Coronary atherosclerosis in outlier subjects at the opposite extremes of traditional risk factors: Rationale and preliminary results of the Coronary Atherosclerosis in outlier subjects: Protective and novel Individual Risk factors Evaluation (CAPIRE) study

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Although it is generally accepted that cardiac ischemic events develop when coronary atherosclerosis (coronary artery disease [CAD]) has reached a critical threshold, this is true only to a first approximation. Indeed, there are patients with severe CAD who do not develop ischemic events; conversely, at the other extreme, individuals with minimal CAD may do. Similar exceptions to this paradigm include patients with diffuse CAD with a low risk factor (RF) profile and others with multiple RFs who develop only mild or no CAD.

Therefore, the CAPIRE project was designed to investigate whether the specific study of these extreme outlier populations could provide clues for identification of yet unknown risk or protective factors for CAD and ischemic events.

In the CAPIRE study, 481 subjects without previous symptoms or history of ischemic heart disease and normal left ventricular systolic function undergoing coronary computed tomography angiography have been selected based on coronary computed tomography angiography findings and cardiovascular RF profile. Therefore, in the whole population, 2 extreme outlier populations have been identified: (1) subjects with no CAD despite multiple RFs, and (2) at the opposite extreme, subjects with diffuse CAD despite a low-risk profile.

Each subject has been characterized by clinical, anatomical imaging variables of CAD and baseline circulating biomarkers. Blood samples were collected and stored in a biological bank for further advanced investigations.

The project is designed as a prospective, observational, international multicenter study with an initial cross-sectional analysis of clinical, imaging, and biomolecular variables in the selected groups and a longitudinal 5-year follow-up. (Am Heart J 2016;173:18-26.)

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Background

Atherosclerosis is a systemic pathological process associated with important clinical acute complications (acute coronary syndrome, fatal and nonfatal ST-elevation myocardial infarction, sudden death) that are not strictly related to its extent and severity. The first clinical manifestation of ischemic heart disease is variable and characterized by acute events in about 50% of cases.[1] Approximately half of all acute coronary events occur in patients without prior cardiac symptoms or diagnosis.[2]

The early identification of individuals at risk is desirable to activate a more effective preventive strategy. Currently, the risk of future clinical events of ischemic heart disease is

currently assessed by using integrated multifactorial prediction models (ie, Framingham Risk Score, PROCAM, SCORE, Pooled Cohort Equations), integrating nonmodifiable risk factors (RFs) such as age, gender, and a family history of early ischemic heart disease, along with modifiable conventional RFs such as arterial hypertension, hypercholesterolemia, smoking, and diabetes mellitus.[3–5]

Although risk assessment by current recommended scores based on traditional RFs is useful to assess the mean cardiovascular risk in a given population, it has limitations concerning individual variability.[6,7] Indeed, many individuals who develop acute coronary events would be classified at low or intermediate risk by the traditional RF-based approaches.[8–13] Conversely, many individuals with an adverse RF profile remain asymptomatic for years, thus suggesting the presence of protective mechanisms.[14]

A meta-analysis including 122,458 patients who experienced acute coronary events confirmed that the risk proportionally increased with RF number, but approximately 15% men and 20% women did not have any of 4 conventional RFs.[13] Similarly, the analysis of the CHA, MRFIT, and FHS trials revealed that patients exposed to at least 2 RFs had a higher risk of cardiac death; however, 70% of subjects with the same risk profile died of other nonischemic causes.[14] Therefore, the exposure to several RFs is not enough to account for clinical events in all subjects, and still unknown environmental and genetic factors may play a role in the pathophysiology of coronary artery disease (CAD).

These findings, despite the significant population attributable risk of conventional RFs,[15] support the need to search novel RFs reflecting different atherogenic aspects to improve cardiovascular event prediction[16] and identify new and accurate biomarkers[17] for subjects at higher risk, who are potential targets for personalized prevention programs. In addition, the identification of yet unknown protective factors may suggest specific therapeutic strategies.

The complex relationship among traditional RFs, CAD and clinical manifestation of ischemic heart disease is the object of the CAPIRE study. This study sought to analyzed the outlier populations at the opposite extremes for the presence of coronary computed tomography angiography (CCTA)–documented CAD and traditional cardiovascular RFs.

