

The Prospective Studies of Atherosclerosis (Proof-ATHERO) consortium: Design and rationale

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Short Title: Design and rationale of the Proof-ATHERO consortium

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Abstract

Atherosclerosis – the pathophysiological mechanism shared by most cardiovascular diseases – can be directly or indirectly assessed by a variety of clinical tests including measurement of carotid intima-media thickness, carotid plaque, ankle-brachial index, pulse wave velocity, and coronary artery calcium. The Prospective Studies of Atherosclerosis (Proof-ATHERO) Consortium (<https://clinicalepi.i-med.ac.at/research/proof-athero/>) collates de-identified individual-participant data of studies with information on atherosclerosis measures, risk factors for cardiovascular disease, and incidence of cardiovascular diseases. It currently comprises 74 studies that involve 106,846 participants from 25 countries and over 40 cities. 21 studies recruited participants from the general population (n=67,784), 16 from high-risk populations (n=22,677), and 37 as part of clinical trials (n=16,385). Baseline years of contributing studies range from April 1980 to July 2014; the latest follow-up was until June 2019. Mean age at baseline was 59 (standard deviation: 10) years and 50% were female. Over a total of 830,619 person-years of follow-up, 17,270 incident cardiovascular events (including coronary heart disease and stroke) and 13,270 deaths were recorded, corresponding to cumulative incidences of 2.1% and 1.6% per annum. The consortium is coordinated by the Clinical Epidemiology Team at the Medical University of Innsbruck, Austria. Contributing studies undergo a detailed data cleaning and harmonisation procedure before being incorporated in the Proof-ATHERO central database. Statistical analyses are being conducted according to pre-defined analysis plans and use established methods for individual-participant data meta-analysis. Capitalising on its large sample size, the multi-institutional collaborative Proof-ATHERO consortium aims to better characterise, understand, and predict the development of atherosclerosis and its clinical consequences.

Keywords: Prospective studies · Consortium · Individual-participant data · Atherosclerosis · Repeat measurements · Cardiovascular disease

Introduction

Cardiovascular diseases (CVD) are the most common cause of death and disability worldwide. According to recent estimates from the Global Burden of Disease Study, about 18 million people die of CVD in a year, which account for over 30% of all global deaths [1]. The pathophysiological mechanism shared by many CVD is atherosclerosis, a gradual and progressive hardening and narrowing of the arteries over the course of life. Initial atherosclerotic alterations can be found as early as in young adulthood [2, 3] and involve endothelial dysfunction, inflammation, and deposition of fat [4]. Advanced atherosclerotic lesions are characterised by formation of atherosclerotic plaque that can destabilise, rupture or fissure, and can ultimately lead to acute vessel occlusion or formation of a local thrombus with dislocation into distal arteries and thereby clinical sequelae [4].

Clinical and subclinical atherosclerosis can be directly or indirectly assessed using a range of different clinical tests which are simple, safe, and non-invasive, and therefore amenable for use in large-scale studies (**Fig. 1**). One of the imaging techniques for atherosclerosis most frequently used is the assessment of carotid intima-media thickness (cIMT). Using B-mode high-resolution ultrasound, the distance between the adventitia-media interface and the intima-lumen interface of the carotid arterial wall is quantified. Spatial resolution of this imaging technique is approximately 50 μm axially and 200 μm laterally. Ultrasound-based cIMT is considered as a marker of the early stage of atherosclerosis. It is related to unfavourable levels of traditional cardiovascular risk factors [5, 6] and has been shown to be in good accordance with “true” cIMT determined in histological studies [7]. Furthermore, increased cIMT has been associated with increased risk of cardiovascular events [8, 9].

Other scalable and commonly available measures to ascertain vessel wall pathology and dysfunction include carotid plaque [10, 11], ankle-brachial index [12], pulse wave velocity [13], and the coronary artery calcium score [14–16] (**Fig. 1**). As reviewed recently [17], these measures have several strengths and weaknesses. cIMT, carotid plaque, ankle-brachial index, and pulse wave velocity are non-invasive and cost-effective markers, which are therefore relatively easy to implement in large clinical studies. However, disadvantages include measurement error and lack of standardisation in measurement protocols for cIMT, specificity of ankle-brachial index [12], and the error associated with the measurement of travelled distance for pulse wave velocity [18]. The coronary artery calcium score directly quantifies presence of calcification in coronary arteries [19]. In contrast to the other mentioned markers, coronary

artery calcification is assessed with computed tomography, which is more costly and exposes the study participant to radiation, thereby limiting large-scale assessments.

