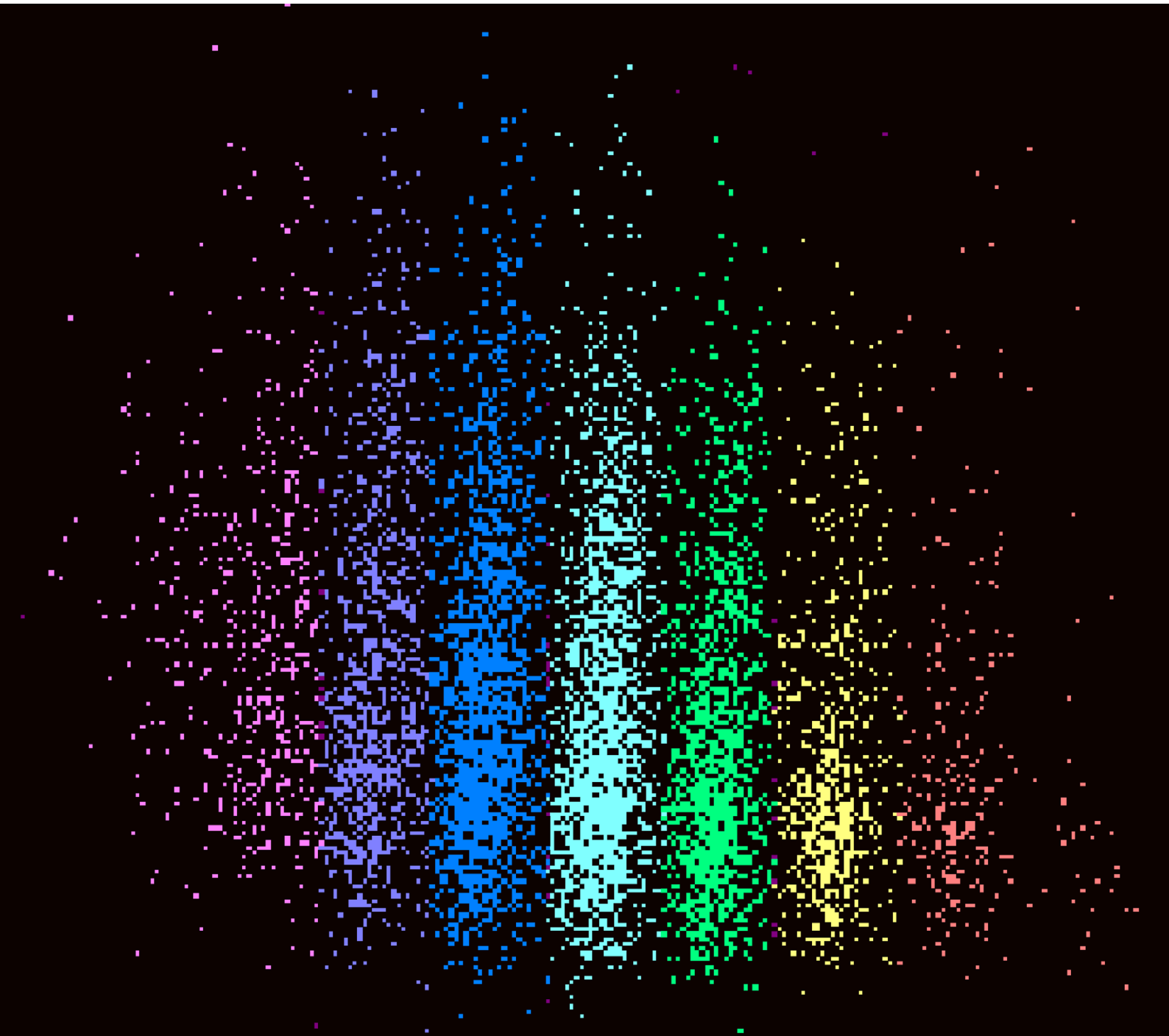
ISSN 2785-7115

OPEN ACCESS

EAJ European Atherosclerosis Journal

www.eathj.org

Volume 1 · Issue 2 · August 2022



OPEN ACCESS
European Atherosclerosis Journal
www.eathj.org
Volume 1 · Issue 2 · August 2022

Quarterly periodical
Registration Court N. 180
del 21.09.2021

Contents

European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly journal covering all topics within atherosclerosis and cardiovascular disease areas. The European Atherosclerosis Journal is an official journal of SITeCS (Società Italiana di Terapia Clinica e Sperimentale - Italian Society for Experimental and Clinical Therapeutics).

European Atherosclerosis Journal

Guidelines for Authors

European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly journal. Papers must be submitted exclusively using the online submission system. To submit your paper, go to the link https://eathj.org/index.php/eaj/about/submissions

AUTHORSHIP AND AUTHOR CONTRIBUTIONS

An Authorship/Author Contributions statement, that specifies the contribution of every listed author (conception, writing, editing, and revision) is required for all types of papers. Please include this statement in your manuscript before submitting it.

CHANGES TO AUTHORSHIP

Authors must provide a definitive list of authors at the time of the original submission. Any change (addition, deletion, or change of order) in the author list after initial submission must be agreed upon by all authors and approved by the Editor-in-Chief. While the Editor-in-Chief considers the request of change, publication of the manuscript will be withheld; in the case that the manuscript has already been published in an issue, any requests approved by the Editor-in-Chief will appear as a corrigendum.

ORCID

ORCID is mandatory for corresponding authors at the time of the submission; all the other authors are encouraged to include ORCID information. Read more about ORCID at https://orcid.org.

ARTICLE TYPES

European Atherosclerosis Journal will consider different article types, including original research papers, reviews, methodology papers, editorials, letters to the Editor, viewpoints, congress/conference reports.

Article categories

Length of article, abstract, figures, and number of references for each category of paper:

	Review	Original Paper	Methodology papers	Editorials, letters to the Editor/viewpoints, congress/conference reports
Abstract maximum length	250 words	250 words	150 words	–
Manuscript maximum length	6000 words	5000 words	3000 words	1500 words
Figure/Table number	6	5	3	2
References	100	60	30	15

Flexibility on word count may be offered after discussion with the Editor-in-Chief.

ORIGINAL RESEARCH PAPERS

Original Research papers include **Basic Research** papers**,** reporting results of original research using cell cultures or animal models, **Clinical and Population Research** papers**,** reporting results from observational, interventional, and genetic studies in humans, and **Translational Research** papers, reporting results of research from both bench-to-bedside and bedside-to-bench.
Original research papers should not exceed 5000 words (including legends to figures and tables), 5 figures/tables, and 60 references. Additional information, tables, and figures will be published as Supplementary material.

REVIEW ARTICLES

Review articles must focus on topics of major interest in basic, clinical, or translational research. Publication of review articles in this Journal is by invitation only. However, the Journal will consider for publication also unsolicited review articles from authors who have contributed substantially to the field. Authors who have not been invited to submit a Review by the Editor-in-Chief or an Editorial Board member should send a letter of interest, an abstract, and a cover letter listing previously published papers on the selected topic to the Editorial Office, whether the topic is of interest for the Journal All those who significantly contributed to the conception, execution, and revision of the review should be listed as co-authors. All review articles (either invited or unsolicited) will undergo a regular peer review process. The following word limits apply: abstract 250 words, main text 6000 words, 6 figures/tables, and 100 references. Authors are encouraged to include a "mechanism/overview" figure and one bullet point box highlighting the main key points.

METHODOLOGY PAPERS

Methodology papers describe novel methods, relevant modifications, or novel applications of established methods in experimental, clinical, or epidemiological research in the field of atherosclerosis. The following word limits apply: abstract 150 words, main text 3000 words (including legends to figures and tables), 3 figures/tables, and 30 references.

EDITORIALS, LETTERS TO THE EDITOR, AND VIEWPOINTS

Editorials are contributions written upon invitation from the Editor-in-Chief or a member of the Editorial Board by expert authors on a recent Original Research Paper of particular interest (published in *European Atherosclerosis Journal* or other journals). Editorials should not exceed 1500 words and 15 references; 1 figure/table is encouraged.
Letters to the Editor and **Viewpoints**. If you have specific issues that you wish to raise concerning work published in European Atherosclerosis Journal, please submit your opinion as a Letter to the Editor or Viewpoint. Papers must not exceed 1500 words (including references), 15 references, and 2 figures/tables. The inclusion of novel data (Viewpoint) will increase the chance of acceptance. The author(s) of the commented manuscript will have the opportunity to respond, and the response will be published in the same issue of the Journal. Please submit these papers to the Editor-in-Chief (Prof. Alberto Corsini, Editor@eathj.org)

CONGRESS/CONFERENCE PROCEEDINGS

Congress/Conference reports are accepted for publication in *European Atherosclerosis Journal* and must be structured as follows: 1) authors and contact details (postal address of all authors and email address of the corresponding author); 2) name of the conference and name of the organizing society); 3) conference dates and venue, and website address if available; 4) topics covered by the conference; 5) conflict of interest statement concerning the congress (e.g. sponsorship). The word count of the entire report (items 1 through 5) must not exceed 1500 words, and 1 figure/table can be included.

MANUSCRIPT PREPARATION

General information. Prepare the manuscript text using a Word processing package and submit it in this format. Do not submit your manuscript as a PDF. Indicate on the title page the word count and the number of figures and/or tables included in the paper. Submitted manuscripts must not exceed 5000 words for Original paper, 6000 words for Reviews, 3000 words for Methodology papers, and 1500 for other article categories, excluding references, tables, and figures. Oxford English spelling should be used.
Title page. The title page must include 1) the title, 2) the name (given name and family name) of all authors and their affiliations (where the work was done), 3) the e-mail address and contact details of the corresponding author. Include also a short title (max 40 characters).
Abstract. The abstract should not exceed 250 words (150 words for Methodology papers).
Keywords. A maximum of 6 keywords should be submitted.
Main text. The manuscript must contain the following sections: Introduction, Methods, Results, Discussion, Conflict of interest (mandatory), Funding (if applicable), Author contribution (mandatory), Acknowledgements (if applicable), References. Abbreviations must be defined when first used in the text.
Tables. Tables must be submitted as Word files, with titles and legends. Tables can be included in the same file as the main text or in a separate file. All tables must have a title and be numbered consecutively and in the order they appear in the text.
Figures. Figures must be numbered consecutively and in the order they appear in the text. Figures must have a title and a legend with a brief description. Figures must be suitable for high-quality reproduction. Submit figures in file(s) separated from that of the main manuscript. Any number exceeding that indicated for a specific article category should be designated as supplementary material.
Acknowledgments. This section should acknowledge any significant contribution of individuals who did not qualify for authorship.
Conflict of interest. All authors must declare any potential conflicts of interest (please refer to the ICMJE guidelines). A conflict of interest statement must be included in the submitted manuscript. If no conflict exists, please state that 'The Author(s) declare(s) that there is no conflict of interest'. An International Committee of Medical Journal Editors (ICJME) disclosure of potential conflicts of interest (COI) form to be submitted for each author and must be received when a revised manuscript is submitted.
Source of funding. A detailed list of all funding sources for the submitted work must be given in a separate section ("Funding"). The list must contain the full funding agency name and grant number(s).
Declaration of Helsinki. Paper reporting results of research involving human subjects must contain a declaration that it complies with the Declaration of Helsinki, that the research protocol has been approved by the local ethics committee, and that informed consent has been obtained from all involved subjects (or their legally authorized representative).
References. In the main text, indicate references by number(s) in square brackets and number them in the order cited. All references must be reported at the end of the article in the Reference section using the Vancouver style. Use of DOI is required.
Supplementary data. The material that is not included in the main text of the manuscript can be made available as supplementary data.
Permissions information. Written permission must be obtained for use of copyrighted material from other sources. If tables and figures included in the manuscript are original and have not been previously published, authors must include the following statement: "The authors declare that all images and figures in the manuscript are original and do not require reprint permission"

REVIEW OF MANUSCRIPTS

All manuscripts submitted to European Atherosclerosis Journal will be evaluated by the Editorial Board. Manuscripts will be returned to authors if they do not meet submission requirements. Manuscripts that pass the first stage will undergo a regular peer review process.
After acceptance, the corresponding author will be contacted by the editorial office. Page proofs will be sent by e-mail to the corresponding author, who should check carefully for any changes or typographic errors. Corrected proofs must be returned to the editorial office within 3 working days.

European Atherosclerosis Journal
www.eathj.org

EAJ 2022;2:37-40
https://doi.org/10.56095/eaj.v1i2.11

FindMyLipidClinic.com: A global Directory of lipid clinics and patient organisations to improve dyslipidemia care

Christophe A.T. Stevens[1], Alexander R.M. Lyons[1], Kanika I. Dharmayat[1], Julia Brandts[1], Antonio J. Vallejo-Vaz[1,2,3], Magdalena Daccord[4], Kausik K. Ray[1]

[1] *Imperial Centre for Cardiovascular Disease Prevention, Department of Primary Care and Public Health, School of Public Health, Imperial College London, London, United Kingdom*

[2] *Department of Medicine, Faculty of Medicine, University of Seville, Sevilla, Spain*

[3] *Clinical Epidemiology and Vascular Risk, Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/ Universidad de Sevilla/CSIC, Sevilla, Spain*

[4] *FH Europe - The European FH Patient Organizations Network - Star House, Star Hill, Rochester, Kent, United Kingdom*

ABSTRACT

Information available on lipid clinics and patient support and advocacy groups, such as location and services provided, is limited or unknown to patients with dyslipidemia and their family members who may also be affected, and non-specialist clinicians, hindering accessibility to appropriate healthcare. To overcome this, the European Atherosclerosis Society Familial Hypercholesterolemia Study Collaboration (EAS FHSC) led by Imperial College London has, in collaboration with the European FH Patient Network (FH Europe), developed FindMyLipidClinic.com, a global Directory of lipid clinics and patient support groups in 29 common languages. Since its launch in 2020, around 4,000 visitors have conducted 12,000 searches across 1,100 locations, which may have retrieved up to 124 lipid clinics and 29 patient groups currently listed in 39 and 27 countries, respectively. However, clinics and patient organisations not currently listed are encouraged to join this directory, and it would also benefit further from collaborations with other existing directories able to contribute.

