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Article

Association of Per- and Polyfluoroalkyl Substance Exposure with Coronary Stenosis and Prognosis in Acute Coronary Syndrome

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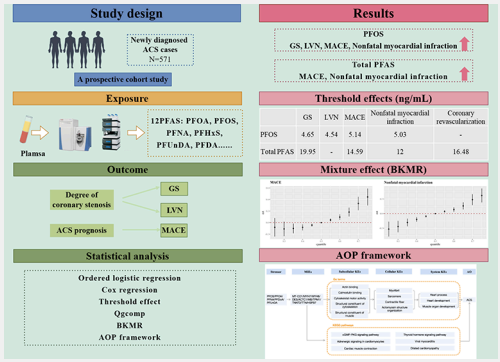
ABSTRACT: Per- and polyfluoroalkyl substances (PFAS) have been associated with an increased risk of acute coronary syndromes (ACS), but the influence on the degree of coronary stenosis and prognosis is unclear. This study enrolled 571 newly diagnosed ACS cases and investigated the association of 12 PFAS with coronary stenosis severity and prognosis. Coronary stenosis was assessed via Gensini score (GS) and number of lesioned vessels (LVN). Prognosis was estimated by tracking major adverse cardiovascular events (MACE). Statistical analyses included ordered logistic regression, Cox regression, threshold effect models, Bayesian kernel machine regression, and quantile g-computation models. The adverse outcome pathway (AOP) framework was applied to reveal the underlying mechanism. The results showed positive association between perfluorooctanesulfonic acid (PFOS) and coronary stenosis, with an odds ratio (95% confidence interval, CI) of 1.33 (1.06, 1.67) for GS and 1.36 (1.08, 1.71) for LVN. PFOS significantly increased the incidence of poor prognosis, with hazard ratios (95% CI) of 1.96 (1.34, 2.89) for MACE. Threshold effects were observed for PFAS on coronary stenosis and prognosis, with PFOS thresholds of 4.65 ng/mL for GS, 4.54 ng/mL for LVN, and 5.14 ng/mL for MACE, and 5.03 ng/mL for nonfatal myocardial infarction. PFAS mixture exposure increased the occurrence of MACE and nonfatal myocardial infarction. The AOP framework shows that PFAS may impact protein binding, the cytoskeleton, multicellular biological processes, and heart function. In summary, our study revealed the adverse effects of PFAS on the degree of coronary stenosis and prognosis in ACS and identified potentially relevant molecular loci.



KEYWORDS: *Per- and polyfluoroalkyl substances, Acute coronary syndrome, Threshold effect, Mixture analysis, Degree of coronary stenosis, Prognosis*

1. INTRODUCTION

Per- and polyfluoroalkyl (PFAS) are widely prevalent in various industrial and consumer categories, including nonstick coatings, water-resistant textiles, and firefighting foams.[1] Humans are primarily exposed to PFAS through the consumption of contaminated food and water, as well as with products containing these chemicals. Human biomonitoring has shown widespread exposure and accumulation of PFAS in the human body, with perfluorooctanesulfonic acid (PFOS) being one of the most abundant.[2] The global distribution and chemical diversity of PFAS has raised concerns about their potential health risks, including cardiovascular effects.[3] PFAS exposure can cause adverse cardiovascular effects, including atherosclerotic plaque formation, hypertension, and disorders of lipid metabolism.[4−7]

Previous research on cardiovascular disease has identified acute coronary syndrome (ACS) as one of the most prevalent acute cardiovascular events. ACS encompasses a series of cardiovascular conditions associated with a sudden reduction in intracoronary blood flow, which ultimately leads to myocardial ischemia and, in severe cases, infarction.[8] At present, ACS is one of the leading causes of death from cardiovascular disease.[9,10] Nonetheless, in recent years, the influence of environmental factors on ACS has garnered increasing attention, such as the PFAS mentioned above.[11] Previous studies have demonstrated the association between PFAS and ACS risk. In a case-control study, we observed that being exposed to PFOS and PFOA was associated with a greater risk of ACS, with odds ratio (OR) (95% confidence interval, CI) of 1.77 (1.15, 2.72) for PFOS and 1.51 (1.07, 2.15) for PFOA, respectively.[12] Although the role of PFAS as a risk factor for ACS is established, the role of PFAS congeners and its mixtures in the degree of coronary stenosis and prognosis of ACS remains undefined. Although the role of PFAS as a risk factor for ACS is established, several critical

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questions remain unanswered, particularly regarding the impact of PFAS on the severity and outcomes of ACS. This prompted us to focus on the influence of PFAS exposure on the degree of coronary stenosis and the prognosis of ACS patients.

The degree of coronary stenosis is directly related to the severity of ACS. Evidence shows that exposure to PFAS could potentially promote coronary artery stenosis and atherosclerotic plaque formation by inducing an inflammatory response and platelet accumulation.[13,14] Studies have shown a connection between PFAS and diseases including diabetes, hyperlipidemia, and hypertension, all of which might worsen the coronary stenosis of ACS.[15] Given these connections, an investigation into the impact of PFAS on coronary stenosis is essential for understanding how environmental exposures may influence the progression of ACS. Furthermore, after the initial episode of ACS, the probability of major adverse cardiovascular events (MACE) was elevated. MACE is a key indicator of prognosis following ACS. It is a composite outcome of serious cardiovascular events including cardiovascular death, recurrent myocardial infarction, ischemic stroke, or unplanned repeat revascularization procedures, leading to a deterioration in prognosis. Several studies have indicated that PFAS may disrupt coronary blood flow and oxygenation through endothelial cell injury and vascular dysfunction, potentially increasing the risk of MACE.[16,17] However, there is a lack of studies investigating the impact of PFAS on prognosis, i.e., the occurrence of MACE, in patients with ACS.

Humans are simultaneously exposed to complex mixtures of PFAS in everyday life, yet most previous research has predominantly utilized conventional single-chemical evaluations. Advanced statistical methods such as Bayesian kernel machine regression (BKMR) and quantile g-computation (qgcomp) now allow assessment of complex interactions and cumulative effects in PFAS mixtures.[18,19] Although some studies have shown a linear dose−response trend between PFAS and health outcomes, emerging evidence shows a nonlinear relationship marked by the presence of a threshold level of exposure that triggers significantly intensified biological changes.[20] The precise definition of thresholds is essential to characterize risk, especially in susceptible populations, and to provide guidance on health protection from PFAS. Current threshold studies have examined the relationship between PFAS and depression and metabolic syndrome, as well as its impact on immunosuppression in specific populations.[21−23] Given our previous findings on PFAS and ACS, investigating potential thresholds for PFAS effects on coronary stenosis and prognosis in ACS patients is important for precise prevention and improved patient management.

To further elucidate the biological mechanisms of PFAS-induced cardiovascular effects, the adverse outcome pathway (AOP) framework provides a valuable tool. The AOP is an innovative risk assessment tool to integrate and delineate toxicological effects within biological tissues.[24] It follows a standardized structure comprising a molecular initiating event (MIE), key events (KEs), and an adverse outcome (AO), as outlined by Ankley et al.[24] Toxicologists have employed the AOP framework to evaluate the biological pathways through which chlorinated organophosphorus flame retardants induce lung function deterioration[25] and arsenic exposure contributes to the onset of nonalcoholic fatty liver disease,[26] aiding in threat and risk assessment. By employing the AOP framework to PFAS exposure and ACS, we can systematically predict the occurrence and progression of ACS, better understand the underlying molecular mechanisms, and establish a more robust basis for risk assessment.

We have found an association between PFAS exposure and the risk of ACS, but it is unclear whether PFAS exposure further affects prognosis by influencing the degree of coronary stenosis in patients with ACS. Therefore, in the present study, we explore the potential relationship between PFAS and the degree of coronary stenosis and prognosis in patients with ACS. The study encompassed an evaluation of mixture effects, threshold effects, and molecular mechanisms between PFAS with degree of coronary stenosis and prognosis in ACS. The AOP framework is used to investigate the underlying molecular mechanisms. We hypothesized that PFAS would exhibit a threshold effect on the degree of coronary stenosis at baseline and adverse cardiovascular outcomes during follow-up.

2. METHOD

2.1. Study Participants

This study consecutively enrolled patients at the Second Hospital of Hebei Medical University in Shijiazhuang, Hebei, China, between January 2022 and August 2022. The inclusion criteria for participants were as follows. 1) patients aged between 18 and 80 years; 2) patients whose ACS has begun no more than 24 h before admission; 3) patients devoid of any prior ACS diagnosis;[27] 4) patients with no history of occupational exposure. Occupational exposure is defined as current or past employment in industries known for high PFAS use or production, including fluorochemical manufacturing, firefighting services using PFAS-containing foam, and facilities producing or using PFAS based products.[28] The exclusion criteria comprised malignancy, heart valve disease, cardiomyopathy, history of coronary artery bypass surgery and percutaneous coronary intervention, acute hepatic, renal failure severe diseases like serious infectious. Acute coronary syndromes are comprised of acute myocardial infarction (ICD-10, code I21) and unstable angina (ICD-10, code T20.0).[29] Two professional cardiologists administered diagnostic examinations for ACS according to the European Society of Cardiology (ESC) 2020 guidelines for ACS based on the patient's clinical symptoms, myocardial enzymes, and electrocardiographic changes.[30]

Originally, 642 patients with newly diagnosed ACS were enrolled. After excluding 14 patients with malignancy, 6 patients with heart valve disease, 15 patients with cardiomyopathy, 4 patients with acute liver and kidney impairment, 24 patients with renal insufficiency, and 8 patients with severe disease, 571 participants were eventually included in this study. The number of participants met the required sample size (Text S1). After their discharge, ACS patients were longitudinally followed for about one year. Specific follow-up and prognostic outcomes are elucidated in Section "2.2.2.2. Evaluation of the Prognosis of ACS". All participants have provided written consent to participate in the study. The study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University (No., 2021-R510). All subjects signed an informed consent form.