According to the 2019 European Society of Cardiology Guidelines for the diagnosis and management of chronic coronary syndromes, atherosclerotic plaque detection by carotid artery ultrasound, assessment of coronary artery calcium score with computed tomography, and measurement of the ankle-brachial index may be considered as risk modifiers in cardiovascular risk assessment in asymptomatic subjects [19]. Because atherosclerosis typically develops over a long period of time and only causes symptoms at an advanced stage, these measures are important tools in clinical practice to quantify atherosclerosis burden and might help inform treatment decisions.

The Prospective Studies of Atherosclerosis (Proof-ATHERO) consortium is an international consortium that brings together individual-participant data from prospective cohorts with detailed information on atherosclerosis, covariates, and incidence of CVD outcomes. The present report provides a description of broad aims of the Proof-ATHERO consortium and the principal methodology involved in collating, harmonising, and analysing study data.

Design

Objectives

Capitalising on its large sample size and the comprehensive information available, the overarching aims of the Proof-ATHERO consortium are to: (i) better characterise the natural history, communalities, and differences of different atherosclerosis measures; (ii) to provide novel insight into the determinants of atherosclerosis development and progression; and (iii) to investigate clinical consequences of atherosclerosis. In contrast to prior reports in individual studies, the large-scale data of Proof-ATHERO enables the study team to conduct power-demanding analyses, including (i) characterisation of atherosclerosis trajectories over time; (ii) determination of the shapes of associations (e.g. linear vs. curvilinear vs. threshold effects); (iii) study of potential effect modifiers (e.g. age, sex, medication, or different lifestyle factors such as smoking habit); (iv) direct comparisons of the added predictive value of different atherosclerosis measures over and beyond assessment of conventional risk factors; and (v) reliable evaluation of atherosclerosis measures as surrogate markers for clinically manifest

CVD endpoints. Overall, Proof-ATHERO aims to analyse world-wide available data to deliver results based on the highest scientific evidence.

Inclusion criteria

Prospective cohorts are eligible for inclusion in the Proof-ATHERO consortium if they were observational studies or clinical trials that: (i) have assessed one or more atherosclerosis measures (i.e. cIMT, carotid plaque, ankle-brachial index, pulse wave velocity, and coronary artery calcium) repeatedly (i.e. at two or more time points); (ii) have ascertained comprehensive information on CVD risk factors (e.g. lifestyle, blood-based markers, history of disease, and medication intake); and (iii) have recorded incident CVD outcomes using well-defined criteria.

A crucial foundation for the Proof-ATHERO consortium was provided by the PROG-IMT project [20]. This initiative led by Matthias Lorenz at the Goethe University at Frankfurt am Main had collated and analysed individual-participant data on the progression of cIMT and, for instance, yielded milestone publications on the association of cIMT progression with future CVD risk in the general population [8], in people with type-2 diabetes [21], and in people at high cardiovascular risk [22]. When the PROG-IMT project was completed in 2017, a majority of contributing studies (83%) decided to continue the fruitful collaboration as part of the Proof-ATHERO consortium and to jointly investigate scientific questions which go beyond the initial aims of the PROG-IMT project. The commitment by these studies gave a unique head-start to the Proof-ATHERO consortium and enabled efficient data accrual at the beginning of the initiative.

Identification and incorporation of new eligible studies is ongoing and we invite researchers to contact the coordinating centre if they wish to contribute to the Proof-ATHERO consortium.

Atherosclerosis measures

Data have been sought from investigators on carotid ultrasound parameters, ankle-brachial index, pulse wave velocity, and coronary artery calcium at baseline and any subsequent re-examinations during follow-up. Atherosclerosis measures assessed by the individual studies are summarised in **Table 1**. Parameters based on carotid ultrasound are being collected systematically on up to twelve sites (common carotid artery, carotid bifurcation, and internal carotid artery; left and right side; near and far wall) and include cIMT, vessel diameter, presence of plaques (yes vs. no), number of plaques, plaque thickness (height in mm), plaque area in a

longitudinal view (in mm²), and plaque morphology according to the Gray-Weale classification [23]. The methodologies which studies used to cIMT and carotid plaque are summarised in **Table S2** and **Table S3**, respectively.