Received 4 July 2022; accepted 31 August 2022

Keywords
Dyslipidemia;
Atherosclerosis;
Global Directory;
Lipid Clinics;
Patient Care and Support;
FindMyLipidClinic.com

Introduction

Lipoprotein metabolism disorders, dyslipidemia, are widely established as a major risk factor for cardiovascular disease (1, 2), yet they are often diagnosed and treated late, and therefore management of these conditions is frequently suboptimal. For example, familial hypercholesterolemia (FH), the most common autosomal dominant condition, characterised by high levels of low-density lipoprotein cholesterol in blood, has an estimated prevalence of 1:311 worldwide (3) and is often diagnosed late, on average at age 44 years (4). FH patients are often identified by opportunistic screening, rather than by the more cost-effective cascade testing of family members (5). Moreover, once they are diagnosed, less than 3% of FH patients on lipid-lowering therapy achieve the acceptable cholesterol targets recommended by clinical guidelines (4). It is likely that such inadequacies of care are partly attributable to a lack of information available on specialist care and lipid clinics to non-specialist clinicians and patients, for referrals for appropriate diagnosis and treatment.

The difficulty in locating specialist lipid clinics in the absence of a global directory may lead to inequalities in the management of patients with dyslipidemia, as well-informed practitioners may be better equipped to refer patients to the necessary care. Furthermore, this can lead to more collaborative and shared decision making as healthcare delivery moves towards person-centred care. Patient advocacy groups, such as FH Europe, the European FH Patient Network (fheurope.org), aim to raise awareness of dyslipidemia among patients, their relatives, policymakers, and clinicians (6, 7), and could potentially reduce these inequalities by guiding patients and non-specialist clinicians toward appropriate specialists. To our knowledge, the few lipid clinic directories available were limited to the Family Heart

Corresponding Author
Mr. Christophe A.T. Stevens: christophe.stevens@imperial.ac.uk

Foundation in the United States (8), the Fundación Hipercolesterolemia Familiar in Spain (9) and the European Atherosclerosis Society [EAS] (10). However, these initiatives are limited by geography and information provided, which restricts their utilities. Inherited dyslipidemias know no borders and the relatives of newly diagnosed patients, who may also potentially be affected by the disease, may reside in different regions not covered by these directories. Ability to filter clinics by services provided to children and/or adults is also lacking in all these initiatives despite being instrumental to identifying clinics able to implement more sophisticated screening programs such as the combination of universal early childhood screening and reverse cascade testing of adults relatives advocated by FH Europe.

Methods

Recently, Imperial College London has launched FindMyLipidClinic.com an online global directory of both lipid clinics and patient support groups drawn from the resources of the EAS Familial Hypercholesterolemia Study Collaboration (FHSC) and FH Europe, respectively. FindMyLipidClinic.com uses advanced mapping techniques to connect patients, their relatives and non-specialist clinicians with specialist clinicians and/or patient support groups so patients may receive the care they need to manage dyslipidemia in convenient locations (**Figure 1**).

FindMyLipidClinic.com is available in 29 languages increasing worldwide accessibility. The web application embeds innovative "Bing Maps" search and mapping functionalities that have kindly been made available to Imperial College London by Microsoft through a non-profit licence (**Figure 1**). FindMyLipidClinic.com also enable its users to filter specialist clinics according to the services offered to adults and children patients, which include but are not limited to cascade testing, genetic testing, genetic counselling, lipoprotein(a) [Lp(a)] testing, imaging, nutritional advice, and lipoprotein apheresis (**Table 1**). Patient support groups generally provide free services to patients including but not limited to sharing of information on diagnosis and management of their condition via webinars, helplines, and information leaflets. However, FindMyLipidClinic.com does not allow its users to filter patient support groups based on these services.

FindMyLipidClinic.com is a non-commercial initiative that is committed to respecting the privacy of its users. As a result, the web application does not store any personal information of its users and is compliant with the European General Data Protection Regulation (GDPR).

Results

FindMyLipidClinic.com is growing rapidly and currently contains 124 specialist lipid clinics across 39 countries most of which are part of the EAS FHSC global network of dyslipidemias specialists, and 29 patient advocacy groups across 27 countries mostly contributed by

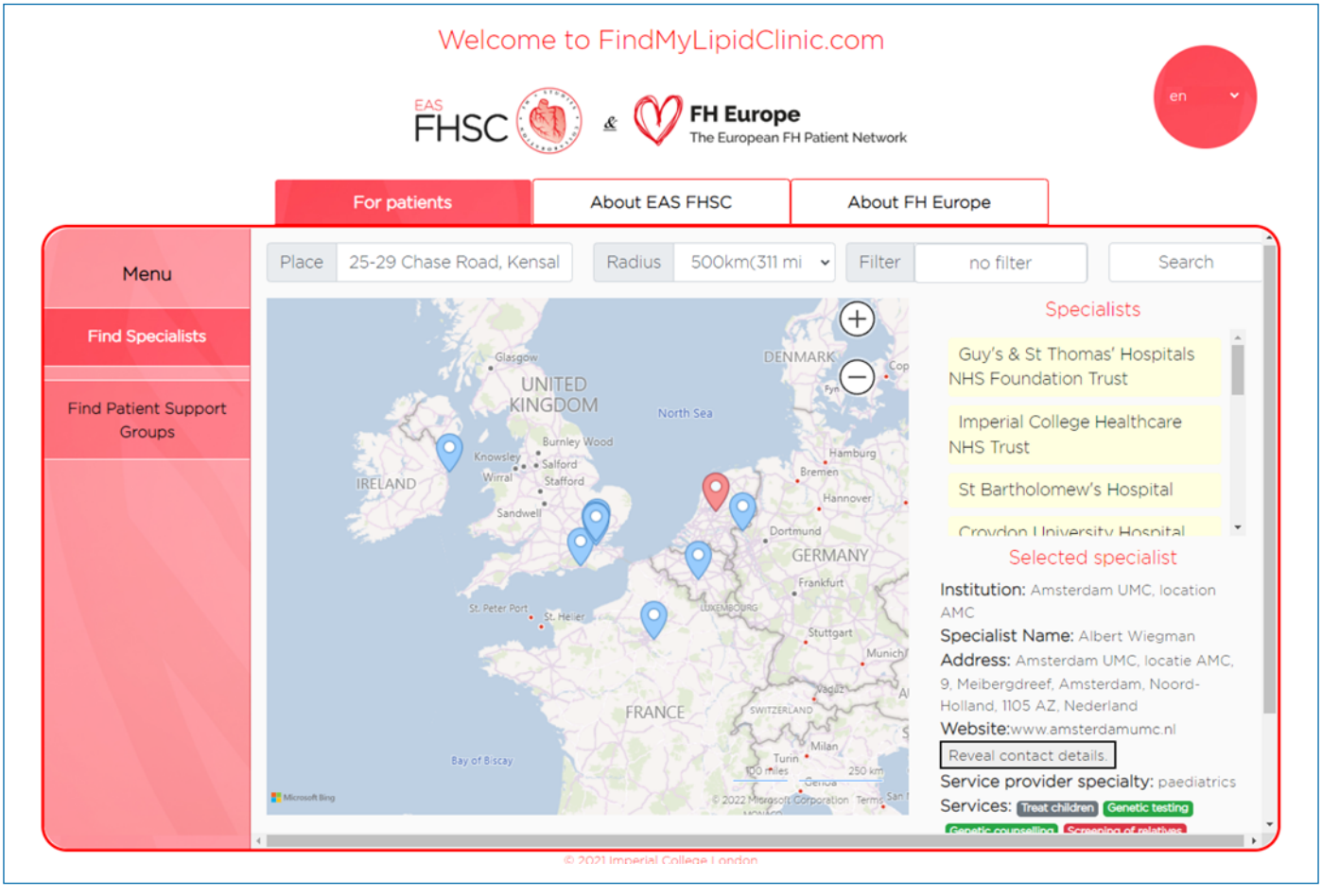


Figure 1 | Screenshot of FindMyLipidClinic.com's main page that allows its users to search for and filter clinics and to view the detailed information of a selected clinic.

Table 1 | Characteristics of clinics listed on FindMyLipidClinics.com.

Clinics Characteristics	Count and Proportion *n (%)*
Patients Treated	
Adults	108 (87%)
Children	58 (47%)
Screening, Testing & Counselling	
Cascade Screening	109 (88%)
Lp(a) Testing	88 (71%)
Genetic Testing	70 (56%)
Genetic Counselling	67 (54%)
Imaging	
Ultrasound	102 (82%)
Echography	97 (78%)
Computed Tomography (CT) scan	72 (58%)
Angiography	64 (52%)
Magnetic Resonance Imaging (MRI)	62 (50%)
Imaging	
Dietitian advice	75 (60%)
Apheresis on site	39 (31%)
Apheresis on referral	33 (27%)

The European FH Patient Network (FH Europe). The map in **Figure 2** shows the location of the clinics listed in the worldwide directory as well as countries where at least one known patient organisation is listed to date.

Over the last 18 months, 4,000 unique visitors of this directory have searched for lipid clinics and patient advocacy groups ~12,000 times across over 1,100 unique locations. Due to the infancy of this ongoing project many countries do not yet have either a lipid clinic or patient support group listed, even in areas such as the United States of America, Canada, Mexico, Australia where the number of potential users appears to be high (**Figure 2**).

Discussion

FindMyLipidClinic.com and other similar directories rely on a high volume of listings across as many world regions as possible to successfully guide patients to the specialist care required to manage their condition. Lipid specialists and patient organisations that are not already listed are therefore encouraged to list their clinics on FindMyLipidClinic.com. Similarly, administrators of other directories are invited to collaborate, where possible, by referring their listed clinics and patient organisations to our global directory. The simple steps outlined on https://findmylipidclinic.com/#/clinician-interface/listmyclinic describe how to take part in this initiative. Information/data about publicly available lipid clinics and patient support organisations on FindMyLipidClinic.com is legally limited to use via the website only and may not be shared otherwise.

Ongoing dissemination of this initiative relies on a Google Ad Grant campaign that displays the link to FindMyLipidClinic.com to users searching for dyslipidemia-related terms such as 'hypercholesterolemia', 'lipid' and 'lp(a)' and their combinations. Unfortunately the Google algorithm determines when the link is displayed to Google users and depends on many factors out of our control. The campaign is active in 29 languages and has no geographical limits. Yet, dissemination through Google alone is far from enough and we call

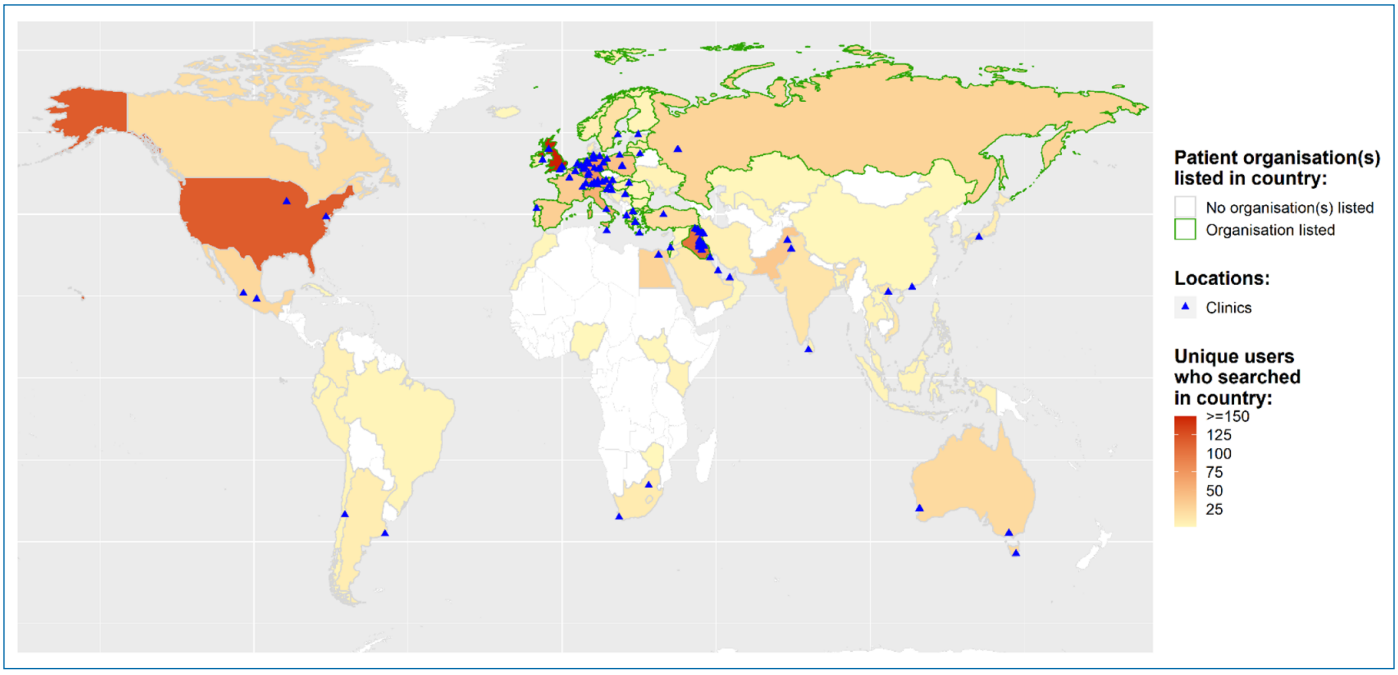


Figure 2 | Location of clinics and patient organisations listed on FindMyLipidClinic.com and number of searches by country.

on readers to disseminate the initiative within their network using their preferred means (e.g. social media, emails, meetings, and presentations).

FindMyLipidClinic.com has a number of practical applications which may include: (i) helping patients and non-specialist clinicians to find specialist clinicians who can diagnose and treat dyslipidemia, (ii) help non-specialist clinicians to find their foreign colleagues who can treat relatives of patients with genetic dyslipidemia living in another country, and (iii) helping patients and their family members to seek advice from their closest patient support groups on how to better identify and live with a dyslipidemia. By freely enabling these practical applications to connect patients, their relatives, non-specialist, and specialist clinicians, FindMyLipidClinic.com can contribute to improvements in the identification and management of dyslipidemia worldwide, encourage shared decision-making between people and the services that provide care, and thus diminish the inequality gap between geopolitical and healthcare settings.

