Brain health throughout cancer survivorship: the impacts of anti-cancer therapy on cerebrovascular regulation and pathology

by

Britton Charles Scheuermann

B.S., Miami University, 2018
M.S., University of Toledo, 2021

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Kinesiology
College of Health and Human Sciences

KANSAS STATE UNIVERSITY
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Abstract

Cancer survivors have substantially greater risk of cardiovascular morbidity and mortality compared to the general population, including a 52% greater risk of heart failure and a 22% greater risk of stroke in some reports. The rates of development of chronic disease, even without immediately fatal endpoints, are similarly worrisome. Addressing both "hard" cardiovascular disease events and the incident development or exacerbation of chronic disease remains critical in the care of cancer survivors. This is even more true today with the increasingly rapid pace of progress in oncology – specifically, the introduction of immune checkpoint inhibitors.

One of the primary concerns addressed in the present dissertation is neurotoxicity, experienced by many cancer survivors as a consequence of both the burden of cancer and due to adverse off-target effects of anti-cancer treatment. This neurotoxicity can manifest in a variety of ways, including cognitive decline, cerebral pathologies like encephalitis or reversible cerebral vasoconstriction syndrome, or in severe cases, stroke or intracranial hemorrhage. Despite the serious and harmful nature of these toxicities, investigations into overall brain health in cancer survivors are limited compared to the research dedicated to other organ systems.

Therefore, in this dissertation, we present a series of four studies aimed at investigating cerebral and cerebrovascular health in cancer survivorship. Our first investigation was a systematic review and meta-analysis examining the associations between two local cerebrovascular regulatory functions (cerebrovascular reactivity, cerebral autoregulation) and one systemic vascular characteristic (arterial stiffness) with cerebral small vessel disease. Subsequently, we aimed to determine if there were impairments in cerebrovascular reactivity in cancer survivors compared to cancer-free controls. We then combined evidence from the first two studies to form a base of evidence suggesting that cancer survivors were likely to be at risk

for cerebral small vessel disease. This led to our last two studies focused on a novel class of anti-cancer treatment, called immune checkpoint inhibitors (ICI). Our third study uses Mendelian randomization and human genetic information to explore relationships between exposure to ICI treatment and the risk of developing cerebral small vessel disease. Finally, our fourth study expands on the previous study's findings with single-cell RNA-sequencing of vascular tissue from patients who did or did not receive ICI treatment. This patient tissue-derived information is combined with genomic and epigenetic analyses to investigate biological processes and pathways linking ICI exposure to cerebral small vessel disease, and to subsequently identify possible therapeutic options. It is our hope that this dissertation may provide insight into the cerebrovascular complications that exist in cancer survivorship, as well as their potential causes and consequences.

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Approved by:

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Carl J. Ade

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There is no chance that I would be at this point in my career if it weren't for my family. My dad, who finished his own training as a physiologist when I was only a few years old, has been a continual source of support, instruction, and guidance in more ways than I can count. My mom has been incredible, and without her, my journey as a scientist and member of society would have been a lot more of an uphill struggle. Finally, my sister has been an amazing source of joy with her upbeat attitude, and watching her progress in her own journey has always been foundational in developing my own resolve. There's no way to put into words what they mean to me, so I will keep it short instead: thank you, genuinely.

Dedication

This dissertation is dedicated to my late grandfather, Ron Scheuermann. I've always been fortunate to be surrounded by a loving immediate and extended family, even as I struggled to find my own path and made mistakes along the way. Before I started my PhD training, Grandpa Scheuermann passed away with a combination of multiple conditions, including cancer and a history of strokes and coronary artery disease. That day, and the continuing memory of his unwavering strength and compassion throughout my entire life, made my path clear moving forward. Every day is a new opportunity to work with my amazing friends and colleagues and change things for the better, the way that Grandpa Scheuermann did for his community.

Preface

Chapter 2 of the present dissertation is a published, peer-reviewed manuscript. All other chapters have been, or will be, submitted to scientific journals similarly. They are therefore written in published or publication-ready format. Chapter 2 has been reproduced with permission from the publisher. The citation is listed below, along with mock citations for the remaining chapters to provide credit for the co-authors and collaborators.

Chapter 2

Scheuermann, B. C., Parr, S. K., Schulze, K. M., Kunkel, O. N., Turpin, V. R. G., Liang, J., & Ade, C. J. (2023). Associations of cerebrovascular regulation and arterial stiffness with cerebral small vessel disease: a systematic review and meta-analysis. *Journal of the American Heart Association*, *12*(23), e032616.

Chapter 3

Scheuermann, B., Hammond, S., Parr, S., Turpin, V.R., Kunkel, O., Baranczuk, A., Schulze, K., Bell, T., Smith, A., Edwards, O., Sanders, S., McGatlin, K., Bailey, H., Ade, C. Cerebral Macro- and Microvascular Regulation in Cancer Survivorship. *In Preparation and Submission.*

Chapter 4

Scheuermann, B., Hakeem, H., Amr, B., Radhi, F., Ade, C. The Effects of Breast Cancer and Immune Checkpoint Inhibition on Cerebral Small Vessel Disease Burden: A Mendelian Randomization Analysis. *In Review at Circulation.*

Chapter 5

Scheuermann, B., Ade, C. Circulatory Links between Immune Checkpoint Inhibitors and Cerebral Small Vessel Disease: Integration of Single Cell RNA-Sequencing and Multiomic Mendelian Randomization. *In Preparation and Submission for Circulation Research.*

Chapter 1 - Physiology and Medicine in the Chronic Disease

"I have criticized the present-day teaching of physiology, anatomy, and pathology, but I want now to urge with all the emphasis I can that whatever may be their present shortcomings, the group of preliminary sciences represented by physiology, anatomy, pathology, and pharmacology form the future basis of practical medicine…The medical art which is not grounded on these sciences is bound to become more and more of an anachronism."

John Scott Haldane, M.D., F.R.S. *The Relation of Physiology to Medicine*.

Edinburgh Medical Journal (1918).

As the quote above suggests, the observations and predictions of J.S. Haldane at the turn of the 20th century extended far beyond his seminal contributions to understanding the roles of gases in physiology.[1] His treatise implored educational institutions to consider adopting the emerging model in the United States, where "…there was everywhere considerable knowledge of, and enthusiastic belief in, scientific methods…"[1] that led to accelerated advances in both basic and clinical investigation. One of the preeminent physician-physiologists of today, Dr. Michael Joyner, recently pointed out that an understanding of coronary physiology was the driving force behind the 60-70% reduction of coronary artery disease mortality in the last half-century.[2] In the face of concerns regarding stagnating progress or progress plagued by unanticipated challenges[3-5], returning to foundational integrative physiology to guide research and practice may be warranted. In my opinion, nowhere is this more evident than in the context of cardio-oncology – a field of practice and research aimed at understanding the cardiovascular

complications of cancer and anti-cancer treatments with the goal of improving cancer survivorship outcomes.[6]

Cancer Survivorship and Chronic Disease

"As we move from an earlier time when few cancers were treated successfully to the point when virtually all of them will be cured, we are passing through an uncharted middle ground, which in many aspects remains primitive."