2.2. Data Collection

2.2.1. PFAS Measurement. Approximately 2 mL of blood was drawn from each participant on a fasting basis prior to coronary angiography and centrifuged at 3000 × g for 10 min to extract the plasma, and subsequently frozen at −80 °C until

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analysis. The method of determining PFAS concentrations in plasma was described in our previous work.[12] Specifically, 200 μL of plasma was mixed with 4 ng of mass-labeled standards of perfluorooctanoic acid (PFOA). Afterward, an addition of 400 μL acetonitrile containing 1% formic acid was introduced, followed by a vertexing period of 1 min and a 10 min sonication step. The resultant supernatant was then passed through a 0.22 μm filter membrane, thereby preparing it for subsequent analysis. A total of 12 PFAS were measured in this study, including PFOA, PFOS, perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS).

Before performing the analysis, the solvent and analysis vessel were inspected and no measurable PFAS was detected. For quality control purposes, quality control (QC) samples were assessed. For every 20 samples, one QC sample was run, and the results were deemed acceptable if the fluctuation in this sample was less than 15%. The details of the instrumental analysis and methodological validation are detailed in the Supporting Information (Text S2, Table S1, Table S2).

2.2.2. Assessment of Outcomes. *2.2.2.1. Evaluation of the Degree of Coronary Stenosis of ACS.* Coronary angiography provides accurate visualization of coronary artery stenosis and atherosclerotic plaque burden. Coronary angiograms were obtained using an angiography system (Philips, The Netherlands) and quantitative coronary angiographic analysis of atherosclerotic plaques and luminal stenoses was performed using a companion software system according to standard operating procedures.[31] The coronary angiograms were analyzed and evaluated by two experienced cardiologists. We quantified the degree of coronary stenosis in ACS using the Gensini score (GS) system and the number of lesioned vessels (LVN).[32,33] The GS is a widely used assessment index that has been extensively validated in the Chinese population.[34] GS provides a comprehensive angiographic assessment that considers not only the degree of coronary luminal stenosis, but also the location of the lesion. The cumulative GS score, weighted by stenosis severity and vessel segment coefficients, provides a comprehensive picture of the overall status of coronary artery disease. In addition, LVN, which is the number of major coronary arteries with angiographic stenosis ≥50%, is another widely used metric to assess the degree of coronary stenosis. The LVN is simple and easy to use in clinical practice. It provides a quick and effective measure of the extent of disease and demonstrates the full complexity of ACS.

In the Gensini scoring system, a lesion is defined as significant when it causes ≥1% reduction in luminal diameter. The severity is scored as follows: 1%−25% obstruction receives a score of 1, with the score doubling as the severity of obstruction progresses through 25%−50%, 50%−75%, 75%−90%, 90%−99%, and 100% diameter reduction, corresponding to scores of 2, 4, 8, 16, and 32, respectively.[35] These scores are subsequently multiplied by the coefficients designated for each coronary artery and its corresponding segments. The GS for each participant is obtained by accumulating all the scores. Based on the GS, we stratified the study participants into four groups.[36] Participants presenting no coronary artery stenosis were classified as GS 0. Those with a GS greater than zero were further divided into three groups (trichotomies): GS 1, with scores equal to or less than 17; GS 2, with scores between 18 and 40; and GS 3, comprising scores above 40. Treating GS as a categorical variable, improves the applicability and interpretability of the degree of coronary stenosis in ACS in clinical and research settings, simplifies statistical analysis, and provides a practical approach to assessing the degree of coronary stenosis of ACS.[34]

Coronary vessel stenosis is categorized as single, double, or multiple based on significant narrowing in the main coronary arteries: single vessel stenosis involves one artery, double vessel stenosis involves two, and multiple vessel stenosis involves more than two.[37] The predominant coronary arteries comprise the left main trunk, left anterior descending artery, left circumflex artery, and right coronary artery.[38] In accordance with GS's treatment, we also subsequently analyzed LVN as a categorical variable. For this investigation, we categorized the study participants into four groups, depending on the number of major coronary arteries exhibiting stenosis.[39,40] These groups comprised LVN 0, representing no coronary artery stenosis; LVN 1, signifying single-vessel stenosis; LVN 2, denoting double-vessel stenosis; and LVN 3, indicating multivessel stenosis. GS and LVN are important indicators for a comprehensive evaluation of the degree of coronary stenosis in patients with ACS. Each possess distinct advantages in assessing the degree of coronary stenosis, and the combination of the two measures provides a complementary quantitative assessment of the degree of coronary stenosis in the ACS.

2.2.2.2. Evaluation of the Prognosis of ACS. Twelve months after discharge, ACS patients were followed up, mainly through telephone interviews and review of medical records. During the follow-up period, 154 cases were lost to follow-up and 417 cases were eventually available for statistical analysis, resulting in a follow-up rate of 73%. The median duration of follow-up in this study was 16.4 months (interquartile range, IQR 14.6−18.0 months). The incidence of MACE served as the main end point in this study, in accordance with the ESC guidelines for the management of acute coronary syndromes.[41] MACE was defined as a composite of cardiovascular death, nonfatal myocardial infarction, stroke, and coronary revascularization (e.g., percutaneous coronary intervention or coronary artery bypass grafting).[42] The secondary end points of this study were nonfatal myocardial infarction and coronary revascularization. Due to the low incidence rate, individual events such as deaths were not analyzed separately.

2.2.3. Covariate Collection. Demographic and clinical data on all participants were gathered by trained researchers. We employed a standardized questionnaire to obtain demographic and other information, including age (continuous), sex (male, female), body mass index (BMI), smoking (yes, no), drinking (yes, no), education level (illiterate, primary to high school, university and above). In this study, the smoking was identified as individuals who had smoked at least 100 cigarettes in their lives, and the nonsmoking was identified as persons who had never smoked or smoked less than 100 cigarettes in their lives. The drinking was identified as having consumed at least 12 alcoholic drinks in someone's lifetime, and the nondrinking was considered as having consumed less than 12 alcoholic beverages. The BMI was computed by dividing the body weight in kilograms by the square of the height in meters (kg/m^2).

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2.3. Statistical Analysis

2.3.1. Descriptive Statistics. Continuous variables were represented as mean ± standard deviation (SD) for normally distributed data and median and IQR for non-normally distributed data. Categorical data, on the other hand, were presented as frequencies (percentages). One-way ANOVA was used for continuous variables that followed a normal distribution, Kruskal−Wallis H-test for those that did not, and the chi-square test for categorical variables. These tests aimed to discern disparities in demographic variables and PFAS exposure levels across the group of GS and LVN. PFAS with a detection rate ≥80% were incorporated into the subsequent statistical study, including PFOA, PFOS, PFNA, PFHxS, PFUnDA, and PFDA. For these six PFAS, concentrations that were below the LOD were substituted with LOD/$\sqrt{2}$. To decrease the skewness of the distribution, the plasma PFAS concentrations underwent a natural Ln transformation in subsequent analyses.

2.3.2. Single Pollutant Analysis. The ordered logistic regression analysis was conducted to evaluate the association between each PFAS and the degree of coronary stenosis of ACS. The OR (95% CI) was computed in relation to the per unit increase in Ln-transformed PFAS concentrations. A test of parallel lines assumption in ordinal logistic regression confirmed the appropriateness of this model ($P > 0.05$), ensuring consistency of the relationships across the degree of coronary stenosis in each category of ACS. In regression models, PFAS was considered as continuous or categorical variable, separately. When treated categorically, plasma PFAS concentrations were divided into tertiles, with the first tertile serving as the reference group. Moreover, a linear trend test was conducted by incorporating the median of each PFAS tertile as a continuous variable. Three distinct regression models were developed, each employing a different strategy for covariates adjustments: crude model, Model 1, and Model 2. The crude model did not adjust for covariates. Model 1 adjusted for age, sex, and BMI. Model 2, perceived as the core model in this analysis, adjusted for smoking, drinking, and education level based on Model 1. Additionally, the stratified analysis was performed, by sex (male, female) and age (<50, ≥ 50). *P*-values were adjusted with the false discovery rate (FDR) method for multiple comparisons.

Survival analyses were performed by applying Cox proportional hazards models to assess the association between PFAS exposure and the occurrence of MACE, myocardial infarction, and coronary revascularization. Hazard ratios (HR) and 95% CI were calculated. Consistent with the ordered logistic regression analysis, we employed three different models and considered PFAS as a continuous or categorical variable. The lost data were uniformly treated as right-censored data. Additionally, a linear trend was also assessed using the median of each PFAS tertile as a continuous variable.

To evaluate the dose−response correlation between PFAS concentrations and the degree of coronary stenosis and prognosis in ACS, a restricted cubic spline regression was implemented. To accurately capture the subtle nonlinear relationship between PFAS and the degree of coronary stenosis, GS and LVN were included in the model as continuous variables. The regression model was furnished with a three-knot restricted cubic spline, with knots positioned at the 25th, 50th, and 75th percentiles.[43] The 25th PFAS concentration was established as the reference point within the model. The model adjusts for covariates consistent with the core model.