Through this novel approach, the study is aimed (1) to detect new protective and susceptibility factors of CAD and ischemic events, thus enabling the definition of specific descriptors (clinical, imaging, and circulating biomarkers) for the still unrecognized individuals at the highest risk; (2) to identify new pathophysiological keys of interpretation of the complex relationship among clinical (including lifestyle habits), anatomical variables of CAD, and circulating biomarkers (cross-sectional phase); (3) to follow up all subjects for a period of 5 years (longitudinal phase) to investigate whether coronary events are more closely correlated with RFs than with coronary atherosclerosis; and (4) to collect and store in a dedicated biological bank blood samples from each selected subject.

Methods

CAPIRE study

The CAPIRE study (ClinicalTrials.gov Identifier: NCT02157662) is part of the GISSI Outlier Project jointly promoted by the Heart Care Foundation Onlus, Italian Association of Hospital Cardiologists (ANMCO) and Mario Negri Institute of Pharmacological Research, Milan. The research strategy of the project is focused on individuals, defined as "outliers," who diverge from the average prevalent behavior predicted by current models.

The CAPIRE study was designed as a prospective, observational, and international multicenter study involving (1) a cross-sectional comparison of clinical, imaging, and biomolecular variables between extreme selected populations and (2) a longitudinal phase in which subjects will be initially followed up for a 5-year period.

Study population

The study population was selected among both male and female subjects aged 45 to 75 years, without any previous clinical manifestations of ischemic heart disease, who underwent 64-slice (or superior) CCTA in the outpatient clinics of the 11 centers involved in the study, because of suspected CAD.

The main indications for CCTA were (1) uninterpretable, equivocal, or contraindicated functional stress test in 44.3% of patients and (2) new-onset chest pain syndrome at low-intermediate pretest likelihood of CAD in 24.7% and other indication (including preoperative evaluation before valve or noncardiac surgery, elevated risk profile, arrhythmias, or atypical symptoms) in 31.0%.

Risk factors and CAD definition. The conventional cardiovascular RFs based on Adult Treatment Panel III[3] and 2013 American College of Cardiology/American Heart Association (AHA) guidelines for cardiovascular prevention[18] were considered for the selection process of the study population.

Risk factors were defined as follows: family history of ischemic heart disease (history of early manifestations of ischemic heart disease in first-degree relatives, with onset <55 years old for men and <65 years old for women), arterial hypertension (history of arterial hypertension, ongoing antihypertensive treatment, or recent observation of blood pressure values >140/90 mm Hg), hypercholesterolemia (total cholesterol values >200 mg/dL or <200 mg/dL with ongoing lipid-lowering medications), diabetes mellitus (fasting plasma blood glucose levels >126 mg/dL or 2-hour values in the oral glucose tolerance test of ≥200 mg/dL or isolated elevation of glycated hemoglobin ≥6.5% or current

use of insulin or oral hypoglycemic agents), and cigarette smoking (current cigarette smoking habit or recent abstention (<1 year).

The source of data for defining cardiovascular RFs was physical examination, anamnestic records, and laboratory tests reported by the subject or documented before CCTA.

After the enrollment, a centrally performed biomarker profile including lipid profile and metabolic markers allowed to refine the assessment of RFs such as diabetes and hypercholesterolemia.

Within enrollment criteria, the choice of risk cutoffs for the different populations based on the number of RFs aims at providing simple enrollment criteria. According to data reported in the literature,[3] most patients without any RFs or one single RF belong to risk group <10% of events at 10 years according to Framingham Study and patients with 3 or more RFs belong to risk group >20% of events at 10 years. Subsequently, for the whole enrolled population, the risk assessment according to different scores (Framingham Risk Score, SCORE, Pooled Cohort Equations) will be carried out.

Diabetes as single RF has been excluded because of evidence of its own higher cardiovascular risk[3] as well as for the development of CAD and being independently associated with the atherosclerotic burden.[19]

Coronary atherosclerotic plaque was defined as any clearly discernible structure that could be assigned to the coronary artery wall and that could be discriminated from surrounding pericardial tissue and epicardial fat. Normal coronary arteries were defined if no atherosclerotic plaque (including focal and eccentric calcified plaques) could be detected in any segment within the coronary artery wall or lumen.