Fitzhugh Mullan, M.D. *Seasons of Survival: Reflections of a Physician with Cancer*. The New England Journal of Medicine (1985).

There is an estimated new diagnosis of cancer every 15 seconds in the United States[7]; a staggering rate that has resulted in approximately 18.6 million U.S. cancer survivors as of January 1st, 2025.[8] Thankfully, the increase in the number of survivors is not solely due to more diagnoses. Recent trends suggest that mortality rates of some of the most common forms of cancer are decreasing, including lung cancer (-4.7%/year), colorectal (-2.0%/year)[9], and breast cancer (-2.0%/year).[10] Improvements in screening and monitoring have contributed to the reduced mortality, but most sources agree that the largest contribution has come from advances in anti-cancer treatment. One of the earliest "success" stories of chemotherapy (after the initial broad testing performed by German scientist Paul Ehrlich[11]) stemmed from the adaptation of mustard gas used in World War 2 after observation of its profound leukopenic properties.[12] Advances in anti-cancer treatment followed rapidly, including the isolation of doxorubicin from cultures of *Streptomyces peucetius*[13] and the synthesis of 5-fluorouracil by Charles Heidelberger and his colleagues.[14] The 21st century has been characterized by a tremendous interest in

developing monoclonal antibodies that target specific tumor-associated antigens[15], such as the use of bevacizumab targeting vascular endothelial growth factor (VEGF) in colorectal and breast cancers.[16] However, these strides in chemotherapy have all been plagued by one critical concern: off-target toxicities.

A phrase heard from Dr. Zachary Clayton at several conferences can summarize one of the major fears of cardiologists, oncologists, and physiologists involved in cancer survivorship: "Today's cancer patient is tomorrow's cardiovascular patients" [*Personal Communications*]. Cancer survivors have substantially greater risk of cardiovascular morbidity and mortality compared to the general population, including a 52% greater risk of heart failure and a 22% greater risk of stroke in some reports.[17] The rates of development of chronic disease are similarly worrisome. Exposure to ibrutinib, a tyrosine kinase inhibitor, led to new onset hypertension in 47.2% of treated patients.[18] Androgen suppression therapy for men with prostate cancer impaired glycemic control in 22% of patients.[19] Addressing both "hard" cardiovascular disease events and the incident development or exacerbation of chronic disease remains critical in the care of cancer survivors. This is even more true today with the increasingly rapid pace of progress in oncology – specifically, the introduction of immune checkpoint inhibitors.

Novel Anti-Cancer Therapies: Addressing Benefits and Harms

"Over the past decade, cancer drugs have dominated the accelerated approval pathway, accounting for more than 85% of approvals…The clinical implications cannot be overstated: patients receiving some treatments might be exposed to clinical, financial, and time toxic effects without any meaningful improvements in how long they live or how they feel."

Kristina Jenei, B.S.N., M.Sc.; Christopher Booth, M.D. The Delicate Balancing Act of Accelerated Approval for Cancer Medicines-Speed, Certainty, and Benefit. JAMA Network Open (2025).

The use of immune checkpoint inhibitor (ICI) therapy will likely be touted as one of the greatest successes in oncology in the past two decades, and for good reason. Trials like KEYNOTE-564 in renal cell carcinoma[20] and the CheckMate-141 trial in squamous-cell head and neck cancer[21] have demonstrated 30-40% reductions in mortality compared to traditional chemotherapy regimens. The so-called "new era of immunotherapy survivorship" [22] has been ushered in with 11 approved ICI therapies and an estimated 56% of cancer patients eligible to receive ICI treatment.[23] Despite the excitement for this "era", the notable toxicities associated with ICI administration has not gone unnoticed by the cardio-oncology community. The goal is not to be nay-sayers of incorporating ICI therapies into regimens, but rather to ensure safe and efficacious planning and monitoring of the cancer survivorship phases. In this regard, early reports of ICI-related myocarditis dominated the research into ICI toxicities, but recent investigations have implicated essentially every organ in the human body as potential target.[24] Of particular interest for the present dissertation, neurotoxicity seen after ICI treatment can vary widely in its etiology and presentation, but the potentially fatal outcomes make it a critical gap in our current knowledge.

The Goals of the Present Dissertation

In this dissertation, we aimed to investigate cerebrovascular dysfunction (as a mechanism and manifestation of neurotoxicity) in cancer survivorship. Cerebrovascular dysfunction exists in

a myriad of syndromes, necessitating a more focused approach. Therefore, our first investigation was a systematic review and meta-analysis examining the associations between two local cerebrovascular regulatory functions (cerebrovascular reactivity, cerebral autoregulation) and one systemic vascular characteristic (arterial stiffness) with cerebral small vessel disease (Chapter 2). Subsequently, we aimed to determine if there were impairments in cerebrovascular reactivity in cancer survivors compared to cancer-free controls (Chapter 3). We then combined evidence from Chapters 2 and 3 to form a basis suggesting that cancer survivors were likely to be at risk for cerebral small vessel disease. This led to Chapters 4 and 5, which use unique methods to navigate the current challenges to studying ICIs (i.e., the paucity of data, the novelty of the drugs and lack of follow-up data). Chapter 4 uses Mendelian randomization and human genetic information to explore relationships between exposure to ICI treatment and the risk of developing cerebral small vessel disease. Finally, Chapter 5 expands on Chapter 4 with single-cell RNA-sequencing of vascular tissue from patients who did or did not receive ICI treatment. This patient tissue-derived information is combined with genomic and epigenetic analyses to investigate biological processes and pathways linking ICI exposure to cerebral small vessel disease, and to subsequently identify possible therapeutic options.

It is our hope that this dissertation may provide insight into the cerebrovascular complications that exist in cancer survivorship, as well as their potential causes and consequences. These initial investigations of the ICIs, specifically, will hopefully inform future investigations promoting monitoring and mitigation strategies targeting ICI-associated neurotoxicity. Finally, we hope this dissertation captures the efforts of all members of the research team involved, and most importantly, the dedicated time that numerous cancer survivors

spent with us throughout this process. The devotion of these participants and patients cannot be understated.

"A human being is far more than the mere organism with which medicine and surgery are immediately concerned…If, therefore, medicine is to have more science and less rule of thumb, let us see to it also that with the science goes more humanity, and, where possible, a broader general education, not in the mere dry bones of the humanities, but on living humanistic lines."

John Scott Haldane, M.D., F.R.S. *The Relation of Physiology to Medicine*.
Edinburgh Medical Journal (1918).