There is also an opportunity to understand the accessibility and availability of lipid services per population size and enable policy-makers to allocate resources accordingly. The success of this initiative however depends on adequate and unbiased coverage of patient populations by lipid clinics and patient support groups, both in terms of geographical spread and in the provision of adequate services to identify, treat and support these patients. All lipid clinics, patient advocacy groups and administrators of local directories are invited to list their organisations and/or to disseminate this global initiative within their respective networks.

Acknowledgements

The authors would like to acknowledge the support of the EAS FHSC's and FH Europe's networks of collaborators, who tested and provided feedback on FindMyLipidClinic.com, and the contribution of Microsoft and Google for the provisions of a not-for-profit licence of the Bing Map mapping tool and for advertising credits on Google Ad Grants, respectively.

Kanika I. Dharmayat acknowledges support from a PhD Studentship from the National Institute for Health Research (NIHR) under the Applied Health Research (ARC) programme for Northwest London, United Kingdom (the views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health).

Antonio J. Vallejo-Vaz acknowledges support from the Programa Beatriz Galindo from the Ministry of Universities, Spain, and University of Sevilla, Spain.

Kausik K. Ray acknowledges support from the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre, UK.

Conflict of interest

Christophe A.T. Stevens reports grants from Pfizer, Amgen, Merck Sharp & Dohme, Sanofi-Aventis, Daiichi Sankyo, and Regeneron all outside the submitted work.

Alexander R.M. Lyons reports grants from Pfizer, Amgen, Merck Sharp & Dohme, Sanofi–Aventis, Daiichi Sankyo, and Regeneron all outside the submitted work.

Kanika I. Dharmayat reports grants from Daiichi-Sankyo, Amgen and Regeneron, and personal fees from Bayer and Regeneron; all outside the submitted work.

Julia Brandts reports a grant from AstraZeneca; and has received speaker honoraria from Amgen; all outside of the submitted work.

Antonio J. Vallejo-Vaz reports participation in grants to the European Atherosclerosis Society and Imperial College London from Pfizer, Amgen, Merck Sharp & Dohme, Sanofi–Aventis, Daiichi Sankyo, and Regeneron, during the conduct of the FHSC study; and personal fees for consulting from Bayer and Regeneron; and honoraria for lectures from Ferrer and Akcea, outside the submitted work.

Magdalena Daccord has no conflict of interest to disclose.

Kausik K. Ray reports grants and personal fees from Amgen, Sanofi–Regeneron, Pfizer, Merck Sharp & Dohme, and Daiichi Sankyo; and personal fees from AstraZeneca, The Medicines Company, Kowa, Novartis, Lilly, Algorithm, Boehringer Ingelheim, AbbVie, Silence Therapeutics, Bayer, Esperion, Abbott, New Amsterdam, and Resverlogix, outside the submitted work.

Source of funding

This Imperial College London led project is part of the EAS FHSC initiative supported by multiple investigator-initiated research grants from Pfizer [No: 16157823], Amgen, MSD, Sanofi-Aventis, Daiichi-Sankyo, and Regeneron.

Authorship and Author Contributions

Christophe A.T. Stevens, Alexander R.M. Lyons, Magdalena Daccord, and Kausik K. Ray conceived and wrote the article. All authors contributed to the design of the FindMyLipidClinic.com Directory, critically revised the manuscript, and gave their final approval.

References

1. Wong ND. Epidemiological studies of CHD and the evolution of preventive cardiology. Nat Rev Cardiol 2014; 11:276-89.
2. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Atherosclerosis 2019; 290:140-205.
3. Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of Familial Hypercholesterolemia Among the General Population and Patients With Atherosclerotic Cardiovascular Disease: A Systematic Review and Meta-Analysis. Circulation 2020; 141:1742-59.
4. Vallejo-Vaz AJ, Stevens CAT, Lyons ARM. EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Global perspective of familial hypercholesterolaemia: a cross-sectional study from the EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Lancet 2021; 398:1713-25.
5. Kerr M, Pears R, Miedzybrodzka Z, et al. Cost effectiveness of cascade testing for familial hypercholesterolaemia, based on data from familial hypercholesterolaemia services in the UK. Eur Heart J 2017; 38:1832-9.
6. Payne J, Williams S, Maxwell D, et al. Familial hypercholesterolaemia patient support groups and advocacy: A multinational perspective. Atherosclerosis 2018; 277:377-82.
7. Wilemon KA, Patel J, Aguilar-Salinas C, et al. Reducing the Clinical and Public Health Burden of Familial Hypercholesterolemia: A Global Call to Action. JAMA Cardiol 2020; 5:217-29.
8. The FH Foundation. Familial Hypercholesterolemia, FH. [accessed 16/11/2021]. Available from: www.thefhfoundation.org.
9. Fundación Hipercolesterolemia Familiar. Fundación Hipercolesterolemia Familiar. [accessed 16/11/2021]. Available from: https://www.colesterolfamiliar.org/.
10. Alieva AS, Tokgozoglu L, Ray KK, Catapano AL. Lipid Clinics Network. Rationale and design of the EAS global project. Atheroscler Suppl 2020; 42:e6-e8.

European Atherosclerosis Journal
www.eathj.org

EAJ 2022;2:41-47
https://doi.org/10.56095/eaj.v1i2.13

Metabolic impact of extrahepatic PCSK9 modulation

Lorenzo Da Dalt[1], **Fabrizia Bonacina**[1]

[1]*Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*

ABSTRACT

The Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) protease is a 692 amino acid glycoprotein which belongs to the proprotein convertase family. PCSK9 binds several receptors of the LDL family, including VLDLR and LRP1 but also CD36, driving their lysosomal degradation. Since the beginning of the 21st century a growing body of interest raised around the opportunity to pharmacologically inhibit PCSK9, and most recently, monoclonal antibodies have been successfully tested for the treatment of severe/genetic forms of dyslipidemia. Despite the majority of circulating PCSK9 being produced by the liver, other organs come into play contributing to its production, such as the heart, the pancreas, and the brain. Nonetheless, extrahepatic PCSK9 may exert a local/paracrine and/or autocrine metabolic impact in the homeostatic regulation of cholesterol metabolism, suggesting that, opposite to the liver, in other tissue PCSK9 deficiency or inhibition could contribute to the development of specific organ and tissue dysfunctions.

Keywords
PCSK9;
Pancreas;
Heart;
Liver and Brain

Received 21 July 2022; accepted 31 August 2022

Introduction

The most updated statistics of the American Heart Association (AHA) (1) and European Society of Cardiology (ESC) (2) still account for cardiovascular diseases (CVDs) as the principal cause of death in the United States and around 50% of all the deaths in the European countries in 2019/2020. CVDs arise from a plethora of risk factors, such as smoking, diabetes, and obesity but among them, hypercholesterolemia remains the principal cause of atherosclerotic cardiovascular events (3). Of note, hypercholesterolemia can be genetically determined or a consequence of unhealthy lifestyle and dietary habits. Among the genetic forms, familial hypercholesterolemia (FH), which affects 1:1.000.000 subjects in homozygosis and 1:200/250 in heterozygosis, is the most severe form of hypercholesterolemia associated with a high risk of CVD. FH diagnosis is made by the identification of mutations on *LDLR*, *APOB* or *PCSK9* genes that lead to increased circulating LDL cholesterol (LDL-C) levels from 200 mg/dL to 400 mg/dL. FH subjects present high levels of cholesterol from childhood thus magnifying their CVD risk; therefore, the currently available pharmacological treatments play a crucial role in the long-time reduction of cholesterol levels from a young age to prevent CVD events later in adulthood.

Since the eighties, statins have been considered the gold standard pharmacological treatment for all forms of hypercholesterolemia. Indeed, in large-scale clinical trials, statins, in combination with proper dietary habits and lifestyle corrections, have been shown to reduce cardiovascular (CV) morbidity and promote the regression of atherosclerotic plaque (4). However, for the most aggressive forms of hypercholesterolemia, the development of novel pharmacological treatments in combination with the highest tolerated dose of statins has been crucial as statins are ineffective for some categories of patients.

Among them, the proprotein convertase subtilisin/kexin type 9 (PCSK9) emerged as an interesting potential target, monoclonal antibodies against PCSK9 have rapidly risen in the clinical practice for treating subjects with hypercholesterolemia who do not reach the LDL-C levels recommended by the guidelines (5). PCSK9 became of interest from a cardiovascular point of view for the first time in 2003 (6) when two gain of function (GOF) mutations in the *PCSK9* gene were discovered in French families with autosomal dominant hypercholesterolemia. Later on, in 2005, loss of function (LOF) polymorphisms, responsible for two nonsense mutations (Y142X and C679X), were also identified. From a clinical point of view, PCSK9 LOF is associated with reduced LDL-C and protection from coronary artery disease (7), while PCSK9 GOF leads to the classical familial hypercholesterolemia phenotype (6). These discoveries speeded up the study of PCSK9 inhibition as a potential pharmacological strategy to reduce lipid burden and its possible side effects. Moreover, despite the liver being the most relevant production site of PCSK9, this protein is also produced at considerable levels by the brain, pancreas, heart, kid-

Corresponding Author
Lorenzo Da Dalt: lorenzo.dadalt@unimi.it

neys and also immune cells, and all these extrahepatic districts are therefore involved in the cardiovascular system regulation. Hence, a deep understanding of the extrahepatic role of PCSK9 would shed the light on the potential effects of its modulation in other tissue than the liver, unmasking side effects or innovative pharmacological routes (8).

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)

PCSK9 biology and regulation

PCSK9 is a serine protease with a molecular weight of 62 kDa. PCSK9 was discovered in 2003 as neural apoptosis-regulated convertase 1 (NARC-1) in the brain. PCSK9 belongs to a group of serine proteases that catalyse the hydrolysis of peptide bonds mediating the activation of target proteins. PCSK9 is initially synthesized as a 75 kDa proPCSK9 in the rough endoplasmic reticulum (RER) and is composed of a signal peptide (aa 1-30), a prodomain (aa 31-152), a catalytic domain (aa 153-404), a hinge region (aa 405-454), and the C-terminal domain (aa 452-692) rich in cysteine and histidine (8). The proPCSK9 is then driven to the smooth endoplasmic reticulum where it undergoes an autocatalytic cleavage that facilitates its folding and secretion. Despite this cut, the signal peptide remains attached with hydrogen bound to the catalytic domain of PCSK9 also favouring the proper protein folding (6). This newly formed interaction protects intracellular PCSK9 from protease degradation and allows its delivery/transport to the Golgi apparatus via coat protein complex (COPII) vesicle pathways. The C-terminal domain is crucial during this phase being the binding site between PCSK9 and COPII vesicles, indeed deletion on this part of the protein is associated with reduced PCSK9 release (9). Intracellularly PCSK9 is mainly inactive and needs to be released in the extracellular space to exert its proper function; however, more recent works have hypothesized an intracellular activity of PCSK9 in the endoplasmic reticulum (ER). PCSK9 starts its maturation in the ER where it is under the control of ER protein Glucose Regulated protein 94 (GRP94) which is actively involved in the regulation of PCSK9 and low-density lipoprotein receptor (LDLR) expression. GRP94 binding to the newly formed PCSK9 in the reticulum can prevent PCSK9-mediated LDLR degradation leading to increased ER stress (10, 11). Indeed, ER-retained PCSK9 bound to GRP94 is not able to interact with the LDLR before its secretion thus protecting the complex from degradation (11) (**Figure 1**). This has also been associated with major modification in circulating LDL cholesterol levels that in mice lacking GRP94 are significantly higher, as are the circulating levels of PCSK9. This observation, therefore, suggests a key intracellular function for PCSK9 before its secretion. Then, PCSK9 release from the cell requires its prior internalization into vesicles, a common mechanism shared with the secretion of LDLR. While PCSK9 is rapidly released in the extracellular matrix, LDLR remains attached to the cellular membrane (8).

Upon secretion, the catalytic domain of PCSK9 binds to the epidermal growth factor A (EGF-A) domain on the receptors belonging to the LDLR family, localized on the plasmatic membrane. Once bound to the EGF-A domain, the PCSK9-LDLR complex is internalized in clathrin-mediated endocytosis (12). The low pH characteristic of the late endosome strengthens the binding between PCSK9 and EGF-A and this strong interaction prevents the dissociation and the recycling of the receptor to the cell membrane. In this way, PCSK9 enhances LDLR lysosomal degradation within the cell cytoplasm preventing receptor recycling on the cell membrane (13). In turn, this leads to a reduced LDLR expression on the plasma membrane of hepatocytes with an increase in the plasmatic levels of LDL-C (14).

PCSK9 can also target other receptors, including other members of the LDLR family such as the very low-density lipoprotein receptor (VLDLR) and ApoER2 (15) but it can also interact with CD36 with a similar mechanism. Indeed, PCSK9 deletion associates with an increased expression of CD36 in the liver of experimental models leading to the accumulation of fatty acids and triglycerides within lipid droplets (16).

Given its role in cholesterol and fatty acid metabolism, PCSK9 expression is finely regulated both at the transcriptional and post-transcriptional levels by different metabolic actors.