The choice of CCTA as a selection method for dichotomic outlier populations is based on the complementarity of relevant specific diagnostic performance and selection criteria for the study population.[20] Coronary computed tomography angiography allows for noninvasive assessment of coronary anatomy, providing evidence of the presence, extent, and severity of CAD, vascular wall, remodeling process, and plaque characteristics. The high negative predictive value of CCTA enables to identify subjects with normal coronary arteries in a direct and more accurate manner than other noninvasive methods. On the other hand, regardless of stenosis quantification, CCTA can better detect segments with subclinical disease, thus accurately defining the overall atherosclerotic process.[21]

A 5-coronary-segment involvement cutoff was set to define a diffuse CAD. This choice was based on previously demonstrated prognostic value[22,23] and on the results of CONFIRM registry that showed a strong role of CCTA in the prediction of all-cause mortality at 2-year follow-up, when individuals were partitioned into groups with a segment involvement score (SIS) of ≤5 vs >5.[24]

This population represents the platform to work for further innovative investigations carried out in homogeneous outlier groups selected by the integration of clinical, imaging, and first-line biomarker profiles.

Therefore, based on CCTA result and RF burden (Figure 1), 2 outlier groups have been identified:

- *No-CAD/High-RF*: subjects with no CAD and 3 or more RFs
- *CAD/Low-RF*: subjects with diffuse CAD extended to more than 5 of the 16 segments according to the AHA classification[25] and 0-1 RF with the exclusion of patients with type 1 or type 2 diabetes mellitus as single RF

Thus, using the same criteria, 2 control groups have been selected, consisting of the following:

- *No-CAD/Low-RF*: subjects with no CAD and 0-1 RF with the exclusion of patients with type 1 or type 2 diabetes mellitus as single RF
- *CAD/High-RF*: subjects with diffuse CAD extended to more than 5 of the 16 segments according to the AHA classification[25] and 3 or more RFs

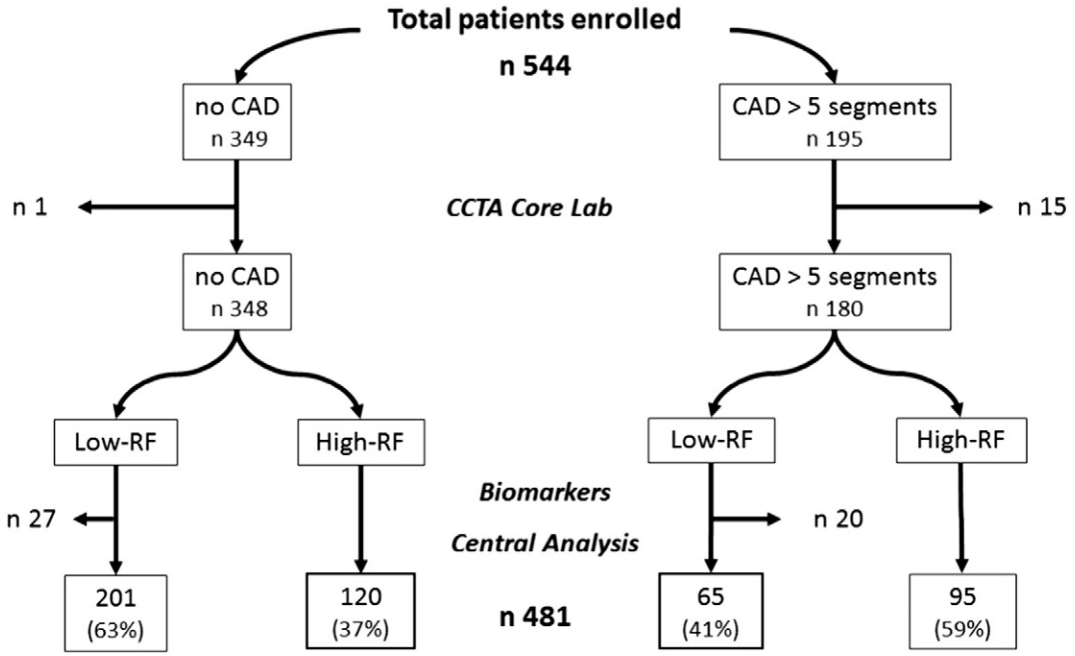
Exclusion criteria were as follows: CCTA did not meet the quality control criteria, previous cardiovascular events (acute myocardial infarction, unstable angina, chronic stable angina, previous percutaneous or surgical coronary revascularization, heart failure), both clinically evident and confirmed by conventional diagnostics; previous heart disorders documented or identified at CCTA, such as dilated cardiomyopathy regardless of etiology, obstructive hypertrophic cardiomyopathy, atrial fibrillation, myocarditis, and inflammatory vascular disease; previous documented acute or chronic peripheral vascular disease (stroke, transient ischemic attack, previous percutaneous, or surgical vascular revascularization; claudication at rest/low-grade effort); and active inflammatory or neoplastic disease.