References

1. Haldane JS. The Relation of Physiology to Medicine. Edinburgh Medical Journal. 1918;20:255.
2. Joyner MJ. Why physiology matters in medicine. Physiology (Bethesda). 2011;26:72-75. doi: 10.1152/physiol.00003.2011
3. Caponigro G, Sellers WR. Advances in the preclinical testing of cancer therapeutic hypotheses. Nat Rev Drug Discov. 2011;10:179-187. doi: 10.1038/nrd3385
4. Yu AF, Ky B. Roadmap for biomarkers of cancer therapy cardiotoxicity. Heart. 2016;102:425-430. doi: 10.1136/heartjnl-2015-307894
5. Keefe DMK, Bateman EH. Potential Successes and Challenges of Targeted Cancer Therapies. J Natl Cancer Inst Monogr. 2019;2019. doi: 10.1093/jncimonographs/lgz008
6. Kostakou PM, Kouris NT, Kostopoulos VS, Damaskos DS, Olympios CD. Cardio-oncology: a new and developing sector of research and therapy in the field of cardiology. Heart Fail Rev. 2019;24:91-100. doi: 10.1007/s10741-018-9731-y
7. Dizon DS, Kamal AH. Cancer statistics 2024: All hands on deck. CA Cancer J Clin. 2024;74:8-9. doi: 10.3322/caac.21824
8. Wagle NS, Nogueira L, Devasia TP, Mariotto AB, Yabroff KR, Islami F, Jemal A, Alteri R, Ganz PA, Siegel RL. Cancer treatment and survivorship statistics, 2025. CA Cancer J Clin. 2025. doi: 10.3322/caac.70011
9. Shiels MS, Lipkowitz S, Campos NG, Schiffman M, Schiller JT, Freedman ND, Berrington de Gonzalez A. Opportunities for Achieving the Cancer Moonshot Goal of a 50% Reduction in Cancer Mortality by 2047. Cancer Discov. 2023;13:1084-1099. doi: 10.1158/2159-8290.CD-23-0208
10. Jatoi I, Sung H, Jemal A. The Emergence of the Racial Disparity in U.S. Breast-Cancer Mortality. N Engl J Med. 2022;386:2349-2352. doi: 10.1056/NEJMp2200244
11. Kaufmann SH. Paul Ehrlich: founder of chemotherapy. Nat Rev Drug Discov. 2008;7:373. doi: 10.1038/nrd2582
12. Adair FE, Bagg HJ. Experimental and Clinical Studies on the Treatment of Cancer by Dichlorethylsulphide (Mustard Gas. Ann Surg. 1931;93:190-199. doi: 10.1097/00000658-193101000-00026
13. Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem. 2009;16:3267-3285. doi: 10.2174/092986709788803312

14. Heidelberger C, Griesbach L, Cruz O, Schnitzer RJ, Grunberg E. Fluorinated pyrimidines. VI. Effects of 5-fluorouridine and 5-fluoro-2'-deoxyuridine on transplanted tumors. Proc Soc Exp Biol Med. 1958;97:470-475. doi: 10.3181/00379727-97-23777

15. Shuptrine CW, Surana R, Weiner LM. Monoclonal antibodies for the treatment of cancer. Semin Cancer Biol. 2012;22:3-13. doi: 10.1016/j.semcancer.2011.12.009

16. Kazazi-Hyseni F, Beijnen JH, Schellens JH. Bevacizumab. Oncologist. 2010;15:819-825. doi: 10.1634/theoncologist.2009-0317

17. Florido R, Daya NR, Ndumele CE, Koton S, Russell SD, Prizment A, Blumenthal RS, Matsushita K, Mok Y, Felix AS, et al. Cardiovascular Disease Risk Among Cancer Survivors: The Atherosclerosis Risk In Communities (ARIC) Study. J Am Coll Cardiol. 2022;80:22-32. doi: 10.1016/j.jacc.2022.04.042

18. Zullig LL, Sung AD, Khouri MG, Jazowski S, Shah NP, Sitlinger A, Blalock DV, Whitney C, Kikuchi R, Bosworth HB, et al. Cardiometabolic Comorbidities in Cancer Survivors: JACC: CardioOncology State-of-the-Art Review. JACC CardioOncol. 2022;4:149-165. doi: 10.1016/j.jaccao.2022.03.005

19. Redig AJ, Munshi HG. Care of the cancer survivor: metabolic syndrome after hormone-modifying therapy. Am J Med. 2010;123:87 e81-86. doi: 10.1016/j.amjmed.2009.06.022

20. Powles T, Tomczak P, Park SH, Venugopal B, Ferguson T, Symeonides SN, Hajek J, Gurney H, Chang YH, Lee JL, et al. Pembrolizumab versus placebo as post-nephrectomy adjuvant therapy for clear cell renal cell carcinoma (KEYNOTE-564): 30-month follow-up analysis of a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2022;23:1133-1144. doi: 10.1016/S1470-2045(22)00487-9

21. Ferris RL, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016;375:1856-1867. doi: 10.1056/NEJMoa1602252

22. Cronin C, O'Sullivan A, Naidoo J. Long-Term Toxicity of Immune Checkpoint Inhibitors-Time to Widen the Lens. JAMA Oncol. 2025. doi: 10.1001/jamaoncol.2024.6809

23. Haslam A, Olivier T, Prasad V. How many people in the US are eligible for and respond to checkpoint inhibitors: An empirical analysis. Int J Cancer. 2025;156:2352-2359. doi: 10.1002/ijc.35347

24. Johnson DB, Nebhan CA, Moslehi JJ, Balko JM. Immune-checkpoint inhibitors: long-term implications of toxicity. Nat Rev Clin Oncol. 2022;19:254-267. doi: 10.1038/s41571-022-00600-w

Chapter 2 - Associations of Cerebrovascular Regulation and Arterial Stiffness with Cerebral Small Vessel Disease: A Systematic Review and Meta-Analysis

Abstract

Background: Cerebral small vessel disease (cSVD) is a major contributing factor to ischemic stroke and dementia. However, the vascular etiologies of cSVD remain inconclusive. The aim of this systematic review and meta-analysis was to characterize the associations between cSVD and cerebrovascular reactivity (CVR), cerebral autoregulation (CA), and arterial stiffness (AS). **Methods and Results:** MEDLINE, Web of Science, and Embase were searched from inception to September 2023 for studies reporting CVR, CA, or AS in relation to radiological markers of cSVD. Data were extracted in predefined tables, reviewed, and meta-analyses performed using inverse-variance random effects models to determine pooled odds ratios (OR). A total of 1611 studies were identified; 142 were included in the systematic review, of which 60 had data available for meta-analyses. Systematic review revealed that CVR, CA, and AS were consistently associated with cSVD (80.4%, 78.6%, and 85.4% of studies, respectively). Meta-analysis in 7 studies (536 participants, 32.9% women) revealed a borderline association between impaired CVR and cSVD (OR:2.26, 95%CI:0.99-5.14; P=0.05). In 37 studies (27,952 participants, 53.0% women) increased AS, per SD, was associated with cSVD (OR:1.24, 95%CI: 1.15-1.33; P<0.01). Meta-regression adjusted for comorbidities accounted for one-third of the AS model variance (R^2=29.4%, $P_{moderators}$=0.02). Subgroup analysis of AS studies demonstrated an association with white matter hyperintensities (OR:1.42, 95%CI:1.18-1.70; P<0.01).