Circulating PCSK9 is almost entirely produced and released by the liver and its production goes through several intracellular modulations in addition to the extracellular furin cleavage. Indeed, its expression is regulated at different levels and by several factors, including diurnal rhythm, hormones, diet, exercise, cholesterol levels, and hypocholesterolemic drugs. Among cholesterol-lowering drugs, statins, by inhibiting HMG-CoA reductase and reducing intracellular cholesterol levels, increase PCSK9 gene expression *in vitro* and *in vivo*. Patients treated with atorvastatin showed a 34% increase in PCSK9 plasmatic levels (17). Statins, by inhibiting HMG-CoA reductase and reducing cholesterol levels, promote the activation of the sterol-regulatory element-binding protein-2 (SREBP2) and SREBP-1c that bind the sterol regulatory elements (SRE) in PCSK9 and LDLR gene promoters and increase their expression (**Figure 1**). PCSK9 promoter contains also other sequences including the highly conserved

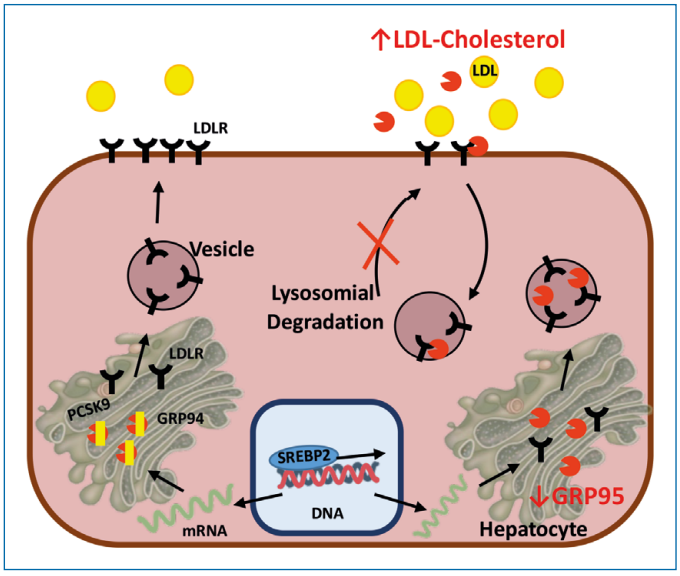


Figure 1 | **Intracellular PCSK9.** The liver is the principal organ involved in PCSK9 production. PCSK9 genetic expression is finely regulated by different transcription factors as SREBP2. PCSK9 is produced as Pre-ProPCSK9 and undergoes different post-transcriptional modification to the mature form. When the mature form reaches the endoplasmic reticulum PCSK9 is packed into vesicle with the LDLR for the release. In the presence of GRP94, PCSK9 is retained into the reticulum leading to an increased recycling of LDLR on the cell membrane. This situation is reverted in the absence of GRP94 and PCSK9 is packed in the same vesicle of the LDLR and can directly mediate vesicle degradation into the lysosome. This is therefore associated with a reduced expression of LDLR on hepatocyte membrane and high cholesterol levels.

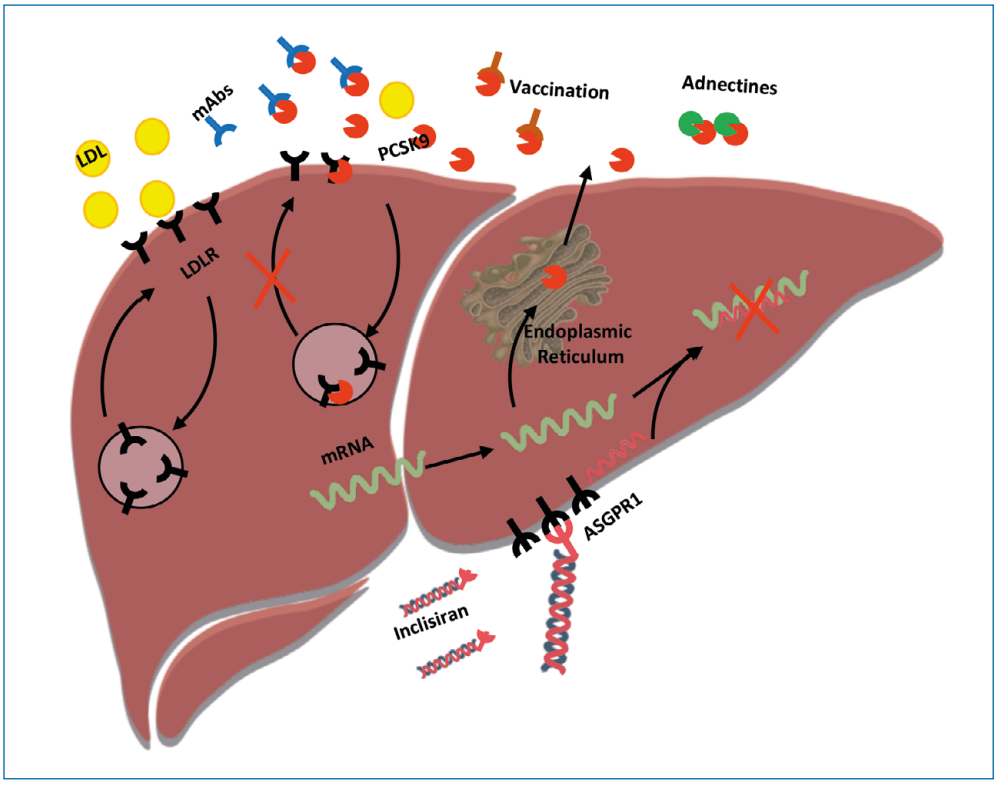


Figure 2 | **Pharmacological approaches for PCSK9 inhibition.** PCSK9 became a pharmacological target in 2015 when monoclonal antibodies have been approved for targeting circulating PCSK9. Circulating PCSK9 can also be targeted by immunization with vaccination that stimulate the production of autoantibodies against PCSK9. Adnectines mimic the 10th domain of fibronectin type III and can bind to circulating PCSK9 avoiding the binding with the LDLR. Other approaches are finalized to inhibit protein transcription of PCSK9 and Inclisiran, a siRNA, modified with three molecules of N-acetylgalactosamine (GalNAc) is selectively driven to the liver by the receptor for asialoglycoproteins (ASGPR).

hepatocyte nuclear factor 1 (HNF-1) and the binding site of transcription factor 1 (SP1) (18). Circulating mature PCSK9 is cleaved by furin producing a truncated protein of about 55 KDa that can still bind the EGF-A domain but is less able to bind the LDLR compared to the full form (19). PCSK9 can also circulate bound to the LDL particles, which contributes to maintaining its uncut form with the highest ability to bind its target (20).

PCSK9 pharmacological treatments

PCSK9 inhibition has been established as the gold treatment for high and very-high CV risk dyslipidemic patients, and many pharmacological approaches are still under development to target different PCSK9 forms.

Monoclonal antibodies (mAbs) targeting PCSK9 (alirocumab and evolocumab) represent the first and most commonly used approach in high-risk patients. Targeting circulating PCSK9 with mAbs was shown to reduce LDL-C, either as monotherapy or in combination with other lipid-lowering drugs, by approximately ~60%. In line with that, cholesterol reduction in subjects already treated with statins showed a reduction of 15% in the incidence of composite cardiovascular death and MI. More recently, a new drug named inclisiran that target PCSK9 hepatic production has been approved for the treatment of high and very high-risk patients. Inclisiran is a small interfering RNA (siRNA), optimized by the conjugation with three molecules of N-acetylgalactosamine (GalNAc) that selectively bind to the receptor for asialoglycoproteins (ASGPR) in the liver (21). Other pharmacological approaches that exploit others pathways include adnectins, synthetic proteins containing the 10th domain of fibronectin type III (22). Adnectins can bind PCSK9 inhibiting the interaction between PCSK9 and the EGF-A domain of LDLR, thus avoiding receptor degradation. Other pharmacological approaches include vaccination with peptide-based immunization that triggers B lymphocytes to produce anti-PCSK9 antibodies, and oral PCSK9 inhibitors (23). The pharmacological targeting of circulating PCSK9 is therefore one of the main therapeutic approaches to strongly reduce cholesterol levels by favouring LDL uptake in the liver and other organs (**Figure 2**). While great efforts have been made to inhibit hepatic PCSK9 production, many studies have been addressed to investigate the possible side effects in extrahepatic tissue metabolism and function.

Extrahepatic effects of PCSK9 inhibition

PCSK9 and the pancreas

Despite the effectiveness in reducing CVD risk, the use of statins has been associated with the development of new-onset diabetes; this has questioned whether other hypocholesterolemic pharmacological strategies, such as PCSK9 inhibitors, would affect pancreatic function and glucose metabolism modulation. This evidence rises the need to test the impact of PCSK9 inhibition on pancreatic function, focusing on endocrine pancreatic β-cells, those involved in insulin production. To deeply investigate the interconnection between PCSK9 inhibition and the risk of developing diabetes, clinical trials with monoclonal antibodies, meta-analysis and mendelian randomization studies have been performed. Despite clinical trials did not show any correlation, meta-analysis and mendelian randomization studies showed a positive correlation between genetic polymorphisms in genes involved in cholesterol metabolism, such as HMG-CoA reductase (confirming the involvement of statins in this pathway), NPC1L1, and PCSK9 with the risk of developing type 2 diabetes (24). For this reason, many studies have focused their attention on the metabolic impact of PCSK9, cholesterol, and lipids selectively in the pancreas. Indeed, lipid metabolism is crucial at the pancreatic level as lipotoxicity has been deeply connected with pancreatic ß-cell dys-

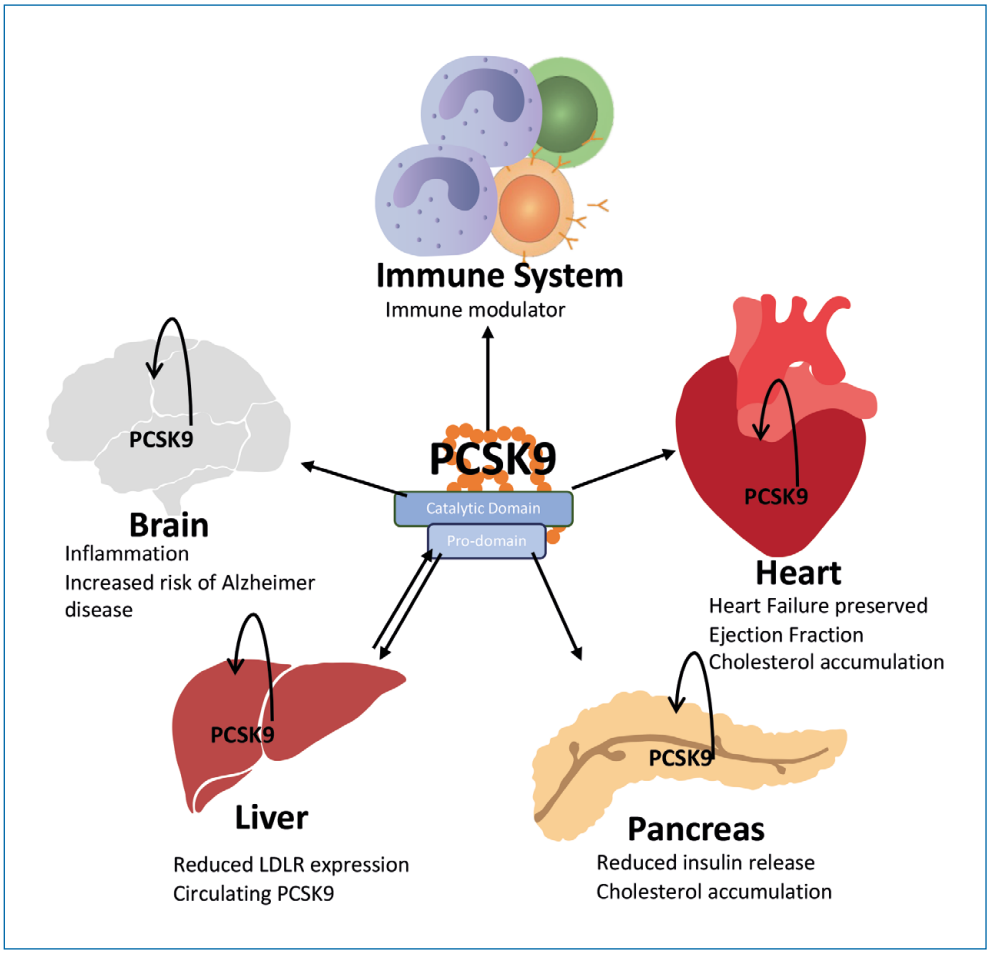


Figure 3 | **Extrahepatic role of PCSK9.** Circulating PCSK9 is mainly produced in the liver where it can modulate LDLR expression. PCSK9 is also produced by many other tissues such as the heart, brain, pancreas and macrophages, where can regulate lipid metabolism and inflammatory response. PCSK9 deficiency could therefore lead to cellular cholesterol accumulation in the pancreas, increasing the risk of diabetes, and in the heart leading to cardio-lypotoxicity and heart failure with preserved ejection fraction. In the brain, PCSK9 deficiency, by affecting apoptotic pathways, plays a role in the modulation of inflammation and the development of Alzheimer disease. PCSK9 has also direct and indirect immunomodulatory functions while PCSK9 inhibition can improve anti-cancer therapy.

function (25). Among lipids, cholesterol accumulation in different cell compartments may affect cellular flexibility leading to the impaired release of insulin granules by β-cells (26). In fact, alterations of the cellular tridimensional structure of β-cells, due to cholesterol accumulation, affect the regulation of membrane calcium channel and the transduction of vesicles containing insulin, with a consequent reduction in insulin release. To note, in transgenic animal models PCSK9 locally produced in the pancreas causes an increased expression of LDLR in pancreatic β-cells leading to cholesterol accumulation and reduced insulin trafficking and release (27). However, other studies have limited the impact of selective β-cell PCSK9 deficiency on glucose intolerance and diabetes development suggesting the possible role of PCSK9 released by other pancreatic islet subpopulations (**Figure 3**).

Cardiac impact of PCSK9 modulation

The heart is the tissue that requires the highest energy to perform continuous contraction. To overcome this need, aerobic metabolism plays a crucial role in the production of adenosine triphosphate (ATP). A healthy heart relies on glycolysis for almost 30% while the remaining part, 70%, is up to fatty acid oxidation (28). Unlike the liver, the heart is less prepared to synthesize fatty acids starting from glucose and amino acids, so the energetic requirement is mainly fed by lipids picked up from the bloodstream. In the heart lipid receptors including VLDLR, LRP1 and LDLR, are the main receptors involved in the uptake of triglyceride (TG)-rich lipoproteins that have been previously metabolized by the lipoprotein lipase (LPL). Scavenger receptors such as CD36 are also involved in the uptake of non-esterified fatty acids, mainly carried by albumin or released by lipoproteins following LPL activity (29, 30). Cardiac lipid metabolism is finely balanced between lipid uptake and mitochondrial oxidation to prevent excess lipid accumulation in the cardiomyocyte. The accumulation of lipids in the heart, and the consequent lipotoxicity, is associated with the development of cardiac dysfunction and heart failure in humans and experimental models. Therefore, these lipotoxic effects contribute to the development of cardiovascular metabolic complications, such as diabetes mellitus and metabolic syndrome. Heart failure (HF) in particular, is a pathological condition in which the heart is no longer able to pump enough blood to support the body's demands (31). More specifically, HF with preserved ejection fraction (HFpEF) is characterized by metabolic changes due to dysfunctional mitochondria that are unable to perform oxidative phosphorylation and generate enough ATP (32). Mitochondrial dysfunction is crucial for different diseases besides HFpEF including atrial fibrillation (33) and diabetic cardiomyopathy (34). In this context, through its ability to modulate LDLR and CD36 expression on the plasma membrane and therefore cellular lipid uptake, PCSK9 plays a key role in the modulation of cardiac function. It has been observed that the lack of PCSK9 in the heart is associated with an increased accumulation of cholesterol in the form of lipid drops and the consequent mitochondrial damage leads to energy depletion in the heart. As a consequence of these alterations, experi-

mental models lacking PCSK9 develop heart failure with preserved ejection fraction (35) (**Figure 3**).