Participant characteristics at the baseline and follow-up surveys

Data on participant characteristics at baseline and follow-up surveys have been sought from investigators on age, sex, ethnicity, socio-economic status, smoking, systolic and diastolic blood pressure, body-mass index, lipid markers (e.g. total cholesterol, high- and low-density lipoprotein cholesterol, triglycerides), markers of inflammation (e.g. C-reactive protein, fibrinogen, leukocyte count), markers of dysglycaemia (e.g. fasting glucose, glycated haemoglobin), use of medication (e.g. antihypertensive, antidiabetic, lipid-lowering medication), and pre-existing diseases (e.g. coronary heart disease, stroke, diabetes, or hypertension). Furthermore, in clinical trials, information on the type of interventions (and dosages, if appropriate) and on adherence to allocated regimens have been collated.

Incident disease outcomes

Data on incident disease outcomes have been collated predominantly on fatal and non-fatal CVD events, including myocardial infarction, angina pectoris, and subtypes of stroke. Details on the ascertainment of prevalent and incident CVD are provided in **Table S4**. Studies assessed prevalent CVD at study baseline using self-report only or supplemented by objective criteria. The vast majority of the studies used objective criteria rather than self-report only for assessing incident coronary heart disease (93%) and incident stroke (90%). Outcomes were classified according to International Classification of Diseases-10 coding (e.g. I20-I25 for coronary heart disease and I61-I69 for stroke) or to study-specific coding systems. In addition, information on cause-specific death has been sought. In 15 studies, cause of death was ascertained based on the death certificate; 44 studies supplemented the death certificate with information from additional sources (e.g. medical records, autopsy findings).

Coordination of the consortium

The Proof-ATHERO consortium is coordinated by the Clinical Epidemiology Team at the Medical University of Innsbruck, Austria. An outline of the processes involved in Proof-ATHERO coordination is provided in **Fig. 2**. Standardised data request forms are sent to eligible studies, inviting them to participate in the initiative. Upon receipt of study data, data cleaning

and harmonisation are performed by a dedicated data management team using a range of tools for detecting inconsistencies and ambiguities in the data. Any queries arising during this process are clarified through direct correspondence with study investigators. Upon completion of the data management process, study data are stored in a central database at the coordinating centre. The data management system of the coordinating centre has been implemented in SAS 9.4. Proposals for analyses can be submitted by all members of the Proof-ATHERO study group (i.e. all named investigators of studies contributing data to Proof-ATHERO) via the consortium's webpage. Upon receipt, proposals are reviewed by a dedicated Proof-ATHERO steering committee, which then allocates resources at the coordinating centre according to resource availability and scientific priority of the project. For contractual reasons, data are stored and analysed exclusively at the Proof-ATHERO Coordinating and Statistics Centres (Medical University of Innsbruck and University of Cambridge). At each step from development of a statistical analysis plan, to the conduct of statistical analyses, and the creation of a manuscript draft, investigators of contributing studies and expert panels are contacted for feedback and comments, therefore making use of the broad and diverse community of experts in the field involved in the initiative.

General approach to statistical analyses

For each scientific project, statistical analyses will be performed according to a pre-specified analysis plan. Statistical analyses will follow established methods in the analysis of individual-participant data [24–29]. Generally, the multi-level structure of data (e.g. multiple cohorts) will be taken into account by combining study-specific estimates using meta-analytical methods or by using mixed regression models with appropriate specification of random effects. Analyses will also involve assessments of between-studies heterogeneity. More details on specific analytical methods will be provided in publications resulting from each scientific project.

Data protection and ethics considerations

All studies contributing data to Proof-ATHERO have previously reported results and have obtained relevant local ethics approval and participants' consent. The data provided by each study remain entirely the property of the principal investigators of that study and are held in confidence by the Proof-ATHERO coordinating centre. To safeguard the identity of individuals at all stages of the analysis and to ensure compliance with data protection legislation and confidentiality guidelines, study data are transferred to the coordinating centre using encrypted

connections. De-identified data are being stored securely in a central database at the coordinating centre, protected by firewalls and accessible only to authorised staff. Participants and collaborating studies have the right to withdraw from the Proof-ATHERO consortium at any time and without giving reasons.