Conclusions: The collective findings of the present systematic review and meta-analyses suggest an association between cSVD and impaired CVR and elevated AS. However, longitudinal investigations into vascular stiffness and regulatory function as possible risk factors for cSVD remain warranted.

Introduction

Cerebral small vessel disease (cSVD) is responsible for roughly one-fifth of ischemic strokes, contributes to 45% of dementias, and is associated with poor outcomes in Alzheimer's disease and cancer, two of the leading causes of death in the United States.[1-3] cSVD in itself is a complex pathology affecting the small (40-200μm) perforating arterioles that supply blood to the subcortical white matter of the brain, manifesting as lesions observed with advanced neuroimaging as described by the STRIVE criteria.[4,5] In addition to the elevated risk for cerebrovascular events, cSVD increases the risk for reduced quality of life and mortality, highlighting the critical importance of identifying risk factors for cSVD to better understand its pathophysiology and inform risk management practices.[6,7] Common risk factors, particularly hypertension, have been the most prominently investigated underlying mechanisms preceding the onset or progression of cSVD.[8-10] However, combining the most common cardiovascular risk factors (e.g., hypertension, smoking, diabetes, hyperlipidemia, and elevated homocysteine) still only explains ~2% of the variance in cSVD.[11]

While the underlying pathophysiology of cSVD is not fully delineated, several studies have suggested that patients with cSVD exhibit vascular vulnerabilities independent of hypertension.[11-13] Interestingly, normotensive patients in these studies often had underlying conditions associated with increased arterial stiffness[14-16], a vascular property of critical importance in health and disease.[17] Stiffening of central (aorta) and conduit (extracranial and intracranial) arteries potentiate small vessel injury in the brain as a result of increased transmission of pulsatile energy[18-20], which may cause or develop in parallel with disruption of local regulatory mechanisms such as cerebrovascular reactivity or cerebral autoregulation.[18,21] Importantly, in a comprehensive review, Wardlaw et al.[12] recently proposed that the

development of cSVD may incorporate the loss of these regulatory functions, suggesting that exploring topics like arterial stiffness and local regulatory mechanisms, such as cerebrovascular reactivity or cerebral autoregulation, could significantly advance our comprehension of cSVD pathogenesis. However, interpretation of the current evidence base is complicated by small sample sizes, varied study methodologies, limited longitudinal studies, and different populations of interest. Therefore, we conducted the present systematic review and meta-analyses to provide an overview of the current evidence for cerebrovascular reactivity, cerebral autoregulation, and arterial stiffness as potential local and global vascular mechanisms associated with cSVD.

Methods

The authors declare that all supporting data are available within the article and its online supplementary files. The analysis was carried out and reported following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Cochrane Collaboration. Additionally, it was registered with the International Prospective Register of Systematic Reviews under the ID number 42023448225.

Search Strategy

Three separate systematic searches of the English literature were performed in the MEDLINE, Web of Science, and Embase electronic databases using the search strategies outlined in the supplementary files (Tables S1-S3) in order to evaluate the relationship between cerebral small vessel disease and cerebrovascular reactivity, cerebrovascular autoregulation, or arterial stiffness. Additional studies were identified through manual searches of reference lists in the publications retrieved. The last database search was performed on September 30th, 2023. There were no *a priori* limitations for constraining searches to specific timeframes; thus, searches included studies published from database inception to the date of the final search. Literature searches and selection of studies were performed by three independent reviewers (B.S., V.T., O.K.), and disagreements were resolved by consensus.

Eligibility Criteria

Studies were considered eligible for the systematic review and meta-analysis if they met the following criteria: (1) full-length publication in a peer-reviewed journal, (2) included participants aged ≥18 years who had brain imaging for detection of cSVD by magnetic resonance

imaging (MRI) and (3) reported markers of cSVD compared against a valid, quantified measure of cerebrovascular reactivity, cerebrovascular autoregulation, or arterial stiffness. While previous reports have assessed cSVD burden using computed tomography (CT)[22-24] and have noted good agreement between CT- and MRI-derived cSVD features, the features detected on CT are limited to only a couple of the full range of markers recommended for quantification by guidelines.[4,25] Research standards currently recommend MRI due to the higher sensitivity and specificity for determining cSVD load[4], and therefore, the present review and meta-analyses exclude studies only using CT. Studies including populations with a previous stroke or other cerebrovascular event (prior transient ischemic attack, intracranial arterial stenosis, etc.) were eligible. The nature of the present research question did not preclude the inclusion of specific study designs. Therefore, cross-sectional or longitudinal studies of any quantitative design were eligible for selection. Studies were excluded if the report was not an original research article; the study was performed using cell, animal, or post-mortem subjects; the study used computed tomography to assess cerebral injury; or cerebral atrophy was the only marker reported. The studies remaining at this step were eligible for qualitative synthesis in the systematic review and were further examined for eligibility for inclusion in quantitative meta-analyses. Studies were not eligible for the subsequent meta-analysis if they did not report an odds ratio (OR) or provide values for the presence of cSVD in the study sample that allowed for the calculation of OR from crude event rates[26]; however, these studies remained eligible for qualitative synthesis in the systematic review.

Exposure Modalities

The primary exposures extracted were local and systemic measures of cerebrovascular reactivity, cerebral autoregulation, and arterial stiffness:

1. Cerebrovascular reactivity is defined as an index of the change in cerebrovascular tone for a given change in the arterial partial pressure of carbon dioxide.[27,28] The two primary means of assessing cerebrovascular reactivity included in the present analysis evaluated cerebral blood flow/velocity during changes in arterial partial pressure of carbon dioxide. The first approach included the breath-hold index (BHI), which calculates cerebrovascular reactivity as the percent change in cerebral perfusion divided by the length of the breath-hold phase. The second approach uses controlled mixtures of inhaled gases and calculates cerebrovascular reactivity as the change in cerebral perfusion divided by the change in arterial carbon dioxide.[29]

2. Cerebral autoregulation is the ability of the brain to adapt vascular tone to maintain a constant cerebral perfusion across a range of perfusion pressures.[30,31] As a non-invasive measurement, cerebral autoregulation is assessed as the change in cerebral perfusion relative to a change in arterial blood pressure. Evaluation of cerebral autoregulation typically examines either spontaneous or induced oscillations in blood pressure and quantification of cerebral autoregulation may include time domain or frequency domain calculations. Frequency domain cerebral autoregulation reports gain and phase values, which estimate the association and time-lag between cerebral perfusion and blood pressure.[32,33] Time domain cerebral autoregulation instead assesses the correlation between blood pressure and cerebral perfusion, providing a correlation coefficient over time-windows lasting at least 2 min.[34]

3. Arterial stiffness is defined as the resistance of the arterial walls to deformation. Arterial stiffness can be a local or regional measure, depending on the method of determination.[17] In the present analysis, the most frequent assessment of arterial stiffness was carotid-femoral pulse wave velocity (cfPWV). Calculated as the ratio of the distance between two sites over the transit time for a pulse wave to reach the sites, cfPWV characterizes global arterial health and structure.[17,20] Two clinically relevant alternative indices of global arterial function (brachial-ankle PWV and Cardio-Ankle Vascular Index) were also reported in the included studies. We also included local (local PWV, β-stiffness index, distensibility coefficient) measures of arterial stiffness in the carotid artery as a conduit vessel along the vascular path in the heart-brain axis. Recent reports of elevated carotid artery stiffness demonstrating an association with cerebral injury justified, including local stiffness measures.[35-37]

Outcomes

The primary outcome of this study is the presence of cSVD, determined in accordance with the standardized STRIVE guidelines as follows[4]:

1. Recent small subcortical infarcts: recent infarction (≤20 mm in diameter) in the territory of a perforating arteriole, occurring within the previous few weeks.