2.3.3. Threshold Effect Analysis. In the present study, an analysis was also performed to investigate potential threshold effects. Typically, there are four models employed for investigating threshold effects, including step, hinge, segmented, and stegmented models.[44] Based on the preliminary fitting of the RCS, the hinge and segmented models were more consistent with our a priori knowledge and data distribution, so we chose these two models to explore the threshold effect. Both the hinge and segmented models are based on segmental regression techniques. The hinge model, a type of segmented linear regression, is designed to identify a threshold within the data. Beyond this threshold, the relationship between the dependent and independent variables changes significantly. The segmented model extends the hinge model by allowing a nonzero slope across the identified threshold, providing greater flexibility to capture the nuances of data relationships. In the framework of generalized linear regression, the mean functions of these two threshold models can be written as follows:

hinge

$$Y = \alpha + \alpha_T Z + \beta[X - \theta]_+$$

segmented

$$Y = \alpha + \alpha_T Z + \beta[X - \theta]_+ + \gamma x$$

where Y is the outcome variable, X is the exposure variable, θ is the threshold parameter, Z is the additional predictor, and $[X - \theta]_+$ denotes the hinge function, equal to $X - \theta$ if $X > \theta$ and 0 otherwise.

The hinge and segmented models can capture and delineate complex nonlinear relationships in the data set more accurately than previous methods of visual assessment. In addition, we performed hypothesis tests on the slopes of these models. If the slope before the turning point was not significantly different from zero (confidence interval not encompassing zero), we consider the use of the hinge model, otherwise, we opted for the segmented model. There are two common methods for determining the turning point: the exact and the smooth method. The primary distinction between these methodologies is in their treatment of the change point. The exact method does not require a starting value at the change point to find the global optimal solution, whereas the smooth method allows for flexibility at the turning point. For this study, the choice of the exact method was due to its ability to provide a globally optimal solution.[45] In this analysis, the degree of coronary stenosis and prognosis were included in the model as categorical variables, with adjustments for covariates consistent with the core model.

2.3.4. Mixture Analysis. Qgcomp and BKMR models were adopted to explore the mixture effect of PFAS on the degree of coronary stenosis and prognosis in ACS. The qgcomp handles nonlinear and heterogeneous exposure-outcome associations, assigning weights to each component within the PFAS mixture to explicate their relative contributions to the degree of coronary stenosis and prognosis. It is worth noting that, irrespective of their directionality, the sum of the absolute values of these weights for both positive and negative values is equal to 1, respectively.[19] The results are presented as OR (95% CI) for the effects on the degree of coronary stenosis and prognosis when the concentration of all compounds within the PFAS mixture increases by a quarter.

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The model adjusts for covariates consistent with the core model.

Although the qgcomp is advantageous in estimating the joint effect on outcomes when all exposures are increased by a quantile concurrently, it falls short in directly depicting dose−response relationships for such mixture effects. Therefore, the BKMR model was adopted to further probe the connection between PFAS mixture exposure and the degree of coronary stenosis and prognosis in ACS. The BKMR model utilizes a kernel function in Bayesian inference to elucidate the intricate associations between exposure variables and health outcomes.[18] The derivation of the dose−response relationship involves comparing all chemicals at a given percentile (escalating from 25 to 75 by 5) with all chemicals at the 50th percentile, ascertained through 10,000 Markov Chain Monte Carlo iterations. Moreover, the BKMR model is capable of pinpointing vital PFAS congeners via an innovative hierarchical variable selection process. The posterior inclusion probability (PIP) of each PFAS, as estimated by the BKMR model, signifies the relative importance of each PFAS to the composite effect of the mixture. The PIP serves as an indicator of the variable's importance, with a maximum value of 1.0. The PFAS congener with a PIP of 0.5 or above is deemed predominant. In this model, the exposure variables were treated as continuous variables, and covariate adjustments were the same as in the core model.

2.3.5. Sensitivity Analysis. Sensitivity analyses were performed to ascertain the robustness of the research findings. ACS conspicuously afflicts an older demographic, whereas individuals younger than 30 years commonly demonstrate a significantly diminished propensity for such disorders.[46] Moreover, individuals with a BMI exceeding 30 kg/m^2 are categorized as obese, a demographic notoriously predisposed to ACS.[47] We executed these analyses after the exclusion of data about individuals under the age of 30 or those presenting a BMI surpassing 30 kg/m^2. By eliminating this younger or obese participants, we focused our research on older or nonobese populations that are more susceptible to ACS.

2.4. Adverse Outcome Pathway Framework Construction

To further explain the underlying mechanism of the effects of PFAS exposure on the degree of coronary stenosis and prognosis in ACS, we conducted a conceptual framework for toxicological research, the Adverse Outcome Pathway (AOP). The AOP provides a consistent structure and terminology for organizing our understanding of ecotoxicology across biological tissue levels, thus facilitating more effective application and integration of diverse information and identification of critical uncertainties and research needs.[48] A series of events constitute the AOP, starting with molecular initiating events (MIEs) and connecting to key events (KEs) at multiple levels (e.g., molecules, cells, tissues, and organs) and ending with adverse outcomes (AO).[48]

2.4.1. Identification of the Potential MIEs. First, PFAS-related genes (PFAS-set) were obtained in CTD using the full names of the 12 PFAS measured in this study on December 2023. Second, ACS-related genes (ACS-set) were constructed with the genes retrieved from Gene Cards (https://www.genecards.org/) with the keyword "acute coronary syndrome". Then, to identify genes with high relevance to the outcome, we used the GeneCards Inferred Functionality Score (GIFtS) and relevance score (GIFtS ≥ 20, relevance score ≥20).[25] Finally, the gene landscape, specifically the overlap of genes between the PFAS-set and the ACS-set, was examined in the Genotype-Tissue Expression (GTEx) Portal (https://gtexportal.org/home/). Potential MIEs were identified by focusing on genes exhibiting a high abundance in heart tissue compared to other tissues; for instance, genes ranking in the top 10 for expression across various tissues, with a particular emphasis on the heart.

2.4.2. Identification of the Potential KEs. The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment were conducted using R package "clusterProfiler" to elucidate phenotypes (MIEs) establishing connections between PFAS and ACS. Following this, the R-annotated phenotypes underwent further refinement, with a focus on identifying those highly correlated with heart disease, thus pinpointing potential KEs.

2.4.3. Construction of the AOP Framework. We manually searched GO items of the potential KEs in the AmiGO 2 database (http://amigo.geneontology.org/amigo) to explore the ancestor chart, and ranked in subcellular, cellular, and system levels.[49] Further, based on empirical and literature evidence, the hierarchical and biological relationships of KEs were identified, and an AOP framework was constructed.

3. RESULT

3.1. Population Characteristics

Table 1 delineates the baseline demographics of patients afflicted by ACS. These participants, with an age distribution centered around 55.9 ± 10.6 years, of whom 63.3% were male. Description of the basic characteristics within the research group, stratified by the GS and LVN, are elucidated in Table S3 and Table S4. The disparities are discernible across demographics within the group of GS and LVN, including age, sex, and smoking. The follow-up results showed that the incidence of MACE was 15.83%, including 5.80% for nonfatal myocardial infarction and 7.19% for coronary revascularization, as shown in Table 1.

Table 1. Baseline Demographic Characteristics and Longitudinal Follow-Up of the ACS Study Cohort[a]

Variables	Total (n = 571)
Age, years, mean ± SD	55.9 ± 10.6
Sex, n (%)	
Male	363 (63.6)
Female	208 (36.4)
BMI, kg/m^2, mean ± SD	26.3 ± 3.2
Educational level, n (%)	
Illiterate	144 (25.2)
Primary to high school	309 (54.1)
College and beyond	118 (20.7)
Smoking, n (%)	
Yes	229 (40.1)
No	342 (59.9)
Drinking, n (%)	
Yes	201 (35.2)
No	370 (64.8)
Prognosis follow-up, n (%)	
MACE	66 (15.83)
Nonfatal myocardial infarction	20 (5.80)
Coronary revascularization	30 (7.19)

[a]Abbreviations: SD: standard deviation; BMI: body mass index; MACE: major adverse cardiovascular event.

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Table 2. Results of the Association between PFAS and GS[a]

			Categorical PFAS levels			
	OR (95% CI)	*P*-FDR	T1	T2	T3	P_{trend}-FDR
Crude[b]						
PFOA	0.89 (0.68, 1.16)	0.377	ref	0.94 (0.66, 1.35)	0.81 (0.56, 1.16)	0.239
PFOS	**1.56 (1.25, 1.94)**	**<0.001**	ref	1.25 (0.87, 1.79)	**1.98 (1.38, 2.85)**	**<0.001**
PFNA	1.10 (0.90, 1.35)	0.354	ref	1.18 (0.82, 1.68)	1.19 (0.83, 1.70)	0.572
PFHxS	1.16 (0.98, 1.36)	0.076	ref	1.27 (0.88, 1.82)	1.46 (1.02, 2.09)	0.054
PFUnDA	0.89 (0.79, 1.01)	0.082	ref	0.83 (0.58, 1.18)	0.93 (0.65, 1.34)	0.748
PFDA	0.95 (0.80, 1.13)	0.548	ref	0.83 (0.58, 1.20)	1.03 (0.72, 1.47)	0.866
Total PFAS	**1.47 (1.09, 1.97)**	**0.011**	ref	1.35 (0.94, 1.94)	**1.54 (1.08, 2.21)**	**0.024**
Model 1[c]						
PFOA	0.91 (0.69, 1.19)	0.648	ref	0.88 (0.61, 1.27)	0.81 (0.56, 1.17)	0.363
PFOS	**1.34 (1.07, 1.68)**	**0.016**	ref	1.14 (0.79, 1.64)	**1.61 (1.11, 2.34)**	**0.012**
PFNA	1.07 (0.86, 1.33)	0.700	ref	1.17 (0.81, 1.68)	1.06 (0.74, 1.53)	0.918
PFHxS	1.02 (0.86, 1.21)	0.804	ref	1.14 (0.79, 1.64)	1.11 (0.77, 1.62)	0.812
PFUnDA	0.90 (0.79, 1.03)	0.167	ref	0.83 (0.58, 1.20)	0.97 (0.67, 1.40)	0.961
PFDA	0.98 (0.82, 1.17)	0.798	ref	0.87 (0.60, 1.25)	1.02 (0.71, 1.47)	0.802
Total PFAS	1.28 (0.94, 1.73)	0.149	ref	1.26 (0.87, 1.82)	1.31 (0.91, 1.89)	0.247
Model 2[d]						
PFOA	0.91 (0.69, 1.20)	0.594	ref	0.88 (0.61, 1.27)	0.80 (0.55, 1.15)	0.315
PFOS	**1.33 (1.06, 1.67)**	**0.034**	ref	1.10 (0.77, 1.59)	**1.60 (1.10, 2.31)**	**0.004**
PFNA	1.07 (0.86, 1.34)	0.613	ref	1.17 (0.81, 1.68)	1.05 (0.73, 1.52)	0.905
PFHxS	1.03 (0.87, 1.21)	0.763	ref	1.18 (0.81, 1.70)	1.09 (0.75, 1.59)	0.887
PFUnDA	0.90 (0.79, 1.03)	0.181	ref	0.82 (0.57, 1.18)	0.96 (0.67, 1.40)	0.991
PFDA	0.99 (0.83, 1.18)	0.891	ref	0.86 (0.59, 1.24)	1.02 (0.71, 1.48)	0.847
Total PFAS	1.28 (0.95, 1.74)	0.150	ref	1.25 (0.87, 1.81)	1.30 (0.90, 1.88)	0.254