PCSK9 and the central nervous system

PCSK9 was discovered in the brain as neural apoptosis-regulated convertase-1 (NARC-1) as its mRNA expression is mainly localized in the telencephalon neurons and is lower in a steady state but its expression is up-regulated during apoptosis (6). While the PCSK9-LDLR pathway is crucial all over the body, in the brain PCSK9 modulation mainly involves the ApoER2 receptor that promotes neural apoptosis through the increase in caspase activity (36). Indeed, cholesterol metabolism is complex in the brain and while all around the body cholesterol is mainly transported in LDL, in the brain it is transported in apoE-rich particles. PCSK9 has also been shown to promote apoptotic events through the JNK pathway and the activation of both extrinsic and intrinsic apoptotic pathways (37). Despite this activity, no signs of neurological effects have been reported in carriers of PCSK9 polymorphisms, while circulating PCSK9 – as well as LDL particles – cannot pass the intact blood-brain barrier in physiological conditions, limiting the impact of lipid-lowering therapies as well as pharmacological inhibition of PCSK9. PCSK9 in the brain is expressed mainly by proliferative cells in the adult brain and during the neurodevelopment of the telencephalon and cerebellum (38). As main the player in regulating lipid metabolism and apoptotic pathways, the role of PCSK9 has been investigated in different neuropathologies including Alzheimer's disease but the results are unclear (39). Mouse models of the disease show that PCSK9 may regulate Aβ clearance by controlling the expression of LRP1 and CD36. Concordantly, Pcsk9$^{-/-}$ mice show an upregulation of β-secretase-1 (BACE1) production, suggesting an increased production of β-amyloid (40). In line with this observation in the cerebrospinal fluid of human patients with Alzheimer's disease, higher PCSK9 levels have been reported compared to healthy subjects (39) (**Figure 3**).

Role of PCSK9 in inflammation and immunity

The inflammatory nature of atherosclerotic cardiovascular diseases and the recognition that immune responses are influenced by the modulation of systemic and cellular lipid metabolism has brought attention to the contribution of PCSK9 to the immune-inflammatory processes. Whether this effect is mediated directly by PCSK9 or through its modulation of systemic dyslipidemia is still questioned. Evidence coming from subjects affected by immune-inflammatory diseases suggests a link between PCSK9 and inflammation; indeed, patients with systemic lupus erythematosus (SLE) show an increase in circulating levels of PCSK9 that positively correlates with C-reactive protein (CRP) levels – a highly sensitive but unspecific marker of inflammation (41), while subjects infected with HIV present a positive correlation between the increased levels of PCSK9 and markers of monocyte activation (42). In line with this, also in patients with stable coronary artery disease, the elevation in plasmatic PCSK9 levels was positively correlated with those of CRP (43) and associated with disease severity (44). While the impairment of lipid metabolism, reported also under inflammatory conditions, might guide these effects, inhibition of circulating PCSK9 by a monoclonal antibody – despite dramatically reducing systemic cholesterol levels – had limited impact on systemic markers of inflammation (45), in contrast to statins that, in parallel to LDL-C, reduce plasmatic CRP levels (46); this evidence might suggest that different routes of pharmacological targeting of cholesterol metabolism would differently affect immune-inflammatory response.

Despite this clinical evidence, molecular studies have reported a direct pro-inflammatory effect of PCSK9 on cells typically associated with the atherosclerotic process, such as smooth muscle cells and macrophages. Vascular smooth muscle cells express PCSK9 that regulates LDLR expression in macrophages (47), an effect associated *in vitro* with the release of pro-inflammatory cytokines (48). Furthermore, PCSK9 expressed by bone-marrow-derived macrophages has been shown to accentuate vascular inflammation (49) independently on the modulation of cholesterol levels but instead involving the activation of the toll-like receptor 4 (TLR4)/NF-kB signalling pathway (50).

It is well documented that lipids, and in particular cholesterol, shape adaptive immune responses. Indeed, the polarization of CD4+ T-lymphocytes toward an activated phenotype (T-effector memory cells, TEM) is directly correlated to systemic cholesterol levels and the severity of coronary artery diseases in humans and has been confirmed in mouse models of atherosclerosis (51). To note, dyslipidemia triggers T cell proliferation and expansion of less functional immunosuppressive T regulatory cells (52) also by affecting the reactivity of antigen-presenting cells to prime lymphocytes (53-55). In line with this, immune system humanized mice, where hypercholesterolemia has been induced by PCSK9 overexpression in the liver, show a similar pro-inflammatory phenotype of T-cells (56). Altogether this evidence suggests that the increased levels of systemic cholesterol are more likely to mediate the polarization of adaptive immune response in the context of cardiovascular disease.

Different is the role played by cellular lipid metabolism on the activation of cell-mediated immune-inflammatory response, a field of investigation known as immunometabolism (57) that is particularly relevant in the context of cancer immunotherapy. It has been recently shown that PCSK9 inhibition potentiates checkpoint therapy for cancer by blocking the recycling of major histocompatibility protein class I (MHC I) proteins on the tumour cell surface, thus increasing its expression and promoting robust intratumoral infiltration of cytotoxic T-cells (58). Furthermore, PCSK9 inhibition in tumour cells directly enhances the activation of CD8+ T-cells by increasing the expression of LDLR that in this context favours the recycling of the T-cell receptor and its intracellular signalling. These findings broaden the role of PCSK9-mediated cholesterol metabolism in the modulation of T-cell response by increasing the expression of the LDLR; to note, LDLR has been recently involved in the immunometabolic response of CD8+ but not CD4+ T-cells (unpublished data), suggesting that the axis PCSK9 inhibition-LDLR expression could be directly targeted to selective T-cell subsets.

Conclusions

The gradual increase in the use of PCSK9 inhibitors in clinical practice raises interest in the possible side effects of circulating protein inhibition. Recent approaches of PCSK9 gene silencing partially exclude the possibility of side effects due to the hepatic selective targeting through ASGR1-mediated uptake. However, concerns are still made about extrahepatic modulation of lipid metabolism. Indeed, PCSK9 is also expressed in other tissues and the characterization of subjects with loss-of-function mutations of PCSK9 has allowed studying the effects of whole protein deficiency. For example, while the risk of developing type 2 diabetes has been reported in subjects with PCSK9, this has not been confirmed in patients treated with inhibitors of circulating PCSK9. This apparent discrepancy was explained by *in vivo* and *in vitro* studies that addressed the role of locally produced PCSK9 in different tissues including the pancreas, heart, and brain. Being PCSK9 crucial for apoptotic pathways modulation its role has been investigated in the field of immunity and cancer showing the possibility of PCSK9-mediated immune modulation. Even

though many works have been focused on investigating the extrahepatic impact of PCSK9 inhibition information is still incomplete and many questions are left unanswered.

Conflict of interest

All authors have no conflict of interest to disclose

Authors' contributions

All authors have made equal intellectual contributions to the writing of this manuscript. All authors read and approved the final manuscript.

Source of fundings

This project was partially self-founded and received fundings from Fondazione Cariplo 2019-1560 (FB)

Permission Information

The authors do hereby declare that all illustrations and figures in the manuscript are entirely original and do not require reprint permission.

References

1. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation. 2022; 145(8):e153-639.
2. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE, et al. European society of cardiology: Cardiovascular disease statistics 2019. Eur Heart J. 2020; 41(1):12-85.
3. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science (80-). 1986; 232(4746):34-47.
4. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170 000 participants in 26 randomised trials. Lancet. 2010; 376(9753):1670-81.
5. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Eur Heart J. 2020; 41(1):111-88.
6. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003; 34(2):154-6.
7. Norata GD, Garlaschelli K, Grigore L, Raselli S, Tramontana S, Meneghetti F, et al. Effects of PCSK9 variants on common carotid artery intima media thickness and relation to ApoE alleles. Atherosclerosis. 2010; 208(1):177-82.
8. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Bélanger Jasmin S, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A. 2003; 100(3):928-33.
9. Cunningham D, Danley DE, Geoghegan KF, Griffor MC, Hawkins JL, Subashi TA, et al. Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. Nat Struct Mol Biol. 2007; 14(5):413-9.
10. Poirier S, Mamarbachi M, Chen WT, Lee ASS, Mayer G. GRP94 Regulates Circulating Cholesterol Levels through Blockade of PCSK9-Induced LDLR Degradation. Cell Rep. 2015; 13(10):2064-71.
11. Lebeau P, Platko K, Al-Hashimi AA, Byun JH, Lhoták Š, Holzapfel N, et al. Loss-of-function PCSK9 mutants evade the unfolded protein response sensor GRP78 and fail to induce endoplasmic reticulum stress when retained. J Biol Chem. 2018; 293(19):7329-43.
12. Poirier S, Mayer G, Poupon V, McPherson PS, Desjardins R, Ly K, et al. Dissection of the endogenous cellular pathways of PCSK9-induced low density Lipoprotein receptor degradation. Evidence for an intracellular route. J Biol Chem. 2009; 284(42):28856-64.
13. Wang Y, Huang Y, Hobbs HH, Cohen JC. Molecular characterization of proprotein convertase subtilisin/kexin type 9-mediated degradation of the LDLR. J Lipid Res. 2012; 53(9):1932-43.
14. Lipari MT, Li W, Moran P, Kong-Beltran M, Sai T, Lai J, et al. Furin-cleaved proprotein convertase subtilisin/kexin type 9 (PCSK9) is active and modulates low density lipoprotein receptor and serum cholesterol levels. J Biol Chem. 2012 Dec; 287(52):43482-91.
15. Poirier S, Mayer G, Benjannet S, Bergeron E, Marcinkiewicz J, Nassoury N, et al. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J Biol Chem. 2008.
16. Demers A, Samami S, Lauzier B, Des Rosiers C, Sock ETN, Ong H, et al. PCSK9 Induces CD36 Degradation and Affects Long-Chain Fatty Acid Uptake and Triglyceride Metabolism in Adipocytes and in Mouse Liver. Arterioscler Thromb Vasc Biol. 2015.
17. Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. J Lipid Res. 2008; 49(2):394-8.
18. Dong B, Singh AB, Shende VR, Liu J. Hepatic HNF1 transcription factors control the induction of PCSK9 mediated by rosuvastatin in normolipidemic hamsters. Int J Mol Med. 2017 Mar; 39(3):749-56.
19. Benjannet S, Rhainds D, Hamelin J, Nassoury N, Seidah NG. The proprotein convertase (PC) PCSK9 is inactivated by furin and/or PC5/6A: Functional consequences of natural mutations and post-translational modifications. J Biol Chem. 2006; 281(41):30561-72.
20. Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. J Biol Chem. 2013 Mar; 288(12):8279-88.
21. Peters DT, Henderson CA, Warren CR, Friesen M, Xia F, Becker CE, et al. Asialoglycoprotein receptor 1 is a specific cell-surface marker for isolating hepatocytes derived from human pluripotent stem cells. Dev. 2016; 143(9):1475-81.
22. Chandler PG, Buckle AM. Development and Differentiation in Monobodies Based on the Fibronectin Type 3 Domain. Cells. 2020; 9(3).
23. Catapano AL, Pirillo A, Norata GD. New Pharmacological Approaches to Target PCSK9. Curr Atheroscler Rep. 2020; 22(7).
24. Schmidt AF, Swerdlow DI, Holmes M V., Patel RS, Fairhurst-Hunter Z, Lyall DM, et al. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. Lancet Diabetes Endocrinol. 2017; 5(2):97-105.
25. Ye R, Onodera T, Scherer PE. Lipotoxicity and B cell maintenance in obesity and type 2 diabetes. J Endocr Soc. 2019; 3(3):617-31.
26. Perego C, Da Dalt L, Pirillo A, Galli A, Catapano AL, Norata