2. Lacune: approximately circular, fluid-filled cavity between 3-15 mm in diameter.

3. White matter hyperintensity: hyperintense regions identified on T2-weighted sequences, not located within subcortical grey matter or the brainstem.

4. Perivascular spaces: fluid-filled spaces that follow a typical course of a blood vessel, with a diameter typically less than 3 mm. In the basal ganglia, perivascular spaces may be enlarged (up to 10-20 mm).

5. Cerebral microbleeds: Small (often 2-5 mm in diameter, but may range up to 10 mm) areas of signal void seen on T2*-weighted MRI, but not seen on FLAIR, T1-weighted, or T2-weighted sequences.

Significant inconsistency has been noted with the reporting of cerebral lesions.[38] To ensure thorough systematic literature searches, the alternate terminology for each cSVD marker reported by Wardlaw et al. was also incorporated into the search strategies.[4] We included studies that described 'silent cerebral infarcts' due to the common use of this term to describe neuroimaging markers of cSVD[38]; the cSVD subtype that these studies analyzed was determined by consensus review of the study methods and comparison against the criteria laid out in the STRIVE guidelines.[4] Brain atrophy alone did not qualify a study for inclusion since cerebral atrophy itself is not a specific marker of cSVD[4,25] and due to inconsistent reporting.[39] Additionally, we omitted 'cortical superficial siderosis' as this was more recently incorporated as a marker for cSVD and cerebral amyloid angiopathy and thus has not been extensively investigated or incorporated into summary scoring of cSVD burden.[25]

Literature Screening and Data Extraction

All search results retrieved from the online databases were downloaded to a commercially available research publication and citation management system (Endnote, Clarivate Analytics). Literature processing began with the removal of duplicates, review articles, conference abstracts, letters to the editor, and case studies. Reviewers (B.S., V.T., O.K.)

independently screened remaining full-text records for eligibility before extracting data from eligible publications. The data extracted from records included factors for study identification (authors, year of publication, journal), study design characteristics (recruitment procedures, study protocols), descriptions of the study samples (sample size, age, sex, past or present cardiovascular or cerebral/cerebrovascular disease, use of medications), details of the exposure or predictor (type of measure, methods of assessment), cerebral small vessel disease markers (along with modality or modalities used for assessment), effect size (OR reported by the study, or calculated from reported crude event rates), and adjustments for covariates. Some studies reported multiple measures of the specific exposure; authors agreed on consensus for extraction in these cases.

Risk of Bias Assessment

The risk of bias was evaluated with the Newcastle-Ottawa Scale[40], in which the quality of each report is assessed on categories of bias, including selection, comparability, and exposure. Studies were predominantly of cross-sectional or case-control design; therefore, we applied the 9-item scale to assess the risk of bias. No study was excluded on the basis of quality alone.

Statistical Analysis

Primary Analyses

All statistical analyses were performed using GraphPad Prism (version 9.0) and the software R (version 4.1.2) with packages *meta* and *metafor*. Consistent with previous meta-analysis approaches, the cSVD subtypes were first pooled for analysis due to the limited number of studies available with each individual subtype.[39,41] When possible we performed subgroup

analyses examining individual subtypes of cSVD to account for differences in the pathogenesis processes when feasible, specifically white matter hyperintensities and lacunes.[5,11,12] Subgroup analyses were also performed by stratifying studies by cerebral region within cSVD subtypes, where feasible, due to variable risk factor contributions depending on location.[5,42] To further explore a potential source of heterogeneity in the studies reporting arterial stiffness measures, we performed a subgroup analysis including only the studies that assessed arterial stiffness as cfPWV, the gold standard approach.[17,20] Finally, subgroup analyses were also performed with the arterial stiffness data to examine the possibility of artery-specific relationships by conducting separate analyses on the studies that measured central artery stiffness (cfPWV or MRI-derived aortic PWV) or carotid stiffness (local carotid PWV, carotid distensibility coefficient, or carotid β-stiffness).

Extracted data for each predictor variable were divided into continuous or dichotomous before separate analyses were performed. In order to standardize effect sizes for meta-analyses in which the exposure variable was a continuous measure, OR from each study were adjusted by multiplying the natural-log of the OR by the study-specific standard deviation (i.e., adjusted OR = exp[SD * ln(OR)]). If interquartile ranges were provided, standard deviations were calculated as (Quartile 3 – Quartile 1)/1.35, consistent with guidelines.[43,44] If standard deviations were reported by subgroup, pooled standard deviations were calculated as described in the Cochrane Handbook.[43] For arterial stiffness, differences in directionality for the carotid distensibility coefficient (i.e., decreases in carotid distensibility coefficient indicate increases in arterial stiffness, whereas increases in PWV, CAVI, and β-stiffness indicate increases in arterial stiffness) were corrected by taking the inverse of the reported effect sizes as directed by the Cochrane Handbook.[43] Studies that reported risk ratios were included in accordance with the

previous consensus that these are roughly equivalent in smaller effect sizes.[45] For meta-analyses in which the exposure variable was dichotomous, studies that did not directly report an OR but provided sufficient information to generate a 2x2 contingency table were included after calculating the OR as described in the Cochrane Handbook.[43] GraphPad Prism version 9.0 was used to calculate the OR and associated confidence intervals. Given the wide variance in methods of assessing each predictor variable, we proceeded with a random-effects model to minimize potential bias.[46] Heterogeneity was assessed with the Cochran Q test, the Higgin I^2 statistic, and the τ^2 statistic. Forest plots were generated describing the OR, 95% confidence intervals and pooled summary estimates. Effect sizes were considered significant at $P<0.05$. Certainty of evidence for each meta-analysis performed was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system.[47,48]

Secondary Analyses

For the investigation of a temporal relationship between the exposure measures and cSVD, we performed secondary analyses incorporating only the reports with longitudinal study designs where possible. Meta-regression analyses were performed to explore the influence of the three most reported vascular risk factors/diseases (hypertension, diabetes mellitus, and hyperlipidemia) on the outcomes derived from primary analyses. For all secondary analyses performed, the effect size was considered significant if $P<0.05$.