Baseline examination

Patients satisfying the inclusion criteria had a structured interview concerning conventional RFs, medical history, previous cardiovascular examinations, and ongoing therapies. During the enrollment examination, anthropometric data and blood pressure were also recorded as well as a rest 12-lead electrocardiogram, questionnaires on psychosocial factors (Hospital Anxiety and Depression Scale-Anxiety, Hospital Anxiety and Depression Scale-Depression, and Type D Scale-14), and dietary habits.

The whole study population had a peripheral venous blood sample taken at baseline and after 2 years of follow-up. The samples were treated to obtain 0.5 mL whole blood, plasma, and serum aliquots and stored in a freezer at −70°C. Samples of each subject enrolled in the study were collected in a single dedicated biological bank (SATURNE-1; Mario Negri Institute of Pharmacological Research, Milan, Italy).

Figure 1



Graphical representation of the selection criteria of the study population and flow diagram of selection process after CCTA core laboratory evaluation and central biochemical assay. CCTA core laboratory evaluation excluded overall 16 patients because of the low image quality or $1 \leq$ SIS < 5. Central biochemical assay refined the assessment of RFs such as diabetes and hypercholesterolemia, and 47 patients scheduled in the low-RF groups were reclassified and excluded because of their higher RF profile.

Laboratory analysis

All circulating biomarkers were measured in a central laboratory, in a single batch, by personnel unaware of patients' characteristics. Serum creatinine, glycated hemoglobin, and lipids were measured with standard, automated laboratory methods. Plasma levels of pentraxin-3 (PTX3) were measured by in-house sandwich enzyme-linked immunosorbent assay as previously described.[26] Detection limit was 0.1 ng/mL. High-sensitivity C-reactive protein was measured with an automatic immunoturbidimetric method (Beckman-Coulter, Galway, Ireland).

Statistical analysis

Given the pure exploratory strategy and hypothesis-generating purposes using a novel approach focusing on still uninvestigated outlier populations, the definition of population size was difficult to assess using traditional hypothesis–driven statistical methodology.

A preliminary retrospective observational, multicentric analysis has been performed by examining the existing databases of 3 participating centers to collect data about the proportion of patients who met the inclusion criteria during a median time of 1.5 years. This analysis was aimed to assess the prospective study feasibility according to inclusion criteria. The retrospective study indicated that, among 619 subjects with absence of CAD at CCTA, 79 (12.8%) had $\geq$3 RFs (no-CAD/high-RF group), whereas among 157 patients with diffuse CAD, 56 (35.7%) had 0-1 RFs (CAD/low-RF group).

Continuous variables were presented as mean with SD or as median with interquartile range (25°-75°) if more appropriate (nonnormal distribution). Continuous variables normally distributed were compared using the Student t test for independent samples. When the variable distribution was not normal, Mann-Whitney U tests for independent samples were used. The proportion of the categorical variables was compared using a χ^2 analysis or Fisher exact test, as appropriate. A P value $<.05$ was considered statistically significant. Estimated odds ratios and accompanying 95% CIs are presented for each class of the variables that became significant at the univariate analysis. Statistical analysis and graphics were produced with SAS (version 11; SAS Institute Inc, Cary, NC).