[a]Notes: Bold denotes statistically significant associations. Abbreviations: GS: gensini score; *P*-FDR: false discovery rate adjusted *P* value; P_{trend}: *P* for trend; P_{trend}-FDR: false discovery rate adjusted *P* for trend; ref: reference; T1: the first tertile; T2: the second tertile; T3: the third tertile. [b]Crude, unadjusted for covariates. [c]Model 1, adjusted for age, sex, and BMI. [d]Model 2, adjusted for smoking, drinking, and educational level based on Model 1.

3.2. Distribution of PFAS Exposure Levels

Table S5 explicates the exposure levels of plasma PFAS in the study population. Six PFAS were detected at over 80%, with median concentrations ranging from 0.39 to 5.56 ng/mL. Among these, PFOS presents the highest median concentration, followed closely by PFOA. The PFAS exposure levels, stratified by the degree of coronary stenosis of ACS, are provided in Table S6 and Table S7, with PFOS revealing significant variations across the GS and LVN.

3.3. Singular Pollutant Analysis

Table 2 presents the results of the association between PFAS exposure and GS. When PFAS was considered as a continuous variable, PFOS demonstrated a significant positive correlation with GS in the three models with OR (95% CI) of 1.56 (1.25, 1.94), 1.34 (1.07, 1.68) and 1.33 (1.06, 1.67), respectively. In the PFAS tertiles model, PFOS was significantly associated with GS in the highest tertiles relative to the lowest tertiles.

Table 3 shows the results of the association between PFAS exposure and LVN. When PFAS was used as a continuous variable, PFOS was significantly positively associated with the number of lesioned vessels in the three models, with OR (95% CI) of 1.57 (1.26, 1.95), 1.35 (1.07, 1.70) and, 1.36 (1.08, 1.71), respectively. Similarly, in the PFAS tertiles model, a significant association was observed between the highest PFOS tertile and LVN, relative to the lowest tertile.

Table 4, Table 5, and Table S8 show the results of the survival analyses. The results indicated that PFOS and total PFAS were significantly and positively associated with the occurrence of MACE and nonfatal myocardial infarction in all three models. After adjustment for all covariates, the HR (95% CI) for PFOS and total PFAS with MACE were 1.96 (1.34, 2.89) and 2.46 (1.51, 4.01), respectively. The HR (95% CI) for PFOS and total PFAS nonfatal myocardial infarction was 3.86 (2.00, 7.46) and 4.56 (1.99, 10.45), respectively. In the PFAS tertile model, PFOS and total PFAS in the highest tertile were significantly associated with MEAC and nonfatal myocardial infarction compared with the lowest tertile. However, there was no significant association between PFAS and the occurrence of coronary revascularization in the survival analysis.

The results of stratified analyses are shown in Table S9 and Table S10. Sex-stratified analysis reveals that, among males, PFOS levels do not exhibit a significant relationship with GS after adjusting for *P*-values, nonetheless a significant positive correlation is observed with LVN. However, this association is absent in the female. The age-stratified analysis demonstrates a significant positive association between PFOS and GS and LVN in individuals younger than 50 years of age. In contrast, for those aged 50 and older, no significant association between PFOS and GS is identified after *P*-value adjustment.

Figure S1 illuminates the dose−response correlation of PFAS and the degree of coronary stenosis and prognosis. The results reveal nonlinear correlation between several PFAS components and the degree of coronary stenosis and prognosis. Specifically, PFOS, PFNA, PFUnDA, PFDA, and total PFAS showed nonlinear associations with GS (Figure S1A). PFOS and PFUnDA exhibited nonlinear relationships with LVN (Figure S1B). Additionally, six PFAS demonstrated nonlinear associations with MACE and coronary revascularize-

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Table 3. Results of the Association between PFAS and LVN[a]

			Categorical PFAS levels			
	OR (95% CI)	*P*-FDR	T1	T2	T3	P_{trend}-FDR
Crude[b]						
PFOA	0.82 (0.63, 1.07)	0.138	ref	1.03 (0.72, 1.49)	0.76 (0.53, 1.09)	0.096
PFOS	**1.57 (1.26, 1.95)**	**<0.001**	ref	1.14 (0.79, 1.63)	**1.85 (1.29, 2.67)**	**0.001**
PFNA	1.13 (0.92, 1.38)	0.244	ref	1.35 (0.94, 1.94)	1.22 (0.85, 1.75)	0.751
PFHxS	1.21 (1.03, 1.43)	0.021	ref	1.21 (0.85, 1.74)	1.59 (1.10, 2.28)	0.018
PFUnDA	0.88 (0.78, 1.00)	0.052	ref	0.79 (0.55, 1.13)	0.86 (0.60, 1.24)	0.44
PFDA	0.89 (0.75, 1.06)	0.198	ref	0.78 (0.54, 1.12)	0.89 (0.62, 1.28)	0.564
Total PFAS	**1.40 (1.04, 1.88)**	**0.027**	ref	1.17 (0.81, 1.68)	**1.54 (1.07, 2.21)**	**0.022**
Model 1[c]						
PFOA	0.84 (0.64, 1.10)	0.297	ref	0.98 (0.68, 1.42)	0.77 (0.53, 1.12)	0.157
PFOS	**1.35 (1.07, 1.70)**	**0.016**	ref	1.05 (0.73, 1.51)	**1.52 (1.04, 2.21)**	**0.045**
PFNA	1.11 (0.9, 1.37)	0.455	ref	1.33 (0.92, 1.93)	1.10 (0.76, 1.60)	0.887
PFHxS	1.05 (0.89, 1.25)	0.632	ref	1.08 (0.75, 1.56)	1.17 (0.80, 1.70)	0.727
PFUnDA	0.90 (0.79, 1.03)	0.166	ref	0.80 (0.55, 1.15)	0.93 (0.64, 1.35)	0.656
PFDA	0.93 (0.77, 1.11)	0.551	ref	0.81 (0.56, 1.17)	0.89 (0.62, 1.29)	0.664
Total PFAS	1.23 (0.90, 1.66)	0.251	ref	1.07 (0.74, 1.54)	1.31 (0.90, 1.91)	0.273
Model 2[d]						
PFOA	0.84 (0.64, 1.10)	0.351	ref	0.99 (0.68, 1.43)	0.77 (0.53, 1.11)	0.214
PFOS	**1.36 (1.08, 1.71)**	**0.023**	ref	1.04 (0.72, 1.49)	**1.53 (1.05, 2.22)**	0.075
PFNA	1.11 (0.9, 1.38)	0.516	ref	1.33 (0.92, 1.93)	1.12 (0.77, 1.62)	0.805
PFHxS	1.05 (0.89, 1.25)	0.698	ref	1.09 (0.75, 1.58)	1.17 (0.80, 1.70)	0.749
PFUnDA	0.90 (0.79, 1.03)	0.269	ref	0.80 (0.55, 1.16)	0.92 (0.63, 1.34)	0.743
PFDA	0.94 (0.78, 1.12)	0.672	ref	0.82 (0.57, 1.19)	0.91 (0.63, 1.33)	0.851
Total PFAS	1.23 (0.91, 1.67)	0.423	ref	1.06 (0.73, 1.54)	1.31 (0.90, 1.91)	0.362

[a]Notes: Bold denotes statistically significant associations. Abbreviations: LVN: the number of lesioned vessels; *P*-FDR: false discovery rate adjusted *P* value; P_{trend}: *P* for trend; P_{trend}-FDR: false discovery rate adjusted *P* for trend; ref: reference; T1: the first tertile; T2: the second tertile; T3: the third tertile. [b]Crude, unadjusted for covariates. [c]Model 1, adjusted for age, sex, and BMI. [d]Model 2, adjusted for smoking, drinking, and educational level based on Model 1.

tion (Figure S1C and Figure S1D), with total PFAS and PFOS showing nonlinear associations with nonfatal myocardial infarction (Figure S1E). The observed nonlinear dose–response curves indicate that the effects of PFAS on cardiovascular outcomes vary across different exposure levels. The distinct inflection points in many of these curves suggest the existence of critical exposure thresholds, which form the foundation for conducting threshold analyses.

Table 6 shows the results concerning threshold effects, unveiling notable threshold associations between PFAS and the degree of coronary stenosis and prognosis. In the hinge model, thresholds were identified for PFOS and total PFAS in association with GS and nonfatal myocardial infarction. Thresholds were also observed for PFOS in relation to LVN and for six PFAS and total PFAS in connection with MACE. Furthermore, thresholds for PFOA and total PFAS in relation to coronary revascularization were also identified. In the segmented model, thresholds were determined for PFOA, PFNA, PFUnDA, and PFDA with GS and PFNA and PFHxS with coronary revascularization.