Characteristics of contributing studies

As of 24 January 2020, a total of 74 studies involving 106,846 participants are part of the Proof-ATHERO consortium. The designs of contributing studies and key study-level characteristics are shown in **Table 2**. In summary, 21 studies recruited participants from the general population, 16 studies were conducted in patient populations with specific pre-existing diseases (e.g. with diabetes), and 37 studies were randomised controlled trials covering a range of different patient populations. The numbers of people enrolled in these three types of studies were 67,784, 22,677, and 16,385, respectively. Baseline years ranged from April 1980 to July 2014; the last follow-up was in June 2019. Mean age at baseline was 59 years (standard deviation: 10); 50% of participants were female. **Fig. 3** demonstrates the geographical location of contributing studies. Study locations were spread across four continents and are based in 25 countries and over 40 cities. The median duration of follow-up (i.e. the time from baseline to first event or end of follow-up) was 6.1 years (interquartile range: 2.7-10.4). Over a total of 830,619 person-years of follow-up, 17,270 incident CVD events and 13,270 deaths were recorded, corresponding to cumulative incidences of 2.1% and 1.6% per annum, respectively. As Proof-ATHERO evolves further, up-to-date information on contributing studies are being made available on the consortium's webpage at <https://clinicalepi.i-med.ac.at/research/proof-athero/>.

Initial set of hypotheses to be tested

The large sample size and variety of data in Proof-ATHERO will enable us to test several hypotheses that are particularly power-hungry and could therefore not be addressed by previous studies. For instance, it is unclear whether cIMT progression could serve as a surrogate marker for hard cardiovascular outcomes in clinical trials [30–32]. Second, given conflicting results of prior individual studies [33–39], the comparative predictive value of cIMT measurements at different locations of the carotid artery remains to be determined in detail. Third, building on the initial insights of our recent literature-based meta-analysis [40], Proof-ATHERO will characterise in detail the association of cIMT with long-term risk of developing carotid plaque.

In general, as a large-scale consortium of patient-level data, the high statistical power and consistent approach to statistical analysis and outcome definitions of Proof-ATHERO will help to address the aforementioned and other questions more reliably than previously possible.

Strengths and limitations

Proof-ATHERO is a large consortium with a huge amount of data on atherosclerosis applying consistent approaches to data harmonisation and analysis. By inclusion of data from 25 countries and different clinical settings, the generalisability of findings will be of particular value. Our study also has several limitations. First, there were some differences between studies in how they assessed atherosclerosis measures and clinical outcomes. To address this issue, we collect meticulously a variety of study-specific characteristics, enabling us to quantify and better understand the impact of these differences in future analyses. Second, comprehensive data cleaning and harmonisation is a serious, often underestimated challenge. However, we managed to develop a sophisticated data management system that enables to transparently and effectively handle various datasets with different structures provided by the individual studies. Third, the current focus of available data lies on cIMT due to participation of multiple studies previously involved the PROG-IMT consortium [20]. Fourth, there exist several other markers for atherosclerosis, such as the assessment of endothelial function [41] with flow-mediated dilation or peripheral arterial tone, which have not been collected within Proof-ATHERO yet. Since the consortium is designed to continuously collect new data as they become available, coverage of other atherosclerosis markers will be expanded over time.

Conclusion

The Proof-ATHERO consortium is a multi-institutional collaborative project that is coordinated at the Medical University of Innsbruck, Austria. The consortium brings together large-scale data from prospective studies in the field of atherosclerosis. Proof-ATHERO combines data on CVD risk factors, repeat assessments of atherosclerosis, and clinical outcomes with cutting-edge data management and analytical tools. Building on these strengths, Proof-ATHERO will help to better characterise, understand, and predict the development of atherosclerosis and its clinical consequences.

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Author Contributions

L. Tschiderer, L. Seekircher, G. Klingenschmid, and P. Willeit are part of the coordinating centre and are responsible for data management and data analysis of the Proof-ATHERO consortium. L. Tschiderer and L. Seekircher drafted the manuscript, conducted the analyses, and interpreted the data. G. Klingenschmid interpreted the data. M. J. Sweeting provided supervision for statistical analyses. P. Willeit is responsible for the conception and design of the work, drafted the manuscript, conducted the analyses, and interpreted the data. All other authors were responsible for data acquisition. All authors revised the manuscript critically for important intellectual content approved the final version of the manuscript.

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Figure Legends

Fig. 1. Measures for quantifying atherosclerosis.

Fig. 2. Data management and analysis workflow in the Proof-ATHERO consortium.

Fig. 3. Location of studies contributing data to the Proof-ATHERO consortium as of 24 January 2020. Full study names and references are provided in **Table S1**.