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Table. Baseline characteristics of the outlier groups

	No CAD		P	CAD		P	*P* between outlier groups CAD/LR vs no CAD/HR
	Low-RF	High-RF		Low-RF	High-RF		
n (%)	201 (41.8)	120 (24.9)		65 (13.5)	95 (19.8)		
Demographic							
Age (y), mean (SD)	58.2 ± 8.6	58.7 ± 8.0	.60	64.2 ± 7.5	63.2 ± 6.9	.37	<.0001
Sex, M/F (%)	49.8:50.2	42.5:57.5	.21	93.8:6.2	71.6:28.4	.0005	<.0001
RFs							
Family history of IHD (%)	7.0	65.0	<.0001	7.7	60.0	<.0001	<.0001
Arterial hypertension (%)	21.4	85.8	<.0001	30.8	91.6	<.0001	<.0001
Dyslipidemia (%)	47.8	96.7	<.0001	43.1	94.7	<.0001	<.0001
Current smoking (%)	4.5	46.7	<.0001	6.2	57.9	<.0001	<.0001
Diabetes (%)	0	24.2	<.0001	0	37.9	<.0001	<.0001
BMI (kg/m^2), mean (SD)	25.1 ± 3.7	27.4 ± 3.9	<.0001	26.8 ± 4.1	28.1 ± 4.6	.051	.34
Systolic BP (mm Hg), mean (SD)	125.1 ± 13.5	129.1 ± 14.6	.017	128.4 ± 13.3	135.2 ± 16.1	.0037	.75
Laboratory data							
Total cholesterol (mg/dL), mean (SD)	195.2 ± 39.3	205.4 ± 46.5	.046	188.0 ± 31.8	181.7 ± 45.0	.30	.003
LDL cholesterol (mg/dL), mean (SD)	119.3 ± 32.5	125.0 ± 35.9	.15	120.0 ± 28.9	111.5 ± 36.9	.106	.3
HDL-C (mg/dL), mean (SD)	55.2 ± 16.0	51.7 ± 14.7	.043	45.0 ± 9.7	45.6 ± 12.5	.744	.0003
Triglycerides (mg/dL), median (IQR)	73 (54-105)	111 (78-184)	<.0001	104 (72-142)	113 (84-168)	.146	.22
Glycated Hb (%), median (IQR)	3.4 (3.2-3.8)	3.6 (3.2-3.9)	.0154	3.4 (3.2-3.9)	4.0 (3.4-4.6)	<.0001	.07
C-reactive protein (mg/L), median (IQR)	1.2 (0.5-2.7)	1.7 (0.9-4.5)	.002	1.5 (0.5-4.6)	2.4 (0.8-5.9)	.26	.5
PTX3 (ng/mL), median (IQR)	3.2 (2.1-4.8)	2.6 (1.8-4.7)	.18	3.4 (2.5-5.7)	3.0 (2.0-4.9)	.085	.06
Serum creatinine (mg/dL), mean (SD)	0.80 ± 0.16	0.80 ± 0.19	.97	0.91 ± 0.2	0.85 ± 0.18	.07	.0008

Values are expressed as mean ± SD, median (IQR, 25°-75° percentile) or no. (%).
LR, low-risk; HR, high-risk; M/F, male-to-female ratio; IHD, ischemic heart disease; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; IQR, interquartile range; Hb, hemoglobin.

Foundation Onlus, in the years 2009 and 2010, from the fundraising campaigns "Accendi il tuo cuore."

Results

Based on the results from a preliminary retrospective analysis, a total of 544 consecutive patients were recruited in the study from January 2011 to June 2013. The on-site preliminary selection, based on CCTA angiograms and on the presence of RF, allowed to select a whole study population with a mean age of 60.2 ± 8.4 years and a predominance of men (58.5%).

Two "outlier" groups were identified: 120 (22.1%) patients with absence of CAD despite 3 or more RFs (no-CAD/high-RF group) and 93 (17.1%) patients with diffuse CAD and 0-1 RF excluding diabetes as single RF (CAD/low-RF group). The control groups included 229 (42.1%) patients with no CAD and 0-1 RF excluding diabetes as single RF (no-CAD/low-RF group) and 102 (18.7%) patients with diffuse CAD and 3 or more RFs (CAD/high-RF group).