Sensitivity Analyses

The presence of "small study effects" was examined using funnel plots, as described by the test of Egger et al.[49,50] Sensitivity analyses were conducted using a leave-one-out approach,

in which the pooled treatment effects were iteratively calculated after excluding each study one at a time and recording the pooled OR, confidence interval, and P-value. We performed further sensitivity analysis by re-calculating the pooled effect size using fixed-effects analyses in order to investigate the impact of the number of studies quantitatively synthesized on the precision of τ^2 estimates.[50]

Results

Systematic Review and Qualitative Synthesis

Cerebrovascular Reactivity and Cerebral Small Vessel Disease

Our initial database and manual search of references identified 479 records assessing the relationship between cerebrovascular reactivity and cSVD, which was reduced to 429 preliminary records after excluding duplicates (Figure S1). Abstract screening and exclusion of non-original research, records lacking full-text, or records that did not quantitatively assess a relationship between cerebrovascular reactivity and cSVD resulted in 46 publications for qualitative synthesis in the systematic review. Of the 46 articles, 3 assessed recent small subcortical infarcts[51-53], 8 assessed the presence of lacunes[54-61], 7 assessed enlarged perivascular spaces[52,57-59,62-64], 9 assessed cerebral microbleeds[52,54,56-61,65], 41 assessed white matter hyperintensities[51,52,54,56-61,64-95], and 5 assessed a total cSVD score.[58,59,65,82,96] The total population across the included reports consisted of healthy/aging individuals with or without vascular risk factors (70% of studies), neurocognitive or neurodegenerative disorders (22%), non-cSVD cerebrovascular disease (20%), and those with prior diagnoses of cSVD (39%). All included studies used only MRI for determining the presence of radiological markers of cSVD. MRI was also the primary modality in the studies included (61% of included studies) for assessing cerebrovascular reactivity, with the remaining studies using transcranial Doppler (39% of included studies). Moreover, diverse techniques were used, including different MRI assessment methods like blood-oxygen level dependent (BOLD MRI) (39% of included studies) and arterial spin labeling (ASL MRI) (13% of included studies), examined various transcranial Doppler target vessels such as the middle cerebral artery and posterior communicating artery, and employed different approaches to induce cerebrovascular hyperemia, such as the CO_2 challenge

(56.5% of included studies), acetazolamide or dipyridamole (13% of included studies), and breath-holds (28% of included studies).[29,97-99]

Of the included studies, 39 were cross-sectional in design, with 32 reporting a significant association between cerebrovascular reactivity and cSVD.[51-54,56-59,62,63,65-70,72-77,80-86,91,92,94] Twenty-one of the cross-sectional studies were case-control studies[51-53,58,63,65-71,75,77,78,80,81,83,86,87,91]. The remaining 18 studies were prospective or retrospective cohort studies.[54-57,59,62,72-74,76,79,82,84,85,92-95] In 203 leukoaraiosis patients, Bian et al.[91] demonstrated that patients exhibiting more severe white matter hyperintensities (Fazekas grades II and III) had a significantly reduced cerebrovascular reactivity compared to those with lower severity. In another larger cohort study, Staszewski et al.[58] reported that in 120 subjects, the lowest tertile of cerebrovascular reactivity had a 19-fold greater likelihood of having cSVD compared to the highest tertile. This is supported by Silvestrini et al.[53], who found that each 0.1 decrease in BHI was associated with a 2-fold greater risk of cSVD. Contrary to these findings, Bisschops et al.[55] found that cerebrovascular reactivity had no relationship with radiological markers of cSVD. This was the only study in the systematic review to focus solely on participants with internal carotid artery occlusion, which has been previously shown to alter transcranial Doppler assessments of cerebral hemodynamics.[100,101]

Of the 7 longitudinal studies[60,61,64,88-90,96], only 2[60,90] did not find an association between cerebrovascular reactivity and progression of cSVD, with follow-up times ranging from 1 to 7 years after the initial assessment of cerebrovascular reactivity. These longitudinal studies primarily focused on white matter hyperintensities; for example, Liem et al.[61] divided 38 participants into two groups by the median cerebrovascular reactivity and reported that in participants with a high cerebrovascular reactivity at baseline, the increase in white matter

hyperintensity volume over 7.1 years was 0.37 percent compared to 2.9 percent in those with a low cerebrovascular reactivity. This relationship was supported by Staszewski et al.[96], who determined in 60 cSVD patients and 20 healthy controls that the participants with a progression of cSVD had a significantly lower cerebrovascular reactivity at baseline. Conversely, Moreton et al.[60] found no association between baseline cerebrovascular reactivity and development of incident cSVD in 14 subjects over a two-year follow-up. In the work by Smolinski et al.[90], similar results to Moreton et al.[60] were found when the measures of cerebrovascular reactivity were compared to cSVD progression in a group of 43 multiple sclerosis patients in remission.

Cerebral Autoregulation and Cerebral Small Vessel Disease

Our initial database and manual search of references identified 254 records assessing the relationship between cerebral autoregulation and cSVD, which was reduced to 202 preliminary records after excluding duplicates (Figure S2). Abstract screening and exclusion of non-original research, records lacking full-text, or records that did not quantitatively assess a relationship between cerebral autoregulation and cSVD resulted in 14 publications for systematic review. Of the 14 articles, 4 assessed recent small subcortical infarcts[102-105], 1 assessed the presence of lacunes[106], 2 assessed enlarged perivascular spaces[106,107], 3 assessed cerebral microbleeds[106-108], 8 assessed white matter hyperintensities[106,107,109-114], and 4 assessed the total cSVD score.[106,107,110,115] All included studies were cross-sectional in design. The total population consisted of healthy/aging individuals with or without vascular risk factors (57% of included studies), previously diagnosed cSVD (36%), cerebrovascular events (36%), and patients undergoing cardiac surgery (7%). All included studies used only MRI for determining the presence of radiological markers of cSVD. Only one included study did not use transcranial

Doppler ultrasound to assess cerebral autoregulation; Meyer et al.[104] calculated blood flow from clearance curves after bolus infusion.

All[102-104,107-112,114,115] but three[105,106,113] studies demonstrated a significant association between cerebral autoregulation impairments and greater cSVD burden. Six studies were case-control studies[102,103,105,107,108,113] and eight studies were prospective or retrospective cohort studies.[104,106,109-112,114,115] In a study of 346 patients undergoing cardiopulmonary bypass, Nomura et al.[110] reported that cSVD was associated with a 3-fold greater odds of impaired cerebral autoregulation. Liu et al.[107] analyzed 113 cSVD patients and 83 cSVD-free controls and similarly found a significantly reduced cerebral autoregulation in the cSVD patients. Multivariate regression models adjusted for age, sex, and heart rate revealed that cerebral autoregulation in the total population was independently associated with individual cSVD markers (except lacunes) and a total cSVD score. Guo et al.[102] extended the findings from the above studies by following up the patients after initial cross-sectional analysis, and demonstrated that the observation of impaired cerebral autoregulation in the cSVD groups was diffuse and persisted for 6 months after the initial lacunar infarction. Contrary to most studies included, both Wu et al.[106] and Xiong et al.[105] reported no association between cerebral autoregulation and any radiological markers of cSVD. However, both studies used approaches to determining cerebral autoregulation that differed from those previously discussed.[116,117]

Less than half of the studies[103,106,110-112,114] accounted for vascular risk factors either in baseline comparisons or with statistical methods. Five[103,110-112,114] of the 6 studies that adjusted for risk factors found that the relationship between cerebral autoregulation and cSVD persisted, while one[106] instead found only a relationship between the vascular risk factors (hypertension and diabetes mellitus) and cSVD.