GD. Cholesterol metabolism, pancreatic β-cell function and diabetes. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2019.
27. Da Dalt L, Ruscica M, Bonacina F, Balzarotti G, Dhyani A, Di Cairano E, et al. PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: The role of the low-density lipoprotein receptor. Eur Heart J. 2019; 40(4):357-68.
28. Schulze PC, Drosatos K, Goldberg IJ. Lipid use and misuse by the heart. Circ Res. 2016.
29. Bharadwaj KG, Hiyama Y, Hu Y, Huggins LA, Ramakrishnan R, Abumrad NA, et al. Chylomicron- and VLDL-derived lipids enter the heart through different pathways: In vivo evidence for receptor- and non-receptor-mediated fatty acid uptake. J Biol Chem. 2010.
30. Carley AN, Bi J, Wang X, Banke NH, Dyck JRB, O'Donnell JM, et al. Multiphasic triacylglycerol dynamics in the intact heart during acute in vivo overexpression of CD36. J Lipid Res. 2013 Jan; 54(1):97-106.
31. Schwinger RHG. Pathophysiology of heart failure. Cardiovasc Diagn Ther. 2021; 11(1).
32. Kumar AA, Kelly DP, Chirinos JA. Mitochondrial Dysfunction in Heart Failure with Preserved Ejection Fraction. Circulation. 2019; 139(11):1435-50.
33. Pool L, Wijdeveld LFJM, de Groot NMS, Brundel BJJM. The role of mitochondrial dysfunction in atrial fibrillation: Translation to druggable target and biomarker discovery. Int J Mol Sci. 2021; 22(16).
34. Jubaidi FF, Zainalabidin S, Mariappan V, Budin SB. Mitochondrial dysfunction in diabetic cardiomyopathy: The possible therapeutic roles of phenolic acids. Int J Mol Sci. 2020; 21(17):1-24.
35. Da Dalt L, Castiglioni L, Baragetti A, Audano M, Svecla M, Bonacina F, et al. PCSK9 deficiency rewires heart metabolism and drives heart failure with preserved ejection fraction. Eur Heart J. 2021 Aug; 42(32):3078-90.
36. WU Q, TANG Z-H, PENG J, LIAO L, PAN L-H, WU C-Y, et al. The dual behavior of PCSK9 in the regulation of apoptosis is crucial in Alzheimer's disease progression (Review). Biomed Reports. 2014;2(2):167-71.
37. Kysenius K, Muggalla P, Mätlik K, Arumäe U, Huttunen HJ. PCSK9 regulates neuronal apoptosis by adjusting ApoER2 levels and signaling. Cell Mol Life Sci. 2012 Jun; 69(11):1903-16.
38. Seidah NG, Mayer G, Zaid A, Rousselet E, Nassoury N, Poirier S, et al. The activation and physiological functions of the proprotein convertases. Int J Biochem Cell Biol. 2008; 40(6-7):1111-25.
39. Adorni MP, Ruscica M, Ferri N, Bernini F, Zimetti F. Proprotein Convertase Subtilisin/Kexin Type 9, Brain Cholesterol Homeostasis and Potential Implication for Alzheimer's Disease. Front Aging Neurosci. 2019; 11:120.
40. Jonas MC, Costantini C, Puglielli L. PCSK9 is required for the disposal of non-acetylated intermediates of the nascent membrane protein BACE1. EMBO Rep. 2008; 9(9):916-22.
41. Fang C, Luo T, Lin L. Elevation of serum proprotein convertase subtilisin/kexin type 9 (PCSK9) concentrations and its possible atherogenic role in patients with systemic lupus erythematosus. Ann Transl Med. 2018; 6(23):452-452.
42. Zanni M V., Stone LA, Toribio M, Rimmelin DE, Robinson J, Burdo TH, et al. Proprotein Convertase Subtilisin/Kexin 9 Levels in Relation to Systemic Immune Activation and Subclinical Coronary Plaque in HIV. Open Forum Infect Dis. 2017; 4(4).
43. Zhang Y, Zhu CG, Xu RX, Li S, Guo YL, Sun J, et al. Relation of circulating PCSK9 concentration to fibrinogen in patients with stable coronary artery disease. J Clin Lipidol. 2014;8(5):494-500.
44. Li S, Zhang Y, Xu RX, Guo YL, Zhu CG, Wu NQ, et al. Proprotein convertase subtilisin-kexin type 9 as a biomarker for the severity of coronary artery disease. Ann Med. 2015; 47(5):386-93.
45. Bohula EA, Giugliano RP, Leiter LA, Verma S, Park JG, Sever PS, et al. Inflammatory and cholesterol risk in the FOURIER trial. Circulation. 2018; 138(2):131-40.
46. Clearfield M. C-reactive protein levels and outcomes after statin therapy. Curr Atheroscler Rep. 2006; 8(1):8-9.
47. Ferri N, Tibolla G, Pirillo A, Cipollone F, Mezzetti A, Pacia S, et al. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. Atherosclerosis. 2012; 220(2):381-6.
48. Ricci C, Ruscica M, Camera M, Rossetti L, MacChi C, Colciago A, et al. PCSK9 induces a pro-inflammatory response in macrophages. Sci Rep. 2018; 8(1).
49. Giunzioni I, Tavori H, Covarrubias R, Major AS, Ding L, Zhang Y, et al. Local effects of human PCSK9 on the atherosclerotic lesion. J Pathol. 2016; 238(1):52-62.
50. Tang ZH, Peng J, Ren Z, Yang J, Li TT, Li TH, et al. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. Atherosclerosis. 2017; 262:113-22.
51. Ammirati E, Cianflone D, Vecchio V, Banfi M, Vermi AC, De Metrio M, et al. Effector memory T cells are associated with atherosclerosis in humans and animal models. J Am Heart Assoc. 2012; 1(1):27-41.
52. Mailer RKW, Gisterå A, Polyzos KA, Ketelhuth DFJ, Hansson GK. Hypercholesterolemia Enhances T Cell Receptor Signaling and Increases the Regulatory T Cell Population. Sci Rep. 2017; 7(1).
53. Westerterp M, Gautier EL, Ganda A, Molusky MM, Wang W, Fotakis P, et al. Cholesterol Accumulation in Dendritic Cells Links the Inflammasome to Acquired Immunity. Cell Metab. 2017; 25(6):1294-1304.e6.
54. Ito A, Hong C, Oka K, Salazar J V., Diehl C, Witztum JL, et al. Cholesterol Accumulation in CD11c+ Immune Cells Is a Causal and Targetable Factor in Autoimmune Disease. Immunity. 2016; 45(6):1311-26.
55. Bonacina F, Coe D, Wang G, Longhi MP, Baragetti A, Moregola A, et al. Myeloid apolipoprotein E controls dendritic cell antigen presentation and T cell activation. Nat Commun. 2018 Aug 6.
56. Li Y, Cam J, Bu G. Low-density lipoprotein receptor family: endocytosis and signal transduction. Mol Neurobiol. 2001 Feb; 23(1):53-67.
57. Norata GD, Caligiuri G, Chavakis T, Matarese G, Netea MG, Nicoletti A, et al. The Cellular and Molecular Basis of Translational Immunometabolism. Immunity. 2015; 43(3):421-34.
58. Liu X, Bao X, Hu M, Chang H, Jiao M, Cheng J, et al. Inhibition of PCSK9 potentiates immune checkpoint therapy for cancer. Nature. 2020; 588(7839):693-8.

European Atherosclerosis Journal
www.eathj.org

EAJ 2022;2:48-53
https://doi.org/10.56095/eaj.v1i1.14

European, Russian, and American Clinical Guidelines on dyslipidemia management – Where do we stand?

Alieva Asiiat, Usova Elena, Reutova Olga

Almazov National Medical Research Centre, 197341 Saint Petersburg, Russia

ABSTRACT

Current clinical guidelines on lipid metabolism disorders are represented by the integration of relevant multicenter observational studies and registries aimed to identify the best strategies in cardiovascular risk stratification, diagnosis, and treatment of dyslipidemias. The approaches outlined in the European, Russian, and American guidelines look relevant to each other despite a range of slightly different postulates, as they all demonstrate a general tendency to the importance of accurate risk stratification of patients and timely action on low-density lipoprotein cholesterol (LDL-C) levels by using effective lipid-lowering therapies.

Keywords
Dyslipidemias;
Clinical guidelines;
Cardiovascular risk;
Dyslipidemia treatment

Received 9 August 2022; accepted 31 August 2022

Introduction

It is well known that cardiovascular diseases (CVDs) are one of the main causes of mortality and disability in all countries across the world. The development of CVDs is associated with both modifiable and unmodifiable risk factors, among which dyslipidaemia plays an essential role. There are specialized documents containing structured information based on scientific evidence on the prevention, diagnosis, and treatment of dyslipidemias, known as clinical guidelines. Many countries stick to the strategy of publishing national guidelines for the management of dyslipidemias, which may reflect some features conditioned by the healthcare system type and ethnic and geographic particularities, but share general concepts. The purpose of this paper is to summarize the key positions of European [1], Russian [2], and American [3] guidelines for the management of patients with dyslipidemias.

Cardiovascular risk stratification and target levels

Risk-based prediction is of strategic importance for choosing the best option for CVDs prevention. There are various charts for calculating cardiovascular risk. Physicians use the systemic coronary risk evaluation (SCORE) chart in Europe and Russia and the ACC/AHA ASCVD Risk Estimator Plus in America.

The ACC/AHA ASCVD Risk Estimator Plus includes a fairly large pool of measures such as sex, age, race, smoking, systolic and diastolic blood pressure, total serum cholesterol, LDL-C and HDL-C, the presence of diabetes mellitus, and information on ongoing therapies (particularly antihypertensive drugs, statins, and aspirin). According to the obtained index, we can define a group of people with low (<5%), borderline (5-7.4%), intermediate (7.5-19.9%), or high (≥20%) 10-year cardiovascular risk. In Europe and Russia, the SCORE chart is generally used, which takes into account five main indicators: sex, age, smoking, systolic blood pressure, and total serum cholesterol, allowing to identify people with low (<1%), moderate (1-5%), high (5-10%), or very high (>10%) 10-year cardiovascular risk. It is worth noting that the SCORE chart undergoes significant changes with each update of the clinical guidelines. Key changes in the most recent guidelines led to introduce an additional age group (from 65 to 70 years) and a decrease in the borderline total cholesterol level from 8 to 7 mmol/L, which is important to improve the screening of patients with clinical suspicion of heterozygous familial hypercholesterolemia. Two new cardiovascular risk charts - SCORE2 (for patients aged 40-69 years) and SCORE2-OP (for patients aged 70 years and older) have been introduced in the current European guidelines for the prevention of CVDs in clinical practice (**Figure 1**) [4].

Corresponding Author
Usova Elena: el.lenkin@yandex.ru

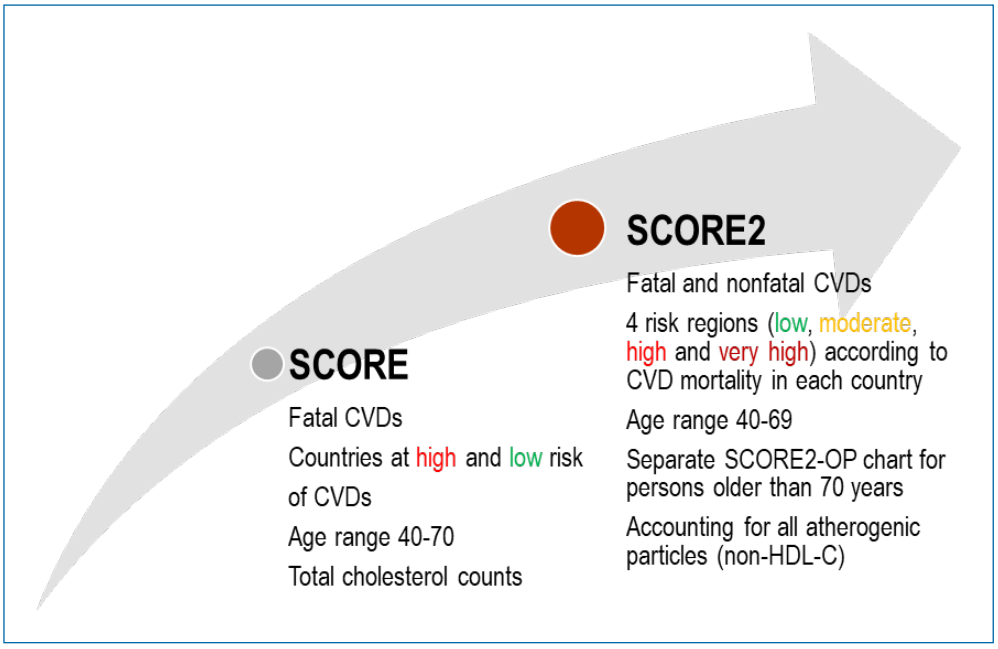


Figure 1 | **The main differences in the risk prevention approach.**

The most significant changes have affected the following items:

1) taking into account regional characteristics (in the SCORE chart, countries were divided into low-risk and high-risk categories only, in the updated charts SCORE2 and SCORE2-OP countries are divided into 4 risk zones: low, moderate, high, and very-high risk);
2) an assessment of the 10-year risk of aggregate fatal and nonfatal adverse outcomes (in the SCORE chart, an additional calculation was required to assess the risk of nonfatal cardiovascular events by multiplying the resulting index by 3 in men, and by 4 in women);
3) a paradigm shift has been implemented with regard to non-high-density lipoprotein cholesterol (non-HDL-C) levels instead of total serum cholesterol levels. In addition, the population was divided into three age groups, indicating the peculiarities of the primary prevention of CVDs. Reasonably, the level of risk by SCORE2 exceeds that obtained with SCORE for the same patient, as the first chart reflects the risk of fatal and non-fatal events while the second one considers only fatal events, unless not using a special index for risk recalculation to include both types of events. Definitely, there is a need for a discussion on how to standardize risk assessment procedures across all future guidelines to streamline work in clinical practice.

In 2018, the American College of Cardiology (ACC) and the American Heart Association (AHA) published guidelines on blood cholesterol control [3]. The updated guideline provides definitions of high- and very high-risk atherosclerotic CVDs with the corresponding recommended LDL-C levels. In 2019, the European Guidelines for the correction of lipid metabolism disorders were published [1] by the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Compared with 2016 guidelines, there are still 4 categories of cardiovascular risk (all with lower LDL-C goals, except for individuals at low risk), but the concept of extreme risk was introduced, for patients with ASCVD who experience a second vascular event within 2 years (not necessarily the same as the first one) despite the background of the maximum tolerated dose of statin, and thus who may be recommended to reduce LDL-C levels <1.0 mmol/L (<40 mg/dL).

According to the VII revision of the Russian clinical guidelines on diagnostics and correction of lipid metabolism disorders to prevent and treat atherosclerosis [2], published in 2020, five categories of cardiovascular risk were identified: extreme (target level of LDL-C <1.0 mmol/L), very high (target level of LDL-C <1.4 mmol/L), high (target level of LDL-C <1.8 mmol/L), moderate (target level of LDL-C <2.6 mmol/L), and low CV risk (target level of LDL-C <3.0 mmol/L). Extreme cardiovascular risk refers to the combination of atherosclerotic CVD with type 2 diabetes mellitus (DM) or familial hypercholesterolemia plus two cardiovascular complications within 2 years despite an optimal lipid-lowering therapy and/or achieved LDL-C levels <1.4 mmol/L. This model likely derives from the clinical practice guidelines of the American Association of Clinical Endocrinologists/American College of Endocrinology, in which an extreme cardiovascular risk group appeared for the first time [5]. Despite variations in the categorization of this risk group, key strategies are similar, since both European and Russian guidelines recommend an LDL-C goal of less than 1.0 mmol/L (<40 mg/dL).

Imaging techniques as a tool for the improvement of cardiovascular risk assessment

An essential place in the clinical guidelines is given to additional methods of examination, allowing a more complete assessment of cardiovascular risk, since the precise estimation of a patient's cardiovascular risk is crucial for choosing an optimal prevention and treatment strategy, including timeline and type of intervention. Current guidelines refer to the use of non-invasive methods for subclinical atherosclerosis assessment in case of borderline cardiovascular risk to identify a risk group more precisely, i.e. to choose an optimal intervention strategy.