In order to verify the extreme and phenotypically homogeneous features of the outlier populations, each CCTA was analyzed by the core laboratory. Glycemic and lipid profiles were also assessed by central analysis to reduce the biased exclusion of RFs only by the anamnestic report.

As shown in Figure 1 after the CCTA core laboratory evaluation, 1 patient of no-CAD/high-RF and 15 patients of CAD/low-RF were excluded. Moreover, central biochemical assay allowed to refine the assessment of RFs such as diabetes and hypercholesterolemia, and 20 patients scheduled in the CAD/low-RF group, in addiction to 27 patients preliminary selected in the no-CAD/low-RF group, were reclassified and excluded because of their higher RF profile. Therefore, the final outlier population consisted of 185 patients: 120 patients in the no-CAD/high-RF group and 65 patients in the CAD/low-RF group. Baseline characteristics of 2 outlier groups are reported in Table.

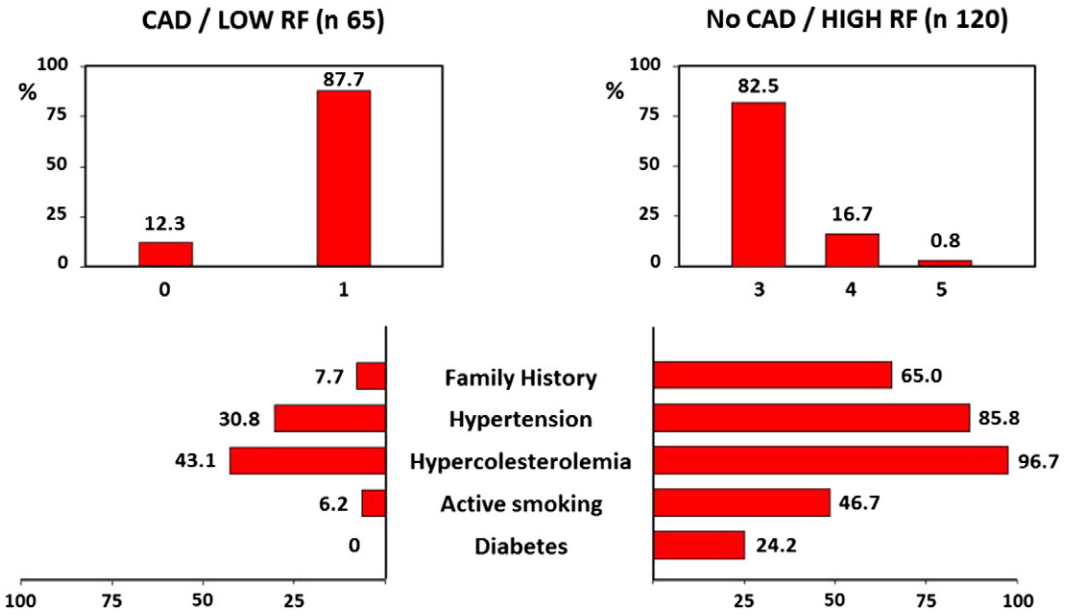
In the CAD/low-RF group, SIS and segment stenosis score were 7.6 ± 2.1 and 11.3 ± 5.0, respectively. Furthermore, CCTA documented obstructive coronary disease with stenosis >50% in 49 (75.4%) patients, of whom 29 (44.6%) had stenosis >70%. The composition of the plaque was mainly calcified plaques (70.5%), whereas noncalcified plaque was only 8.7%.

The CAD/low-RF group was significantly older than the no-CAD/high-RF group (64.2 ± 7.5 vs 58.7 ± 8.0 years, respectively; $P < .0001$), with prevalence of men (93.8% vs 42.5%, respectively; $P < .0001$).

The RF distribution exhibited opposite and extreme features; most of the CAD/low-RF group patients had 1 RF (87.7%) with the exclusion of diabetes as a single RF. In the no-CAD/high-RF group, 3 RFs were reported in 82.5% patients, 4 RFs were reported in 16.7%, and only 0.8% of patients had all the RFs.