Arterial Stiffness and Cerebral Small Vessel Disease

Our initial database and manual search of references identified 878 records assessing the relationship between arterial stiffness and cSVD, which was reduced to 694 preliminary records after excluding duplicates (Figure S3). Abstract screening and exclusion of non-original research, records lacking full-text, or records that did not quantitatively assess a relationship between arterial stiffness and cSVD resulted in 82 publications for systematic review. Of the 82 articles, 12 assessed recent small subcortical infarcts[118-129], 24 assessed the presence of lacunes[21,130-152], 9 assessed enlarged perivascular spaces[118,138,144,145,150,152-155], 23 assessed cerebral microbleeds[37,118,120,123-127,131-134,138,139,144,145,149,150,152,153,156-158], 59 assessed white matter hyperintensities[21,36,118,119,122,123,125-127,130-134,138-142,145,150-152,155,156,159-192], and 8 assessed the total cSVD score.[133,138,139,152,193-196] The total population consisted of healthy/aging individuals with or without vascular risk factors (77% of included studies), previously diagnosed cSVD (6%), cerebrovascular events (8%), cognitive decline or complaints (5%), cardiovascular disease including MI and heart failure (13%), and metabolic syndromes or renal disease (11%). All included studies used only MRI for determining the presence of radiological markers of cSVD. Central or large artery stiffness was the primary assessment (69 studies, or 84%); only 13 studies measured conduit artery stiffness. The most frequently used approach was cfPWV (26 studies, 32%), followed by brachial-ankle PWV (20 studies, 24%).

The majority of studies included in the systematic review were cross-sectional in nature; with 86% of the cross-sectional studies reporting a significant association between arterial stiffness and cSVD.[21,118-121,123-128,130-134,136-145,147,148,150-154,156-158,160,161,163,165-167,169,171-173,176-179,182,183,185-187,190,193-195] Of the 69 cross-sectional studies, 13 were case-control

studies[118,127,128,155,166,168,171,173,177,179,182,192,195] and 56 were prospective or retrospective cohort studies.[21,119-121,123-126,130-154,156-158,160-163,165,167,169,172,176,178,181,183,185-188,190,193,194,196] Using the Framingham Heart Study third generation cohort, Pase et al.[137] demonstrated a significant association between arterial stiffness and white matter hyperintensity volume that was unaffected by adjustment for age, sex, and common vascular risk factors. Van Sloten et al.[143] extended these findings to other cSVD markers in 2058 older participants (mean age of 79.6 years, 59% women), who reported that a one-standard deviation increase in cfPWV increased the risk of subcortical infarcts, enlarged perivascular spaces, and cerebral microbleeds by 27%, 15%, and 9%, respectively. This concept is supported by the data from 1820 subjects analyzed by Cooper et al.[120], who showed that not only was cfPWV related to white matter hyperintensities, but also that in mediation analyses, ~41% of the effect of cfPWV on cognitive function was explained by the relationship between cfPWV and cSVD. In contrast, Rundek et al.[146] did not find any association between arterial stiffness and cSVD in a cohort of 1166 participants of the Northern Manhattan Study (NOMAS). The sample from the study by Rundek et al. was older (71 ± 9 years), 40% were on antihypertensive medication, and 53% were current or former smokers.[146]

Of the 13 longitudinal studies[36,37,122,129,159,164,170,174,175,180,184,189,191] included in the systematic review, 11 of the 13 reported significant associations between arterial stiffness and cSVD. The average follow-up time was 7.0 years from baseline (range 2 – 21.5 years). King et al.[184] demonstrated that arterial stiffness was a significant predictor of white matter hyperintensity progression over 7 years in 1270 subjects, such that a 1% increase in aortic arch stiffness was associated with a 0.3% increase in subsequent white matter hyperintensity volume. Using the gold-standard cfPWV, Rosano et al.[170] found that a higher arterial stiffness predicted the presence of cSVD over a 10-year follow-up, even after multivariate adjustment including

vascular risk factors, incident stroke, incident myocardial infarction, and changes in arterial blood pressure. Ding et al.[37] analyzed the association between baseline carotid artery stiffness and incident cSVD in a sample of 2512 participants from the Age, Gene/Environment Susceptibility (AGES-Reykjavik) study and demonstrated that the risk of incident cerebral microbleeds was 11% higher per standard deviation decrease in baseline carotid arterial strain. This relationship was not attenuated by adjustment for age, sex, arterial blood pressure and other vascular risk factors. Conversely, the study from Suri et al.[189] was one of two reports to find no association between arterial stiffness and cSVD. Specifically, the authors demonstrated no relationship between cfPWV and the progression of white matter hyperintensity volume. Tsao et al.[191] similarly found no association between arterial stiffness and cSVD. Of their 1223 older participants (mean age of 61 years) from the Framingham Heart Study only 28% reported antihypertensive medication use and 9% reported prior diagnoses of diabetes mellitus, both lower than expected age-specific prevalence.[197,198]

Meta-analyses

Six separate meta-analyses were planned before beginning the literature search to determine if cerebrovascular regulatory functions or properties of arterial stiffness were associated with cSVD. Measures were separated by whether the predictor variable was analyzed as a continuous or dichotomous variable. For dichotomous predictors, the cut-off values varied significantly across studies due to differences in study methodology. For cerebral autoregulation specifically, we only found articles reporting OR or crude event rates with cerebral autoregulation as a dichotomized measure.

Cerebrovascular Reactivity and Cerebral Small Vessel Disease

Of the studies included in the qualitative synthesis and systematic review of the association between cerebrovascular reactivity and cSVD, 10 studies were eligible for inclusion in the quantitative meta-analysis. The analyses of these 10 studies included 4 studies (pooled sample n=387, 37.7% women) that utilized a continuous predictor and 7 studies (pooled sample n=536, 32.9% women) that utilized a dichotomous predictor; one study presented cerebrovascular reactivity as both a continuous and dichotomous predictor variable (Table 1). A sample size of 4 studies was deemed too low for quantitative synthesis, therefore, the individual results of these studies are presented qualitatively in Figure 1. Of note, all 4 studies reported a significant association between impaired cerebrovascular reactivity and cSVD. For the dichotomous exposure measure of cerebrovascular reactivity, Cochran Q statistics indicated that there was significant heterogeneity in the dichotomous predictor data (Q=18.62, P=0.005).The Higgin's I^2 value was 67.8% (95% CI: 28.5-85.5%) and τ^2 was 0.82 (95% CI: 0.11-6.44). Shown in Figure 2, meta-analysis of dichotomous cerebrovascular reactivity revealed a borderline significant association between an impaired cerebrovascular reactivity and an increased prevalence of cSVD (Dichotomous Predictor Pooled OR, 2.26; 95% CI: 0.99-5.14, P=0.05).