The importance of subclinical atherosclerosis diagnosis is conditioned by the absence of any clinical symptoms over a long period, however, it reflects the level of the atherosclerosis progression, and thus, very often reveals a necessity for intervention. Several non-invasive diagnostic tools are available, among which the most popular and accessible are duplex scanning of peripheral arteries, quantita-

tive assessment of coronary calcium index by Agatston, and coronary computed tomography angiography.

All clinical guidelines provide a comprehensive commentary on the choice of diagnostic approaches within specific chapters. The American Clinical Guidelines were the first that included coronary calcium testing as a risk assessment tool and paid special attention to a detailed algorithm to select candidates for the procedure, including cost-effectiveness data and its availability across the country. As for the duplex scanning of peripheral arteries, the intima-media thickness (IMT) measurement loses traction from a point of view of its predictive value [6], while atherosclerotic plaque presence is considered an important tool that may increase the level of cardiovascular risk in both European and Russian guidelines. Compared to IMT, atherosclerotic plaques have a stronger association with the development of cardiovascular events in patients without a previous history of coronary artery disease [7] and are stronger predictors of increased risk of major cardiovascular adverse event recurrence in a cohort of patients with a history of CVDs [8]. Reclassification is of value in people identified as being at moderate CV risk by using markers such as CAC score > 100 Agatston units, ankle-brachial index (ABI) < 0.9 or > 1.40, carotid-femoral pulse wave velocity > 10 m/s, or the presence of plaques at carotid or femoral ultrasonography based on the European guidelines. Russian guidelines suggest stratifying the risk of patients based on the percentage of stenosis due to the presence of atherosclerotic plaque, i.e. 25-49% and >50% for high and very-high risk, respectively. The CAC score index classifies patients as being at low (0), moderate (1-10), high (11-100), high/very high (101-400), or very high (>400) risk, based on the number of Agatston units. The ability to use the above-described reclassifiers can provide a more accurate evaluation of the cardiovascular risk category and perform the necessary timely interventions.

Dyslipidemia management

Lifestyle modification comprises the correction of key modifiable risk factors across all the guidelines.

Among drugs for the treatment of dyslipidaemias, statin therapy remains the first-line approach for reducing LDL-C levels. Data collected from large randomized clinical trials of statins (JUPITER (rosuvastatin 20 mg) [9], WOSCOPS (pravastatin 20-40 mg) [10], 4S (simvastatin 20-40 mg) [11], LIPID (pravastatin 40 mg) [12], HPS (simvastatin 40 mg) [13], ASCOT-LLA (atorvastatin 10 mg) [14], CARDS (atorvastatin 10 mg) [15], PROVE IT-TIMI 22 (pravastatin 40 mg and atorvastatin 80 mg [16] and TNT (atorvastatin 80 mg) [17]) have demonstrated a significant reduction in cardiovascular and overall mortality regardless of sex, age, and baseline total cholesterol level. In both European and Russian guidelines, algorithms for the management of lipid metabolism disorders include also cholesterol absorption inhibitors (ezetimibe) (SHARP [18]), fibrates (FIELD [19], Accord [20]), omega-3 PUFAs (GISSI-Prevenzione [21]), and PCSK9 inhibitors (FOURIER [22], ODYSSEY Outcomes [23]). Bile acid sequestrants and nicotinic acid (Coronary Drug Project [24], Stockholm Ischemia Heart Study [25], HDL Atherosclerosis Treatment Study [26]) are indicated in the European and American clinical guidelines, but not in Russian guidelines as these drugs are not currently registered and cannot be used for the treatment of patients with dyslipidemia.

To overcome the problem of achieving recommended LDL-C goals in patients with high pre-treatment levels of LDL-C, the option of using combination therapy is provided by all guidelines, including a moderate or high-intensity regimen of statin therapy combined with ezetimibe and/or a proprotein convertase subtilisin/kexin type 9 inhibitor. The very recent European consensus papers suggest considering a combination therapy as a first-line strategy in very-high risk patients [27, 28] to obtain a higher reduction in LDL-C levels in a shorter time, thus reducing the cumulative cardiovascular risk of these patients.

New approaches for lipid-lowering therapy are being actively introduced. Recently, the first and the only one in the class of lipid-lowering drugs based on small interfering RNA (siRNA) - Inclisiran - was registered in Europe, Russian Federation, and the USA. The registration was based on the results of phase III clinical trials ORION-9, -10 and -11 [29, 30]. Having in a toolbox an innovative drug for the treatment of dyslipidaemia that can be administered twice a year might allow to achieve and maintain LDL-C goals and rise patient compliance.

Conclusion

Despite some discrepancies in the European, Russian and American clinical guidelines, globally, there is a unidirectional vision in the strategy of dyslipidemia management (**Table 1**). The proper use in clinical practice can contribute to earlier detection of patients with lipid metabolism disorders, timely action on atherogenic lipid fractions, and prevention of CVDs and mortality.

Perspectives

There is a range of perspectives on dyslipidemia management related to new risk evaluation strategies and treatment options.

The approach of cardiovascular risk assessment and management based not only on the current 10-year risk scores but also on the concept of cumulative LDL-C burden, deriving from Mendelian randomization studies [31], might be considered as the tool for estimating the magnitude of the clinical benefit that can be achieved by maintaining recommended LDL-C goals and identifying the most effective timing for intervention.

Furthermore, several drugs targeting different lipid fractions, such as LDL-C, triglycerides, and lipoprotein (a) [Lp(a)] have been registered recently or are in phase III clinical trials, which seem very promising for the improvement of lipid disorder management in the near future [32]. Changing the treatment trajectory of atherosclerotic CVDs by reducing LDL-C levels is now possible with bempedoic acid, inclisiran, evinacumab, and the possibility of considering vaccines and genome editing is currently under discussion. Bempedoic acid inhibits the enzyme ATP-citrate lyase, which catalyses the synthesis of a precursor of cholesterol. Several studies have shown its favourable effect in lowering LDL-C and a satisfactory safety profile [33-35]. Inclisiran inhibits PCSK9 protein synthesis by RNA interference. This leads to increased uptake of circulating LDL-C by hepatic receptors and a decrease of LDL-C concentration in blood. The safety of inclisiran and its impact on the prognosis continues to be investigated as part of the ORION clinical research program. The development and research of the monoclonal antibody evinacumab, which specifically binds angiopoietin-like protein 3 (ANGPTL3), an inhibitor of lipoprotein lipase and endothelial lipase, thus reducing levels not only of LDL-C but also triglycerides. The main pool of studies on the efficacy and safety of this drug is concentrated in the group of patients with homozygous hypercholesterolemia and has already demonstrated significant results in reducing atherogenic lipid levels [36, 37]. Developing vaccines and genome editing technologies and investigating their clinical potential might be a promising step in the treatment strategy for patients with dyslipidemias, as confirmed by several studies [38-40].

Special attention to other atherosclerotic targets and reconsideration of the causal role of triglyceride-rich lipoproteins has ensured the de-

Table 1 | Key tools used for the risk assessment and dyslipidemias treatment according to European, Russian, and American clinical guidelines

Parameter	ESC/EAS for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk 2019	Russian guidelines on diagnosis and correction of lipid metabolism disorders in order to prevent and treat atherosclerosis, 2020, VII revision	American guidelines on the Management of Blood Cholesterol 2018 AHA/ACC/AACVPR/AAPA/ ABC/ACPM/ADA/AGS/ APhA/ASPC/NLA/PCNA
CVD risk assessment	SCORE Cardiovascular Risk Chart Sex Age 40-70 years SPB 120-180 mmHg Total cholesterol 4-7 mmol/L	SCORE Cardiovascular Risk Chart Sex Age 45-70 years SPB 120-180 mmHg Total cholesterol 4-7 mmol/L	ACC/AHA ASCVD Risk Estimator Plus Sex Age 20-79 years Race Total cholesterol 130-320 mg/dL HDL cholesterol 20-100 mg/dL LDL cholesterol 30-300 mg/dL SPB 90-200 mmHg DPB 30-140 mmHg Personal history (diabetes, smoking, treatment for hypertension, statin, aspirin therapy)
Imaging in the assessment of cardiovascular risk	Peripheral arterial duplex scan Calcium score CT angiography Carotid–femoral pulse wave velocity	Peripheral arterial duplex scan Calcium score CT angiography	Peripheral arterial duplex scan Calcium score CT angiography
Cardiovascular risk categories	4 risk categories: very high, high, moderate and low However, there is a group of patients who had a second vascular event within 2 years (not necessarily the same as the first) on a maximum tolerated dose of statin	5 risk categories: extreme, very high, high, moderate and low	4 risk categories: very high, high, moderate and low However, 5 risk groups based on the guidelines of the American Association of Clinical Endocrinologists Also, for clinical decision-making in adults of different races/ethnicities, it is reasonable for clinicians to review race/ethnic features that can influence ASCVD risk to adjust the choice of statin or intensity of treatment
Target LDL-C levels based on the level of cardiovascular risk	For patients with ASCVD who experience a second vascular event within 2 years (not necessarily of the same type as the first event) while taking maximally tolerated statin-based therapy, an LDL-cholesterol goal of <1.0 mmol/L (<40 mg/dL) may be considered Very high - <1.4 mmol/L (<55 mg/dL) and decreased ≥50% from baseline; High - <1.8 mmol/l (<70 mg/dL) and ≥50% reduction from baseline; Moderate - <2.6 mmol/l (<100 mg/dL); Low - <3.0 mmol/L (<116 mg/dL)	Extreme risk - <1.4 mmol/L (<55 mg/dL), optimal <1.0 mmol/L (<40 mg/dL); Very high - <1.4 mmol/L (<55 mg/dL) and decreased ≥50% from baseline; High - <1.8 mmol/l (<70 mg/dL) and ≥50% reduction from baseline; Moderate - <2.6 mmol/l (<100 mg/dL); Low - <3.0 mmol/L (<116 mg/dL)	Very high - <1.8 mmol/L (<70 mg/dL); High - <2.6 mmol/l (<100 mg/dL) Reduce levels ≥50% in patients with DM and LDL-cholesterol ≥1.8 mmol/L (≥70 mg/dL) Moderate - clinician-patient risk discussion before starting a statin. Reduce LDL-cholesterol levels by ≥ 30% in patients without DM and LDL-cholesterol levels ≥1.8 mmol/L (≥70 mg/dL) Low - clinician-patient risk discussion
Dyslipidemias management strategies	Lifestyle modification, statins, ezetimibe, PCSK9 inhibitors, fenofibrate, n-3 fatty acids, monacolin, phytosterols, bile acid sequestrants, lomitapide, mipomersen, inclisiran*, lipoprotein apheresis	Lifestyle modification, statins, ezetimibe, PCSK9 inhibitors, fenofibrate, n-3 fatty acids, monacolin, inclisiran*, lipoprotein apheresis	Lifestyle modification, statins, ezetimibe, PCSK9 inhibitors, fenofibrate, n-3 fatty acids, bile acid sequestrants, lomitapide*, mipomersen*, inclisiran*, lipoprotein apheresis

Abbreviations: ASCVD - atherosclerotic cardiovascular disease, CT - computed tomography, DBP - diastolic blood pressure, DM - diabetes mellitus, HDL - high-density lipoprotein, LDL - low-density lipoprotein, PCSK9 - proprotein convertase subtilisin/kexin type 9, SBP - systolic blood pressure, SCORE - systemic coronary risk evaluation.
*- registered in the country, but not presented yet within the clinical guidelines

velopment of drugs aimed at reducing their concentrations and improving the cardiovascular profile of the patient [41]. These drugs include volanesorsen, vupanorsen, pemafibrate, and evinacumab. Volanesorsen, being an antisense oligonucleotide, prevents the translation of apolipoprotein C-III and inhibits lipoprotein lipase. Studies have shown a significant effect in reducing triglycerides [42, 43], however, reactions at the injection site and the possibility of developing secondary thrombocytopenia have been noted. Vupanorsen is also an antisense oligonucleotide, it inhibits ANGPTL3 and leads to improvement of the lipid profile [44]. No changes in platelet levels have been reported, but there remains the possibility of reactions at the injection site. The oral drug pemafibrate, a peroxisome proliferator-activated receptor alpha (PPARα) agonist, is being actively studied as part of the PROMINENT study [45]. This molecule aims to reduce triglyceride and apolipoprotein C-III levels, contributing to the prevention of cardiovascular events, particularly in patients with type 2 diabetes.
Another important lipid particle in terms of the development of atherosclerotic CVDs is Lp(a). Patients with premature, unexplained atherosclerotic cardiovascular events despite optimal LDL-C levels may have increased Lp(a) levels. Drugs aimed at reducing Lp(a) levels are represented by pelacarsen and olpasiran. Pelacarsen is an antisense oligonucleotide that inhibits Lp(a) synthesis. A study of this drug aims to evaluate efficacy, tolerability, and safety in the treatment of hyperlipoproteinemia. Published data demonstrate a sustained reduction in Lp(a) [46, 47], which is favourable for reducing residual cardiovascular risk. N-Acetylgalactosamine-conjugated siRNA, olpasiran, also reduces Lp (a) levels. The efficacy and safety of the drug in patients with Lp(a) >60 mg/dL are currently being evaluated [48]. The results obtained will contribute to the possibility of using targeted drugs in clinical practice.
Beyond that, combination lipid-lowering therapy has to be actively introduced into clinical practice, weighing the benefits versus the risks. The need to implement this approach is analysed in the recent EAS consensus documents [49, 50], and perhaps the future guidelines will convey this concept within the algorithms for the management of patients at high/very-high risk requiring combined lipid-lowering therapy right from the start.

Conflict of interest

The Authors declare that there is no conflict of interest.

Source of funding

This work was financially supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2022-301).

Permissions information

The authors declare that the table in the manuscript is original and does not require reprint permission.