3.4. Mixture Analysis

Figure 1 depicts the relationship between PFAS mixture and degree of coronary stenosis and prognosis in the gqcomp model. The results indicated that the odds of MACE and nonfatal myocardial infarction increased significantly with each order of magnitude rise in PFAS mixture content, with OR (95% CI) of 1.55 (1.08, 2.23) and 2.16 (1.07, 4.35), respectively. Furthermore, the gqcomp model showed that PFOS exhibits the most significant positive impact on the degree of coronary stenosis and prognosis, aligning with the findings of the logistic regression model. However, the PFAS mixture does not show any significant effect on GS, LVN, and coronary revascularization (Table S11), with the OR (95% CI) 0.99 (0.88, 1.13), −0.99 (0.94, 1.05), and 1.09 (0.66, 1.82) for each quartile increase in the mixtures on the overall effect, respectively.

Figure 2 illustrates the relationship between the PFAS mixture and the degree of coronary stenosis and prognosis in the BKMR model. The results showed a significant positive association between PFAS mixtures and nonfatal myocardial infarction. Furthermore, there was a rising trend in the effective value of the PFAS mixture for GS, LVN, MACE, and coronary revascularization over the 50th percentile. The results regarding the selection of PIP variables for each PFAS in the PFAS mixture in the BKMR model are shown in Table S12. The PIP for PFOS was highest for LVN, MACE, and nonfatal myocardial infarction, with PIPs of 0.887, 0.999, and 1.000, respectively.

3.5. Sensitivity Analysis

The results of the sensitivity analysis are presented in Table S13. When participants younger than 30 years of age and those with a BMI greater than 30 kg/m^2 were excluded, respectively, the association between PFAS and degree of coronary stenosis remained consistent with the primary analysis. These results revealed the robustness of the relationship between PFAS and the degree of coronary stenosis.

3.6. Construction and Assessment of the AOP Framework

We obtained a total of 9870 genes related to 12 PFAS from the CTD. As for the genes that contributed to ACS, 6939 genes

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Table 4. Results of the Association between PFAS and the Occurrence of MACE[a]

			Categorical PFAS levels			
	HR (95% CI)	*P*-FDR	T1	T2	T3	P_{trend}-FDR
Crude[b]						
PFOA	1.26 (0.81, 1.95)	0.530	ref	0.56 (0.28, 1.11)	1.25 (0.72, 2.17)	0.600
PFOS	**2.05 (1.41, 2.99)**	**0.001**	ref	0.40 (0.17, 0.98)	**2.61 (1.48, 4.60)**	**0.001**
PFNA	1.11 (0.77, 1.59)	0.810	ref	0.46 (0.23, 0.92)	1.11 (0.65, 1.92)	0.674
PFHxS	1.26 (0.94, 1.69)	0.277	ref	0.95 (0.49, 1.84)	1.69 (0.94, 3.05)	0.172
PFUnDA	1.01 (0.82, 1.25)	0.919	ref	0.43 (0.21, 0.87)	1.18 (0.69, 2.03)	0.972
PFDA	1.03 (0.76, 1.39)	0.919	ref	0.63 (0.32, 1.23)	1.34 (0.76, 2.33)	0.620
Total PFAS	**2.55 (1.58, 4.11)**	**0.001**	ref	0.49 (0.21, 1.15)	**2.76 (1.55, 4.93)**	**<0.001**
Model 1[c]						
PFOA	1.35 (0.87, 2.10)	0.372	ref	0.57 (0.29, 1.13)	1.38 (0.79, 2.40)	0.356
PFOS	**1.96 (1.34, 2.87)**	**0.002**	ref	0.40 (0.17, 0.97)	**2.54 (1.42, 4.55)**	**0.003**
PFNA	1.13 (0.79, 1.62)	0.620	ref	0.48 (0.24, 0.95)	1.16 (0.67, 2.00)	0.591
PFHxS	1.21 (0.90, 1.63)	0.372	ref	0.96 (0.49, 1.86)	1.58 (0.86, 2.88)	0.306
PFUnDA	1.06 (0.85, 1.32)	0.620	ref	0.46 (0.22, 0.94)	1.41 (0.81, 2.46)	0.599
PFDA	1.09 (0.81, 1.46)	0.620	ref	0.69 (0.35, 1.37)	1.49 (0.84, 2.62)	0.356
Total PFAS	**2.50 (1.54, 4.04)**	**0.001**	ref	0.50 (0.21, 1.17)	**2.81 (1.56, 5.06)**	**<0.001**
Model 2[d]						
PFOA	1.30 (0.83, 2.04)	0.444	ref	0.55 (0.28, 1.09)	1.33 (0.76, 2.33)	0.452
PFOS	**1.96 (1.34, 2.89)**	**0.002**	ref	0.41 (0.17, 1.00)	**2.53 (1.41, 4.56)**	**0.003**
PFNA	1.09 (0.76, 1.58)	0.749	ref	0.45 (0.22, 0.90)	1.09 (0.62, 1.89)	0.687
PFHxS	1.19 (0.88, 1.61)	0.444	ref	0.89 (0.46, 1.75)	1.55 (0.84, 2.85)	0.336
PFUnDA	1.04 (0.84, 1.30)	0.749	ref	0.45 (0.22, 0.92)	1.36 (0.78, 2.38)	0.687
PFDA	1.05 (0.78, 1.41)	0.749	ref	0.65 (0.33, 1.29)	1.37 (0.77, 2.44)	0.531
Total PFAS	**2.46 (1.51, 4.01)**	**0.002**	ref	0.50 (0.21, 1.18)	**2.78 (1.53, 5.05)**	**0.001**

[a]Notes: Bold denotes statistically significant associations. Abbreviations: MACE: major adverse cardiovascular event; HR: hazard ratios; *P*-FDR: false discovery rate adjusted *P* value; P_{trend}: *P* for trend; P_{trend}-FDR: false discovery rate adjusted *P* for trend; ref: reference; T1: the first tertile; T2: the second tertile; T3: the third tertile. [b]Crude, unadjusted for covariates. [c]Model 1, adjusted for age, sex, and BMI. [d]Model 2, adjusted for smoking, drinking, and educational level based on Model 1.

were obtained from the Gene Cards database, and 5679 genes had a high relevance with the outcome (GIFtS ≥ 20 and relevance score ≥20). Then, we identified 3186 genes connecting PFAS with ACS. Compared with other tissues, top 10 genes (MT-CO1, MYH7, MYH6, DES, ACTC1, MB, TPM1, TNNT2, TTN, HSPB7) had a high abundance in the heart tissue. Thus, these genes were included as potential MIEs. Then, we obtained 145 phenotypes (including 139 GO enrichment and 6 KEGG enrichment) from the enrichment analysis of potential KEs. The phenotype ranked top 10 from GO enrichment and 6 KEGG enrichment) were defined as potential KEs. Notably, these phenotypes were closely related to myocardial contraction, which was reported to play a vital role in ACS. Subcellular KEs included myofibril, sarcomere, contractile fiber, and actomyosin structure organization. Cellular KEs included muscle organ development, cardiac muscle tissue morphogenesis, cardiac muscle tissue development, and the system KE was heart morphogenesis, heart contraction, and heart process. Furthermore, nine KEGG pathways (cGMP-PKG signaling pathway, adrenergic signaling in cardiomyocytes, etc.) were included (Figure 3).

4. DISCUSSION

In this study, we explored the association between PFAS exposure and the degree of coronary stenosis and prognosis in ACS. The degree of coronary stenosis in ACS was assessed by GS and LVN, and the prognosis of ACS was evaluated by the incidence of MACE, including nonfatal myocardial infarction and coronary revascularization. The results showed a significant impact of PFOS and total PFAS on the degree of coronary stenosis and prognosis. Specifically, PFOS was significantly and positively associated with GS and LVN, and PFOS and total PFAS showed a significant positive correlation with MACE and nonfatal myocardial infarction. We also identified thresholds for correlations between PFOS, total PFAS, and other PFAS congeners and degree of coronary stenosis and prognosis, including GS, LVN, MACE, nonfatal myocardial infarction, and coronary revascularization. These thresholds contribute to understanding the critical levels of PFAS exposure that may exacerbate cardiovascular risks. Moreover, we observed a significant positive effect of PFAS mixtures on the occurrence of MACE and nonfatal myocardial infarction. The AOP framework showed that PFAS could affect protein binding and cytoskeletal, induce multicellular organismal processes, and impair heart function. Our study underlines the influence of PFAS, especially PFOS, on the degree of coronary stenosis and prognosis. These findings improve the understanding of the impact of PFAS on ACS and highlight the need for targeted strategies to reduce the risks associated with PFAS exposure.