As shown in Figure 2, also the RF presence significantly diverged among groups. Patients in the CAD/low-RF group showed hypercholesterolemia (43.1%) and arterial hypertension (30.8%), whereas family history of ischemic

Figure 2



Number of RFs and RF distribution within the 2 outlier groups, CAD/low-RF and no-CAD/high-RF.

heart disease occurred in 7.7% of cases and active smoking in 6.2%.

In the no-CAD/high-RF group, hypercholesterolemia (96.7%), arterial hypertension (85.8%), and family history of ischemic heart disease (65.0%) largely occurred; 46.7% was active smokers; and 24.2% had diabetes.

Regarding anthropometric variables, body mass index mean values were not different between the 2 outlier groups. The mean total cholesterol value was significantly higher in patients in the no-CAD/high-RF group, but mean serum low-density lipoprotein cholesterol and median triglycerides values did not differ between groups. Noteworthy, the treatment with statins was significantly higher in the no-CAD/high-RF group than in the CAD/low-RF group (56.7% vs 12.3%; $P < .0001$).

The mean high-density lipoprotein cholesterol (HDL-C) was significantly higher in the no-CAD/high-RF group (51.7 ± 14.7 mg/dL) than in the CAD/low-RF group (45.0 ± 9.7 mg/dL; $P = .0003$). Furthermore, the outlier populations showed different serum creatinine mean levels even within the reference range (0.91 ± 0.2 [CAD/low-RF group] vs 0.8 ± 0.19 [no-CAD/high-RF group], $P = .0008$). Regarding inflammatory markers, there were no difference between the 2 groups for median levels of C-reactive protein and PTX3.

Discussion

We presented here the study design and rationale of the CAPIRE study developed with the aim to investigate, with an innovative approach focused on the outliers individuals, new protective and RFs regarding the development of diffuse coronary atherosclerosis and the onset of different clinical manifestation of ischemic heart disease.

First, our preliminary results demonstrate that 2 outlier populations can be detected in the clinical field of patients who undergo CCTA for routine clinical reasons. Among individuals without previous cardiovascular history investigated for suspected CAD, CCTA allows to identify 2 relevant outlier populations: (1) subjects with no CAD and multiple RFs, and 2) at the opposite extreme, subjects with diffuse CAD (SIS >5), with higher expected risk of events, despite a low RF burden.

The central analysis of CCTA and biomarkers guaranteed a rigorous control of both imaging and RF inclusion criteria and allowed to allocate outlier populations at the extreme and opposite RF distribution, thus preventing selection bias that characterizes a retrospective analysis.

The baseline clinical characteristic of the study population highlighted the opposite RF burden between the 2 outlier groups. Furthermore, the preliminary univariate and unadjusted analyses allow to identify age, gender, HDL-C, and serum creatinine as distinctive variables among the 2 groups.

During the study, we collected biological samples from outlier population in a Bio-Bank. These samples will be used to identify and characterize novel environmental and genetic RFs through the assessment of known and emerging markers of lipid, metabolic, thrombotic, inflammatory, immunologic, and genetic profile. Advanced "-omic" (genomic, proteomic, and transcriptomic) investigations

could be carried out in homogeneous outlier groups selected by the integration of clinical, imaging, and first-line biomarker profiles.

Our preliminary data combined to previous studies and our retrospective preliminary analysis confirmed the existence of outlier subjects, thus supporting that the presence of RFs was neither a necessary nor a sufficient condition for atherosclerosis development, but unknown, genetic, environmental, and individual factors may play a critical role in disease development. These preliminary findings challenge the generalization of the widely accepted simplistic paradigm of a straightforward relationship between RF and CAD, and thus, they could stimulate new avenues for research.

Several bodies of evidence have suggested that the relationship between RFs and coronary events appears to be valid only to a first approximation in population analyses and in specific subgroups. Autoptic[27] and angiographic[28] studies and analyses with intracoronary ultrasound[19] or coronary artery calcium (CAC) indicated that the relationship among RFs and the extent of coronary atherosclerosis was weak and variable.[29,30]

The individual variability of the relationship between CAD and RFs was also reported using CCTA. Indeed, Faletra et al[31] demonstrated that after CCTA in subjects without previous clinical manifestations of ischemic heart disease, about 18% of patients with CAD did not show any conventional RFs, whereas 12% of subjects with no CAD had at least 3 RFs. Consistently, Johnson et al[32] demonstrated that the individual amount of CAD burden detected at CCTA was not closely related to the National Cholesterol Education Program score risk categories.

