426)	0.0009
CXCL11	C-X-C motif chemokine 11	Current smoker	0.637 (0.504 - 0.807)	0.0002
		Past smoker	0.994 (0.857 - 1.154)	0.94
DPP6	Dipeptidyl aminopeptidase-like protein 6	Current smoker	0.656 (0.519 - 0.830)	0.0004
		Past smoker	0.794 (0.679 - 0.928)	0.004
EDIL3	EGF-like repeat and discoidin I-like domain-containing	Current smoker	1.903 (1.483 - 2.442)	<.0001

protein 3				
			Past smoker	1.270 (1.013 - 1.593) 0.038
FGF-21 panel)	(INF Fibroblast growth factor 21		Current smoker	0.982 (0.782 - 1.233) 0.88
			Past smoker	1.239 (1.066 - 1.439) 0.005
GDF-2	Growth/differentiation factor 2		Current smoker	0.756 (0.596 - 0.959) 0.021
			Past smoker	1.213 (1.038 - 1.418) 0.015
IL1RL2	Interleukin-1 receptor-like 2		Current smoker	0.864 (0.695 - 1.076) 0.19
			Past smoker	0.790 (0.685 - 0.912) 0.001
IL-12B	Interleukin-12 subunit beta		Current smoker	0.552 (0.439 - 0.693) <.0001
			Past smoker	1.038 (0.894 - 1.204) 0.63
KIT	Mast/stem cell growth factor receptor Kit		Current smoker	1.899 (1.464 - 2.462) <.0001
			Past smoker	1.025 (0.864 - 1.216) 0.78
LOX-1	Lectin-like oxidized LDL receptor 1		Current smoker	0.811 (0.622 - 1.059) 0.12
			Past smoker	0.707 (0.589 - 0.848) 0.0002
LYVE1	Lymphatic vessel endothelial hyaluronic acid receptor 1		Current smoker	0.836 (0.648 - 1.078) 0.17
			Past smoker	1.200 (1.022 - 1.409) 0.026
MMP-10	Matrix metalloproteinase-10		Current smoker	1.391 (1.142 - 1.694) 0.001
			Past smoker	1.082 (0.945 - 1.238) 0.25
NCAM1	Neural cell adhesion molecule 1		Current smoker	0.554 (0.420 - 0.731) <.0001
			Past smoker	0.829 (0.696 - 0.988) 0.036
Notch 3	Neurogenic locus notch homolog protein 3		Current smoker	0.612 (0.468 - 0.801) 0.0003
			Past smoker	0.932 (0.784 - 1.107) 0.42

PRSS8	Prostasin	Current smoker	1.477 (1.157 - 1.885)	0.002
		Past smoker	1.031 (0.887 - 1.199)	0.69
RAGE	Receptor for advanced glycosylation end products	Current smoker	0.923 (0.730 - 1.167)	0.51
		Past smoker	0.808 (0.690 - 0.945)	0.008
SCGB3A2	Secretoglobin family 3A member 2	Current smoker	1.401 (1.119 - 1.754)	0.003
		Past smoker	0.862 (0.722 - 1.030)	0.10
ST2	ST2 protein	Current smoker	0.710 (0.571 - 0.884)	0.002
		Past smoker	0.939 (0.817 - 1.078)	0.37
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	Current smoker	2.009 (1.580 - 2.555)	<.0001
		Past smoker	1.029 (0.880 - 1.204)	0.72
TR	Transferrin receptor protein 1	Current smoker	0.733 (0.599 - 0.898)	0.003
		Past smoker	0.870 (0.764 - 0.991)	0.036
TWEAK	Tumor necrosis factor	Current smoker	0.664 (0.518 - 0.852)	0.001
		Past smoker	1.026 (0.875 - 1.202)	0.75
VEGFD	Vascular endothelial growth factor D	Current smoker	1.561 (1.233 - 1.977)	0.0002
		Past smoker	1.248 (1.069 - 1.456)	0.005