In this study, we found that PFOS had a significant effect on the degree of coronary stenosis and prognosis. In the single contaminant analysis, PFOS was significantly and positively associated with the degree of coronary stenosis and prognostic indicators. The OR (95% CI) was 1.33 (1.10, 1.61) for GS and 1.43 (1.16, 1.76) for LVN, and the HR (95% CI) was 1.96 (1.34, 2.89) for MACE and 3.86 (2.00, 7.46) for nonfatal myocardial infarction. The mixture analysis model also identifies PFOS as a major contaminant. According to the qgcomp model, PFOS accounted for the greatest weight of

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Table 5. Results of the Association between PFAS and the Occurrence of Nonfatal Myocardial Infarction[a]

			Categorical PFAS levels			
	HR (95% CI)	*P*-FDR	T1	T2	T3	P_{trend}-FDR
Crude[b]						
PFOA	1.25 (0.58, 2.73)	0.569	ref	0.51 (0.15, 1.69)	1.05 (0.39, 2.81)	0.883
PFOS	**5.05 (2.61, 9.78)**	**0.000**	ref	0.66 (0.11, 3.98)	**5.40 (1.56, 18.67)**	**0.009**
PFNA	1.58 (0.83, 3.03)	0.192	ref	0.41 (0.11, 1.59)	1.47 (0.56, 3.85)	0.433
PFHxS	**1.79 (1.06, 3.02)**	0.070	ref	1.26 (0.34, 4.70)	2.79 (0.89, 8.79)	0.146
PFUnDA	1.40 (0.88, 2.22)	0.192	ref	0.64 (0.18, 2.27)	1.76 (0.64, 4.85)	0.433
PFDA	1.71 (0.91, 3.20)	0.162	ref	0.66 (0.18, 2.32)	1.73 (0.63, 4.76)	0.433
Total PFAS	**5.12 (2.29, 11.47)**	0.000	ref	0.66 (0.11, 3.94)	**5.43 (1.57, 18.75)**	**0.009**
Model 1[c]						
PFOA	1.53 (0.69, 3.41)	0.295	ref	0.53 (0.16, 1.76)	1.31 (0.48, 3.54)	0.597
PFOS	**4.13 (2.11, 8.10)**	**0.000**	ref	0.64 (0.11, 3.80)	**4.57 (1.31, 15.91)**	**0.021**
PFNA	1.70 (0.89, 3.25)	0.124	ref	0.43 (0.11, 1.65)	1.69 (0.63, 4.53)	0.270
PFHxS	1.59 (0.92, 2.73)	0.124	ref	1.20 (0.32, 4.47)	2.17 (0.68, 6.94)	0.226
PFUnDA	1.55 (0.98, 2.45)	0.111	ref	0.76 (0.21, 2.74)	2.66 (0.92, 7.66)	0.226
PFDA	1.78 (1.02, 3.11)	0.096	ref	0.83 (0.23, 3.01)	2.24 (0.80, 6.31)	0.226
Total PFAS	**4.96 (2.12, 11.57)**	**0.001**	ref	0.63 (0.11, 3.80)	**5.20 (1.50, 18.03)**	**0.012**
Model 2[d]						
PFOA	1.48 (0.65, 3.37)	0.350	ref	0.49 (0.15, 1.65)	1.29 (0.47, 3.53)	0.624
PFOS	**3.86 (2.00, 7.46)**	**0.000**	ref	0.66 (0.11, 3.98)	**4.70 (1.35, 16.40)**	**0.019**
PFNA	1.73 (0.90, 3.31)	0.138	ref	0.38 (0.10, 1.49)	1.73 (0.63, 4.75)	0.256
PFHxS	1.52 (0.88, 2.60)	0.152	ref	1.12 (0.30, 4.22)	2.29 (0.71, 7.33)	0.248
PFUnDA	1.51 (0.95, 2.41)	0.138	ref	0.71 (0.20, 2.58)	2.60 (0.90, 7.50)	0.248
PFDA	1.74 (1.00, 3.01)	0.116	ref	0.77 (0.21, 2.89)	2.14 (0.74, 6.14)	0.248
Total PFAS	**4.56 (1.99, 10.45)**	**0.001**	ref	0.67 (0.11, 4.00)	**5.20 (1.49, 18.14)**	**0.014**

[a]Notes: Bold denotes statistically significant associations. Abbreviations: HR: hazard ratios; *P*-FDR: false discovery rate adjusted *P* value; P_{trend}: *P* for trend; P_{trend}-FDR: false discovery rate adjusted *P* for trend; ref: reference; T1: the first tertile; T2: the second tertile; T3: the third tertile. [b]Crude, unadjusted for covariates. [c]Model 1, adjusted for age, sex, and BMI. [d]Model 2, adjusted for smoking, drinking, and educational level based on Model 1.

Table 6. Threshold Effect of PFAS and Degree of Coronary Stenosis and Prognosis[a]

	Threshold (ng/mL)				
PFAS	GS	LVN	MACE	Nonfatal myocardial infraction	Coronary revascularization
PFOA	7.68	-	4.88	-	9.18
PFOS	4.65	4.54	5.14	5.03	-
PFNA	1.07	-	0.84	-	0.77
PFHxS	-	-	1.08	-	0.91
PFUnDA	0.38	-	0.65	-	-
PFDA	0.84	-	0.67	-	-
Total PFAS	19.95	-	14.59	12.00	16.48

[a]Notes: Thresholds identified using the hinge model: PFOS and total PFAS with GS and nonfatal myocardial infarction; PFOS thresholds observed in relation to LVN; Thresholds for six PFAS and total PFAS in connection with MACE; Thresholds for PFOA and total PFAS related to coronary revascularization. Thresholds determined using the segmented model: For PFOA, PFNA, PFUnDA, and PFDA in relation to the GS; For PFNA and PFHxS in relation to coronary revascularization. The model was adjusted for age, sex, BMI, smoking, drinking, and educational level. Abbreviations: GS: gensini score; LVN: the number of lesioned vessels; MACE: major adverse cardiovascular event; -: no data available.

0.64−0.98. Based on the BKMR model, the PIP of PFOS was the highest in all indicators except GS, which was ranked second. The pronounced effects of PFOS may be attributed to its extensive historical use, resulting in higher cumulative exposure, as well as its long half-life and high protein-binding affinity, which contribute to its persistence in the body.[50,51] Moreover, the powerful effects of PFOS on lipid metabolism and inflammatory processes, crucial in the pathogenesis of coronary artery disease, may explain its stronger association with cardiovascular outcomes.[52] The discrepancy between PFOS and PFOA effects, particularly when compared to our previous case control study, may be explained by the lower PFOA concentrations observed in the current study population (median 4.69 ng/mL), which fell below the identified threshold levels for various outcome measures. This suggests that PFOA exposure in our current study may not have reached the critical levels necessary to significantly impact coronary stenosis and prognosis. Despite these differences, both studies consistently highlight the potential role of PFOS as a key PFAS compound associated with adverse cardiovascular outcomes.

Our findings are consistent with several existing epidemiological studies regarding the risk of atherosclerosis and coronary artery calcification. In a cohort study involving 664 adolescents and young adults, PFOS exhibited a significant and positive correlation with carotid intima-media thickness, a marker of subclinical atherosclerosis. This correlation remained significant after adjusting for confounding factors, including traditional cardiovascular risk factors. In addition, a strong correlation between PFOS exposure and the lipid-transporting and -metabolizing lipoprotein E genotype alleles E2 and E3/E3 was observed, with OR (95% CI) of 2.93 (1.16, 7.42) and 1.84 (1.21, 2.81), respectively. These alleles are important genetic markers related to hyperlipidemia, coronary heart disease, and ischemic stroke.[53] Besides, coronary artery calcification is an

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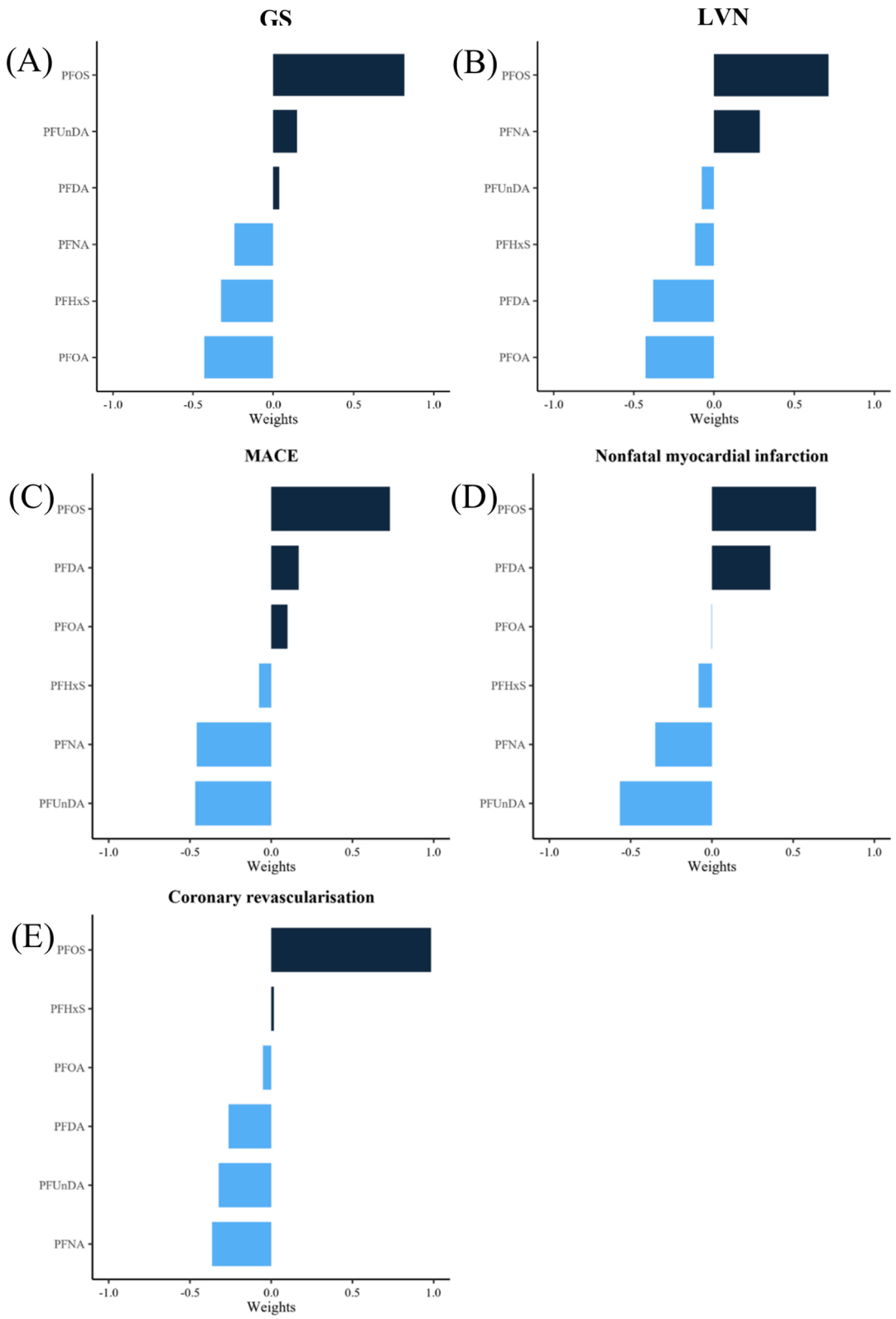