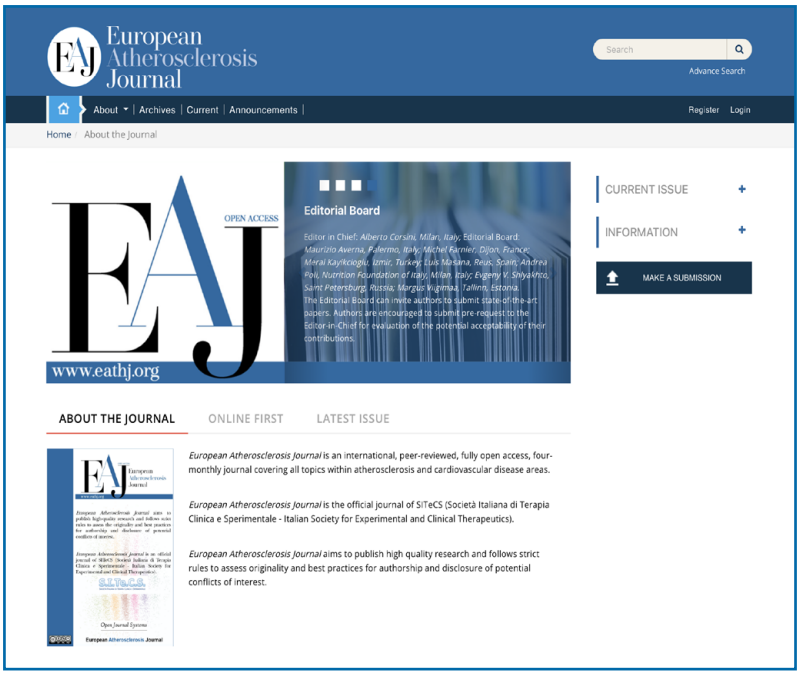
Credit authorship contribution statement

Alieva Asiiat: conception, review, and editing, Usova Elena: writing - original draft, responsible for literature search, Reutova Olga: writing - original draft, responsible for literature search.

References

1. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020 Jan 1;41(1):111-88.
2. Kukharchuk VV, Ezhov MV, Sergienko IV, et al. Diagnostics and correction of lipid metabolism disorders in order to prevent and treat atherosclerosis. Russian recommendations, VII revision. Atherosclerosis and Dyslipidemia. 2020; 1: 7-42.
3. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Jun 18; 139(25):e1082-e1143.
4. Visseren FLJ, Mach F, Smulders YM, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7; 42(34):3227-337.
5. Jellinger, Paul S. American Association of Clinical Endocrinologists/American College of Endocrinology Management of Dyslipidemia and Prevention of Cardiovascular Disease Clinical Practice Guidelines. Diabetes spectrum: a publication of the American Diabetes Association, 2018; 31,3: 234-45.
6. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. Atherosclerosis. 2012 Jan; 220(1):128-33.
7. Ikeda N, Kogame N, Iijima R, et al. Carotid artery intima-media thickness and plaque score can predict the SYNTAX score. Eur Heart J. 2012 Jan; 33(1):113-9.
8. Tada H, Nakagawa T, Okada H, e al. Clinical Impact of Carotid Plaque Score rather than Carotid Intima-Media Thickness on Recurrence of Atherosclerotic Cardiovascular Disease Events. J Atheroscler Thromb. 2020 Jan 1; 27(1):38-46.
9. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008 Nov 20; 359(21):2195-207.
10. Kashef MA, Giugliano G. Legacy effect of statins: 20-year follow up of the West of Scotland Coronary Prevention Study (WOSCOPS). Glob Cardiol Sci Pract. 2016; 2016(4):e201635.
11. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet. 1994 Nov 19; 344(8934):1383-9.
12. Keech A, Colquhoun D, Best J, et al. Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose: results from the LIPID trial. Diabetes Care. 2003 Oct; 26(10):2713-21.
13. Heart Protection Study Collaborative Group. Randomized trial of the effects of cholesterol-lowering with simvastatin on peripheral vascular and other major vascular outcomes in 20,536 people with peripheral arterial disease and other high-risk conditions. J Vasc Surg. 2007 Apr; 45(4):645-654; discussion 653-4.
14. Sever PS, Dahlöf B, Poulter NR, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. Lancet. 2003 Apr 5; 361(9364):1149-58.
15. Colhoun HM, Betteridge DJ, Durrington PN, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet. 2004 Aug 21-27; 364(9435):685-96.
16. Cannon CP, Braunwald E, McCabe CH, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004 Apr 8; 350(15):1495-504.

17. Waters DD, Guyton JR, Herrington DM, et al. Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? Am J Cardiol. 2004 Jan 15; 93(2):154-8.
18. Baigent C, Landray, MJ, Reith, C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet (London, England), 377(9784), 2181-92.
19. Keech A, Simes RJ, Barter P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005 Nov 26; 366(9500):1849-61.
20. Elam M, Lovato L, Ginsberg H. The ACCORD-Lipid study: implications for treatment of dyslipidemia in Type 2 diabetes mellitus. Clin Lipidol. 2011; 6(1):9-20.
21. Hopper L, Ness A, Higgins JP, et al. GISSI-Prevenzione trial. Lancet. 1999 Oct 30; 354(9189):1557.
22. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. N Engl J Med. 2017 May 4; 376(18):1713-22.
23. Schwartz GG, Steg PG, Szarek M, et al. ODYSSEY OUTCOMES Committees and Investigators. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 2018 Nov 29; 379(22):2097-107.
24. Berge KG, Canner PL. Coronary drug project: experience with niacin. Coronary Drug Project Research Group. Eur J Clin Pharmacol. 1991; 40 Suppl 1:S49-51.
25. Carlson LA, Böttiger LE. Ischaemic heart-disease in relation to fasting values of plasma triglycerides and cholesterol. Stockholm prospective study. Lancet. 1972 Apr 22; 1(7756):865-8.
26. Zhao XQ, Morse JS, Dowdy AA, et al. Safety and tolerability of simvastatin plus niacin in patients with coronary artery disease and low high-density lipoprotein cholesterol (The HDL Atherosclerosis Treatment Study). Am J Cardiol. 2004 Feb 1; 93(3):307-12.
27. Ray KK, Reeskamp LF, Laufs U, et al. Combination lipid-lowering therapy as first-line strategy in very high-risk patients. Eur Heart J. 2022 Feb 22; 43(8):830-3.
28. Averna M, Banach M, Bruckert E, et al. Practical guidance for combination lipid-modifying therapy in high- and very-high-risk patients: A statement from a European Atherosclerosis Society Task Force. Atherosclerosis. 2021 May; 325:99-109.
29. Raal FJ, Kallend D, Ray KK, et al. ORION-9 Investigators. Inclisiran for the Treatment of Heterozygous Familial Hypercholesterolemia. N Engl J Med. 2020 Apr 16; 382(16):1520-30.
30. Ray KK, Wright RS, Kallend D, et al. ORION-10 and ORION-11 Investigators. Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol. N Engl J Med. 2020 Apr 16; 382(16):1507-19.
31. Ference BA, Yoo W, Alesh I, et al. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. J Am Coll Cardiol. 2012 Dec 25; 60(25):2631-9.
32. Tokgozoglu L, Orringer C, Ginsberg HN, et al. The year in cardiovascular medicine 2021: dyslipidaemia. Eur Heart J. 2022 Feb 22; 43(8):807-17.
33. Laufs U, Banach M, Mancini GBJ, et al. Efficacy and Safety of Bempedoic Acid in Patients With Hypercholesterolemia and Statin Intolerance. J Am Heart Assoc. 2019 Apr 2; 8(7):e011662.
34. Ballantyne CM, Laufs U, Ray KK, et al. Bempedoic acid plus ezetimibe fixed-dose combination in patients with hypercholesterolemia and high CVD risk treated with maximally tolerated statin therapy. Eur J Prev Cardiol. 2020 Apr; 27(6):593-603.
35. Rubino J, MacDougall DE, Sterling LR, et al. Combination of bempedoic acid, ezetimibe, and atorvastatin in patients with hypercholesterolemia: A randomized clinical trial. Atherosclerosis. 2021 Mar; 320:122-8.
36. Raal FJ, Rosenson RS, Reeskamp LF, et al. Evinacumab for Homozygous Familial Hypercholesterolemia. N Engl J Med. 2020 Aug 20; 383(8):711-20.
37. Reeskamp LF, Millar JS, Wu L, et al. ANGPTL3 Inhibition With Evinacumab Results in Faster Clearance of IDL and LDL apoB in Patients With Homozygous Familial Hypercholesterolemia-Brief Report. Arterioscler Thromb Vasc Biol. 2021 May 5; 41(5):1753-9.
38. Kawakami R, Nozato Y, Nakagami H, et al. Development of vaccine for dyslipidemia targeted to a proprotein convertase subtilisin/kexin type 9 (PCSK9) epitope in mice. PLoS One. 2018 Feb 13; 13(2):e0191895.
39. Fukami H, Morinaga J, Nakagami H, et al. Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia. Cell Rep Med. 2021 Nov 16; 2(11):100446.
40. Chadwick AC, Musunuru K. CRISPR-Cas9 Genome Editing for Treatment of Atherogenic Dyslipidemia. Arterioscler Thromb Vasc Biol. 2018 Jan; 38(1):12-8.
41. Tokgözoğlu L, Libby P. The dawn of a new era of targeted lipid-lowering therapies. Eur Heart J. 2022 Sep 7; 43(34):3198-208.
42. Gouni-Berthold I, Alexander VJ, Yang Q, et al. Efficacy and safety of volanesorsen in patients with multifactorial chylomicronaemia (COMPASS): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Diabetes Endocrinol. 2021 May; 9(5):264-75.
43. Witztum JL, Gaudet D, Freedman SD, et al. Volanesorsen and Triglyceride Levels in Familial Chylomicronemia Syndrome. N Engl J Med. 2019 Aug 8; 381(6):531-42.
44. Gaudet D, Karwatowska-Prokopczuk E, Baum SJ, et al. Vupanorsen, an N-acetyl galactosamine-conjugated antisense drug to ANGPTL3 mRNA, lowers triglycerides and atherogenic lipoproteins in patients with diabetes, hepatic steatosis, and hypertriglyceridaemia. Eur Heart J. 2020 Oct 21; 41(40):3936-45.
45. Pradhan AD, Paynter NP, Everett BM, et al. Rationale and design of the Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes (PROMINENT) study. Am Heart J. 2018 Dec; 206:80-93.
46. Hardy J, Niman S, Goldfaden RF, et al. A Review of the Clinical Pharmacology of Pelacarsen: A Lipoprotein(a)-Lowering Agent. Am J Cardiovasc Drugs. 2022 Jan; 22(1):47-54.
47. Yeang C, Karwatowska-Prokopczuk E, Su F, et al. Effect of Pelacarsen on Lipoprotein(a) Cholesterol and Corrected Low-Density Lipoprotein Cholesterol. J Am Coll Cardiol. 2022 Mar 22; 79(11):1035-46.
48. Olpasiran trials of cardiovascular events and lipoproteiN(a) reduction - DOSE finding study. (18 July 2022, date last accessed).
49. Averna M, Banach M, Bruckert E, et al. Practical guidance for combination lipid-modifying therapy in high- and very-high-risk patients: A statement from a European Atherosclerosis Society Task Force. Atherosclerosis. 2021 May; 325:99-109.
50. Ray KK, Reeskamp LF, Laufs U, et al. Combination lipid-lowering therapy as first-line strategy in very high-risk patients. Eur Heart J. 2022 Feb 22; 43(8):830-3.



European Atherosclerosis Journal **is an international, peer-reviewed, fully open access, four-monthly journal covering all topics within atherosclerosis and cardiovascular disease areas.**

European Atherosclerosis Journal **is an official journal of SITeCS (Società Italiana di Terapia Clinica e Sperimentale - Italian Society for Experimental and Clinical Therapeutics).**

European Atherosclerosis Journal **aims to publish high quality research and follows strict rules to assess originality and best practices for authorship and disclosure of potential conflicts of interest.**

www.eathj.org

Focus and Scope

European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly international journal that publishes papers in the field of atherosclerosis and cardiovascular disease, from basic research to clinical and translational studies. Meta-analysis and systematic reviews will also be accepted. Papers will be considered for publication based on originality and contribution to the field.

The journal will consider for publication original articles (experimental and clinical), review articles, methodology papers, editorials, letters to the Editor, viewpoints, congress/conference reports.

The Editorial Board can invite authors to submit state-of-the-art papers. Authors are encouraged to submit pre-request to the Editor-in-Chief for evaluation of the potential acceptability of their contributions.

Open Access Policy

European Atherosclerosis Journal provides immediate open access to its content on the principle that making research freely available to the public supports exchange of knowledge.

Article Processing Charge

Publishing an article in *European Atherosclerosis Journal* requires the payment of an Article Processing Charge (APC) of € 2,000. There are no charges for submission, rejected articles, or color figures.

Payment can be made by bank transfer. Invoices will be emailed by the editorial assistant shortly after acceptance of the paper to the payment contact provided by the authors at the time of submission.

Peer Review Process

Immediately after submission, the Editor-in-Chief evaluates the manuscript. The Editor can accept the paper at this stage after discussing with members of the Editorial Board.

Rejection at this stage may occur if the submitted manuscript is insufficiently original, has serious scientific flaws, has poor grammar or English language, or is outside the aims and scope of the Journal.

A manuscript that meets the minimum criteria will be reviewed by at least 2 expert reviewers. Suggestions for potential reviewers from the authors are welcome, but it is the Editor's decision whether or not to follow such suggestions.

Reviewers will evaluate the manuscript according to the following criteria:

- Originality (the main results and conclusions must not have been published or under consideration elsewhere);
- Method appropriateness;- Validity of data and clarity of result presentation;
- Conclusions consistency with the evidence and arguments presented;
- Updated and proper references.

Reviewers will not copyedit manuscripts. Language corrections can be suggested during peer review process. The final decision is made by the Editor-in-Chief.

Code of conduct

Editors, authors, and reviewers must follow the best practice in publication ethics. Authors are expected to respect rules on authorship, dual or overlapping submission, plagiarism, figure manipulation, conflicts of interest, and compliance with policies on research ethics. Reviewers and Editors must treat manuscripts impartially and in confidence, and declare any competing interests. The Editor-in-Chief can contact authors' institutions, funders or regulatory bodies, if required. In the presence of a proven misconduct, the Journal will take actions to correct the scientific record, which may include either issuing a correction or paper retraction. If you have any concerns about potential misconduct, please contact the Editor-in-Chief.

Published by SITeCS under a Creative Commons license

OPEN ACCESS
EAJ European Atherosclerosis Journal
www.eathj.org