Recently, Silverman et al[30] in the MESA cohort confirmed the evidence of CAC heterogeneity in individuals at the extremes of traditional risk classification. Indeed, among the population with CAC 0 the absolute risk of coronary heart disease event remained low for a mean of 7.1 years of follow-up, although RFs were associated with higher relative risk. These findings emphasize the challenging issue concerning which protective factors patients with CAC 0 and exposed to multiple RFs have.

In our prospective study, we sought to detect, characterize, and collect either clinical and imaging data or biological samples of these outlier populations in order to investigate still unknown risk or protective factors for coronary atherosclerosis and ischemic events.

Among individuals investigated for suspected CAD, our results demonstrated the ability of CCTA for the identification of 2 relevant populations of outliers: patients who have no CAD despite multiple RFs and those who have extensive CAD with ≤1 RF.

In our study, the outlier groups were characterized by different distributions of age and gender, which became the greatest determinants of both risk and protection for atherosclerotic process, regardless of the modifiable RFs.

In all major cohorts, age became the single strongest RF related to future cardiovascular events and the female gender was a strong protective factor (for the age range considered in the study). Indeed, a minority of women were found in the CAD/low-RF group.

However, the impact of aging and gender in coronary risk prediction models might inadvertently deemphasize new risk or protective factors that can be modifiable early in life and that can greatly affect long-term outcomes. Any future investigation will take into account this issue, and the analysis should be adjusted, in particular for age and gender, in order to detect the added value of new predictive variables.

A first-line biomarker profile including lipid profile, inflammatory, and metabolic markers such as hemoglobin A_{1c} and serum creatinine was assessed to characterize the outlier population with a wide broad of circulating markers as well as to further select biological homogenous subgroups.

The outlier population showed significantly different mean HDL and serum creatinine values, consistently with the well-established association between both HDL and renal function and CAD in large populations. The detection of these significant difference also in a small but homogenous oppositely extreme population further supports the potential value of a study of outlier population as an innovative strategy to investigate multifactorial diseases (eg, ischemic heart disease), particularly for hypothesis-generating purposes.

Indeed, the bias of large and nonhomogeneous populations and the confounding effect of different pathogenetic variables are reduced, whereas the detection of significant individual protective or risk biomolecular markers can be enhanced also in a relatively small-sized population. This approach has recently been proposed for genome-wide association studies to increase study efficiency of complex phenotypes.[33]

Limitations

The principal limitation of the CAPIRE study is its pure exploratory strategy and hypothesis-generating purposes using a novel approach focusing on poorly studied outlier populations. Thus, the definition of the population size is difficult to assess using traditional hypothesis-driven statistical methodology. Indeed, the sample size could be an important limitation of the study. However, as expected, the outlier patients are rare and we reported a feasible approach to study them.

However, the opposite and extreme features of inclusion criteria of the CAPIRE study based on opposite RF burdens and CCTA anatomical disease categories (normal coronary arteries vs SIS >5) with divergent prognostic value can further improve the detection of potential differences among small-sized population.

Another limitation is regarding the initial assessment of risk profile based only on the presence of the traditional RFs. Anyway, according to Adult Treatment Panel III, most patients with 0-1 RF belong to the risk group <10% of events at 10 years according to Framingham Study and patients with 3 or more RFs belong to risk group >20% of events at 10 years.[3]

Conclusion

Analyzing the clinical, imaging, and biomolecular variables of apparently healthy individuals at the extremes for the relationship between traditional RF profile and the presence of CAD documented by CCTA, the CAPIRE study would allow for the investigation of novel protective and susceptibility factors for CAD and ischemic events, distinguishing the causal role of coronary atherosclerosis from that of RFs, independently from age and sex.

Disclosures

M. Magnoni, M. Gorini, T. Mocetti, M.G. Modena, M. Canestrari, S. Berti, G. Casolo, D. Gabrielli, P. Marraccini, S. Masson, R. Latini, A.P. Maggioni, and A. Maseri have no conflict of interest to disclose. D. Andreini is on Speaker Bureau of GE Healthcare.

Appendix

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