Figure 1. Association between PFAS mixture and degree of coronary stenosis and prognosis in the gqcomp model. Notes: (A) GS, (B) LVN, (C) MACE, (D) nonfatal myocardial infarction, (E) coronary revascularization. Negative weights in the model suggest reduced odds of adverse degree of coronary stenosis and prognosis, while positive weights indicate increased odds of degree of coronary stenosis and prognosis. The model was adjusted for sex, age, BMI, smoking, drinking, and educational level. Abbreviations: GS: gensini score; LVN: the number of lesioned vessels; MACE: major adverse cardiovascular event.

indicator of the progression of cardiovascular disease, the extent of which is associated with an increased risk of cardiovascular events, more severe coronary artery stenosis, and poor prognosis.[54] Another study found an association between PFAS and calcification. Researchers evaluated data collected from 666 adults with prediabetes who participated in

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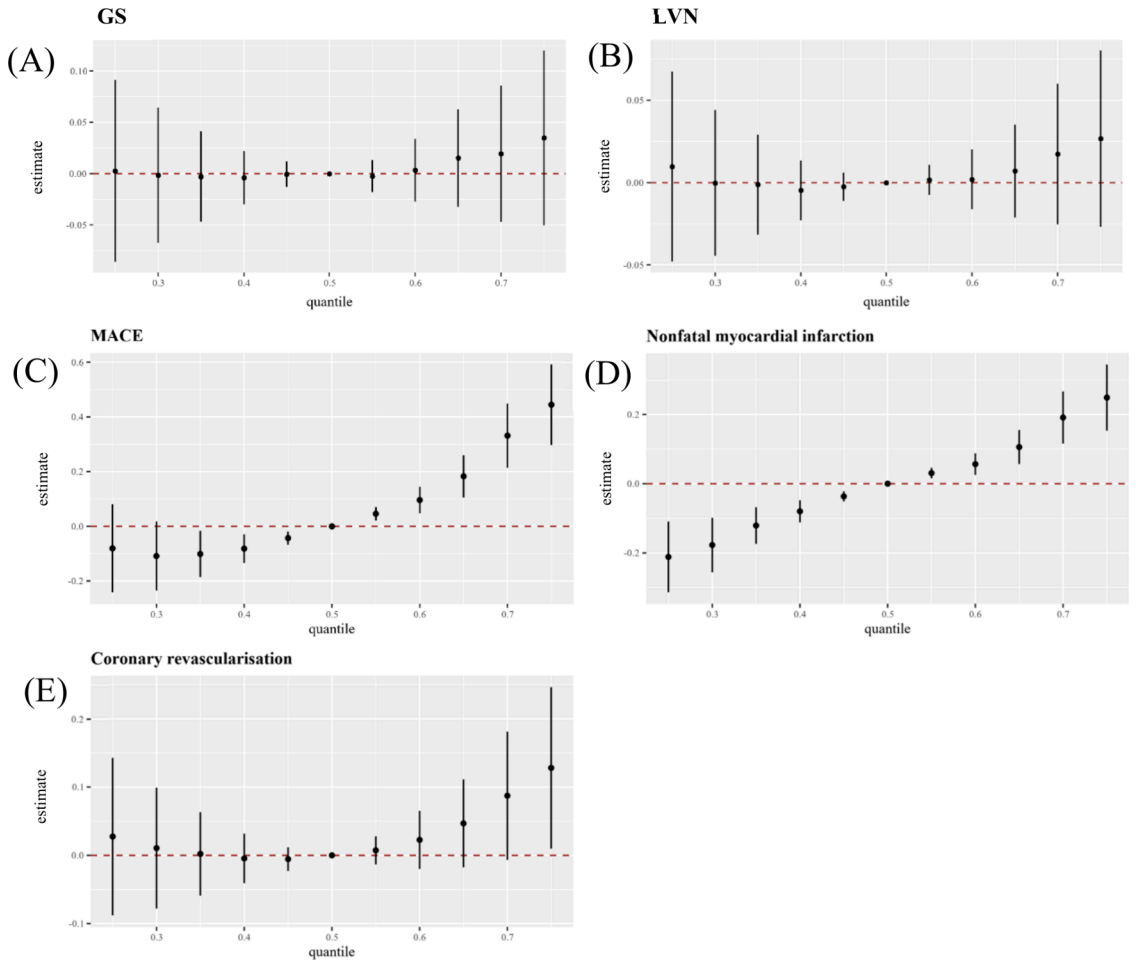

Figure 2. Association between PFAS mixture and degree of coronary stenosis and prognosis in the BKMR model. Notes: (A) GS, (B) LVN, (C) MACE, (D) nonfatal myocardial infarction, (E) coronary revascularization. The figure illustrates the estimated difference in the degree of coronary stenosis and prognosis and 95% credible intervals, comparing all PFAS concentrations at various percentiles (incrementally increasing from the 25th to the 75th percentile by 5) against the baseline 50th percentile. The model was adjusted for sex, age, BMI, smoking, drinking, and educational level. Abbreviations: GS: gensini score; LVN: the number of lesioned vessels; MACE: major adverse cardiovascular event.

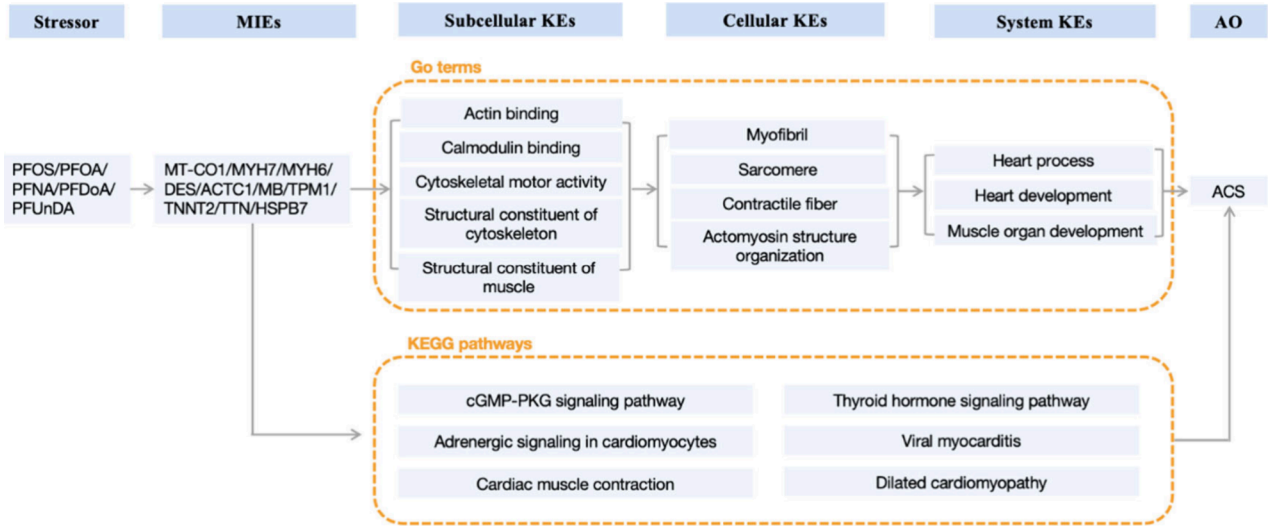

Figure 3. AOP framework from PFAS to ACS. Abbreviations: AOP: adverse outcome pathway; PFAS: Per- and polyfluoroalkyl; ACS: acute coronary syndrome; MIE: molecular initiating event; KE: key event; AO: adverse outcome.

the Diabetes Prevention Program trial. The results indicated a significant positive association between plasma PFOS levels and the risk of coronary artery calcification with OR (95% CI) of 1.67 (1.10, 2.54) after adjusting for confounding factors,

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including sex, age, BMI, race/ethnicity, smoking, education, and diabetes prevention interventions.[55]

However, it is pertinent to highlight that a limited number of studies have yielded inconclusive evidence regarding the association between PFAS and cardiovascular health. For instance, a study conducted by the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) involving 1016 individuals aged 70 in Uppsala revealed no significant correlation between PFAS exposure and intima-media thickness, an established indicator of carotid atherosclerosis, and the development of carotid plaque.[56] While acknowledging the rigors of previous research, our study design differs in important ways that may account for the differences in observed outcomes. Recognition of these differences is critical in interpreting the results of the current study. First, the prevalence of adverse cardiovascular events is not fully reflected by the indicator of carotid atherosclerosis alone. The occurrence of cardiovascular events is also related to the body's coagulation status and whether the plaque is dislodged. In contrast, our study directly employs the end point event ACS as the outcome indicator, Therefore, there is a possibility of discrepancy in the conclusions compared to studies only examining carotid atherosclerosis. Second, the two studies had different age distributions of the population, and age is a factor associated with cardiovascular disease, so the effect of older age on cardiovascular events may have masked the effect of PFAS. Consequently, the generalizability of the findings to these additional aspects of cardiovascular health should be interpreted cautiously. In addition, a nested case-control study revealed no discernible association between PFAS exposure and myocardial infarction. The study incorporated 1528 subjects drawn from two distinct Swedish population cohorts in 2003−2009 and 1997−1999.[57] Notably, the results observed in the Swedish cohort may not be applicable universally to populations with different demographic characteristics, such as the Chinese population.

In our study, we identified significant threshold effects of PFAS on the degree of coronary stenosis and prognosis, suggesting a critical level of exposure above which the risk of degree of coronary stenosis and prognosis increases markedly. Notably, we observed distinct thresholds for individual PFAS compounds, including PFOA and PFOS, as well as for total PFAS exposure. These thresholds varied across different outcome measures, such as the GS, LVN, and MACE. The observed thresholds ranged from approximately 4 to 10 ng/mL for individual PFAS compounds and from 12 to 20 ng/mL for total PFAS. This variability in threshold levels likely reflects the distinct biological properties and mechanisms by which these compounds interact with the cardiovascular system.[58,59] PFAS compounds differ in their carbon chain length, chemical structure, and bioaccumulation potential, which may influence their toxicological profiles and the onset of adverse health effects.[60] For instance, long-chain PFAS (e.g., PFOS, PFOA) are known to persist longer in human tissues, potentially leading to higher body burdens over time. In contrast, shorter chain PFAS may be more readily excreted but could still exert significant health impacts at certain exposure levels. These findings are consistent with the study by Yi et al, which used NHANES data (2005−2018) and found a nonlinear increase in the risk of depression when total PFAS exceeded a threshold of 39.66 ng/mL.[22] Moreover, the study by Cui et al. identified PFAS thresholds associated with reduced lymph node counts in patients with colorectal cancer, including PFOA, PFOS, PFNA, and 6:2 Cl-PFESA, which ranged from 0.07 to 2.23 ng/mL.[61] The different threshold levels suggest that different concentrations of PFAS trigger different biological pathways, with higher thresholds for the degree of coronary stenosis and prognosis compared to immune responses in cancer.

In addition, the findings from this investigation can be compared to the Human Biomonitoring (HBM) values established by the Human Biomonitoring Commission of the German Environment Agency.[62,63] The HBM values are derived from a comprehensive analysis of various health effects associated with PFAS exposure and are below which no adverse health effects are anticipated. For PFOA and PFOS in serum or plasma, the HBM-I values are set at 2 ng/mL and 5 ng/mL, respectively. The HBM-II values are 5 ng/mL and 10 ng/mL for women of childbearing age and 10 ng/mL and 20 ng/mL for all other populations. In general, the HBM-I represents a precautionary threshold below which even sensitive groups are protected, while the HBM-II is intended to protect most of the general population. The threshold levels for PFOA and PFOS identified in our study are close to the HBM range, with the PFOS threshold below the HBM-I value. This indicates that our study is comparable to the HBM evaluation, as it provides precise threshold values for PFAS exposure in relation to a specific health outcome−increased degree of coronary stenosis and poor prognosis. They may be more sensitive to PFOS-induced myocardial injury in patients with ACS. Previous studies may have focused only on doses that cause adverse reactions in healthy people and failed to consider the vulnerable population. Therefore, a lower dose may be more appropriate for the vulnerable population. Furthermore, this study identified a threshold effect for total PFAS. Although the HBM does not explicitly specify limits for total PFAS, this study provides population evidence to support limiting total PFAS in population exposures. It is important to note that our calculation of total PFAS is based on six specific congeners, which may not fully represent the entire spectrum of PFAS exposure. This observation offers fresh insights into the relationship between PFAS exposure and cardiovascular health, and further investigation into the biological basis of this threshold effect is necessary.

Furthermore, in the present study, we explored the effect of PFAS mixtures on the degree of coronary stenosis and prognosis. The consistent findings of both the qgcomp and BKMR models indicate that higher levels of PFAS mixtures are correlated with an increased incidence of MACE and nonfatal myocardial infarction. The absence of significant associations between PFAS mixtures and other degree of coronary stenosis and prognosis indicators could be attributed to two primary factors. First, the susceptibility to PFAS exposure may vary across different degrees of coronary stenosis and prognosis indicators. Specific outcomes such as MACE and myocardial infarction may be more sensitive to PFAS,[64,65] whereas GS and LVN, which were indicators of the degree of coronary stenosis, might be less affected due to their complex pathophysiological basis. Factors including genetic predispositions and existing health conditions, coupled with interactions between PFAS, lipid metabolism, and inflammatory pathways, contribute to this variability.[66−70] Second, the complexity of PFAS mixtures and the diverse effects of their various components may differentially influence the degree of coronary stenosis and prognosis. This complexity can lead to a range of interactions within the mixture, potentially resulting in additive, synergistic, or antagonistic effects on health outcomes. The qgcomp model

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revealed both positive and negative influences of PFAS mixtures on the degree of coronary stenosis and prognosis (Figure 1). Additionally, the interactions of these mixtures with the cardiovascular system, influenced by molecular mechanisms, bioaccumulation, and the persistence of PFAS, may lead to different effects.

These findings imply that the association between PFAS exposure and cardiovascular disease progression may be subject to the influence of additional potential factors. Thus, it is imperative to probe the underlying mechanisms of PFAS to elucidate the connections between PFAS exposure and the degree of coronary stenosis and prognosis in ACS. Based on the data collected from the CTD, GeneCards, and GTEx, we identified the 10 most frequently occurring genes as MIEs. Through GO annotation, we found that MIEs first participate in protein binding and cytoskeletal motor activity, further affecting muscle organ development and heart process, heart development, and finally inducing the occurrence of ACS. Consistent with our results, the authors found that PFAS may affect mitochondrial dynamics and morphology by affecting protein binding, further leading to mitochondrial dysfunction.[71] For example, ablation of the fusion proteins mitofusins 1 and 2 in the hearts of adult mice resulted in severe dilated cardiomyopathy.[72] Experimental evidence also has shown that PFOS can affect the heart process, as PFOS treatment only affects the early stages of cardiac differentiation, promoting cell differentiation into WT1+ epicardial cells.[73] Parallel evidence shows this effect may further result in heart and pericardium, heart bleeding, and pericardium edema, further aggravating the condition of ACS patients.[74−76] Through KEGG, we found that PFAS may induce ACS through the cGMP-PKG signaling pathway, which is involved in mediating contraction of vascular smooth muscle, antiatherosclerosis processes, etc. Previous studies have shown that PFAS exposure can promote the development of atherosclerosis, ultimately leading to poor ACS patient prognosis. PFAS exposure has been shown to increase atherosclerosis in hyperlipidemia mice and ApoE knockout mice.[77] Specifically, PFAS exposure regulates the expression of key genes involved in cholesterol efflux, lipoprotein synthesis, and lipid transport,[78] further activate nuclear transcription factors and peroxisome-activated receptors,[79] which is intrinsically related to the degree of coronary stenosis and poor prognosis of ACS.[80] Additionally,PFAS induces endothelial dysfunction, exacerbates arterial stiffness, stimulates endothelial cell proliferation, increases pro-inflammatory cytokine levels and biomarkers of oxidative damage,[13,81,82] and aggravates the degree of coronary stenosis and poor prognosis by promoting the risk of atherosclerosis progression.

In this study, we scrutinized the correlation between PFAS exposure and the degree of coronary stenosis and prognosis in patients with ACS. First, this is the first study of PFAS and ACS disease the degree of coronary stenosis and prognosis in ACS. We have refined PFAS exposure and the degree of coronary stenosis, prognosis, and molecular biological mechanism considerations, providing population-based research evidence and mechanistic hypotheses for understanding contaminant pathogenesis. Second, the BKMR and qgcomp models were employed to evaluate the effect of the PFAS mixture on the degree of coronary stenosis and prognosis. Third, the threshold effects evaluated in this study provide insight into the significantly increased risk of adverse degree of coronary stenosis and prognosis at specific levels of PFAS exposure, enhancing our understanding of exposure-risk relationships. Nevertheless, our study bears some limitations. First, this study primarily determined PFAS exposure based on single plasma concentrations. While this approach is widely used in epidemiological studies, it may not adequately reflect long-term exposure patterns. Second, the degree of coronary stenosis of ACS was quantified using GS system and LVN. Although these are recognized and widely used measures, they possess inherent limitations such as the lack of a comprehensive risk profile, and both indicators only focus on anatomical and morphological factors. Moreover, our results are contingent on the specific demographic characteristics and exposures of the population we studied. Therefore, caution is advised when extrapolating our findings to populations with different demographic traits or exposures. From a public health perspective, our findings suggest that PFAS exposure may be associated with adverse cardiovascular outcomes. While our study alone cannot establish causality, it underscores the necessity for continued research and vigilant monitoring of PFAS exposure in cardiovascular health contexts. In clinical practice, these results highlight the importance of considering environmental factors alongside traditional risk factors in the assessment and management of ACS.

5. CONCLUSION

In this study, we explored the potential correlation between PFAS exposure and the degree of coronary stenosis and prognosis in ACS. We discovered that exposure to PFOS notably heightened the degree of coronary stenosis and poor prognosis of ACS. Importantly, we detected a significant threshold effect between PFAS and the degree of coronary stenosis and prognosis in ACS, implying that the risks increase substantially beyond a certain exposure level. We also observed significant positive correlations between PFAS mixtures and the incidence of MACE and nonfatal myocardial infarction. Furthermore, we revealed that the mechanism by which PFAS leads to ACS may be related to affecting protein binding and the cytoskeleton, as well as inducing multicellular biological processes. Hence, more experimental research is warranted to elucidate the role of PFAS exposure in ACS and the potential mechanisms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00166.

Sample size calculation details, methodological validation for PFAS detection, quality control data, demographic characteristics by groups, detailed PFAS concentration distributions, additional association analyses including stratified analyses, dose−response curves, mixture effect analyses, and sensitivity analyses (PDF)

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Notes

The authors declare no competing financial interest.

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