Funnel plots were inappropriate for the cerebral predictors, given that each category had less than 10 studies.[199] The leave-one-out analysis conducted with dichotomous cerebrovascular reactivity measures demonstrated fluctuations in both the effect sizes (OR range: 1.58-2.79; number of studies per run=6) and the P-values (range 0.02-0.14), including reporting significance (P<0.05) with the removal of either Bisschops et al.[55] or Tawfik et al.[59] In further sensitivity analyses, the fixed-effect model for dichotomous cerebrovascular reactivity

Table 2-1. Cerebrovascular reactivity (CVR) studies presenting odds ratios or crude event rates.

Study	Assessment Modalities for CVR	cSVD Subtype(s)	Sample (n, % women)	Age (years)	Subject Characteristics	Risk of Bias Score (Max of 9)
Continuous Predictors						
Bakker et al. 1999[84]	TCD	WMH	73, 26.0%	70.2 ± 8.0	Healthy elderly	7
Lee et al. 2022[81]	MRI	WMH	62, 67.7%	Controls: 33.8 ± 8.0, Cases: 34.0 ± 8.1	Migraine patients vs. non-migraine controls	7
Molina et al. 1999 [51]	TCD	RSCCI	92, 30.4%	Controls: 58.3 ± 12.0, Cases: 56.6 ± 13.4	Controls vs. first-ever lacunar infarction patients	8
Silvestrini et al. 2006[53]	TCD	RSCCI	160, 35.6%	Controls: 59.8 ± 9.2, Cases: 67.6 ± 8.7	Controls vs. lacunar stroke patients	8
Dichotomous Predictors						
Bakker et al. 1999[84]	TCD	WMH	73, 26.0%	70.2 ± 8.0	Healthy elderly	7
Bisschops et al. 2003[55]	TCD	Lacunes	70, 27.1%	59, range: (35-71)	Unilateral Occlusion of ICA	7
Deplanque et al. 2013[52]	TCD	RSSCI, WMH, PVS, CMB	162, 27.2%	Controls: 63.2 ± 10.4, Cases: 62.1 ± 11.4	cSVD patients vs. non-cSVD controls	6
Liem et al. 2009[61]	MRI	RSSCI, WMH, CMB	38, 52.6%	Controls: 36.7 ± 8.0, Cases: 42.2 ± 10.0	Controls vs. NOTCH3 Mutation Carriers	7
Palaiodimou et al. 2023[93]	TCD	WMH	23, 43.0%	51 ± 13	Fabry disease patients	8
Staszewski et al. 2019[58]	TCD	Lacunes, WMH, PVS, CMB, cSVD Score	120, 40.0%	Controls: 71.7 ± 3.4, Cases: 71.8 ± 3.4	cSVD patients vs. non-cSVD controls	5
Tawfik et al. 2022[59]	TCD	Lacunes, WMH, PVS, CMB, cSVD Score	50, *	*	Lacunar stroke patients	6

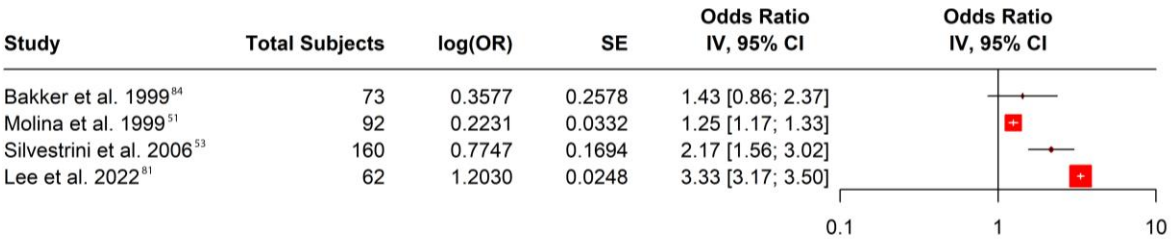


Figure 2-1. Individual study results from studies reporting cerebrovascular reactivity as a continuous exposure measure. Forest plot illustrating the individual effect sizes reported for each study containing continuous cerebrovascular reactivity in relation to cSVD burden. OR, odds ratio; SE, standard error; IV, inverse-variance.

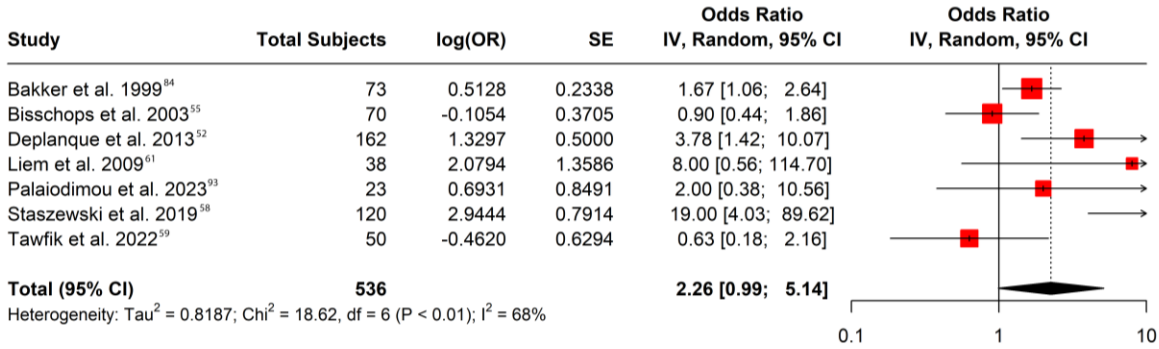


Figure 2-2. Odds ratio results from pooled studies reporting cerebrovascular reactivity as a dichotomous exposure variable. Forest plot illustrating the effect sizes dichotomous cerebrovascular reactivity in relation to cSVD burden. There was a borderline significant association between impaired cerebrovascular reactivity and a greater cSVD burden (OR: 2.26, 95% CI: 0.99-5.14). OR, odds ratio; SE, standard error; IV, inverse-variance.

demonstrated significance (OR: 1.73, 95% CI: 1.25-2.40, P=0.001) in contrast to the borderline significant results of the random-effects analysis.

Cerebral Autoregulation and Cerebral Small Vessel Disease

Of the studies included in the qualitative synthesis and systematic review of the association between cerebral autoregulation and cSVD, 3 studies (pooled sample n=455, 30.1% women) were eligible for inclusion in the quantitative meta-analysis, with all reporting cerebral autoregulation as a dichotomous variable (Table 2). A sample size of three studies was deemed insufficient for quantitative analysis; therefore, the individual results of the studies are presented qualitatively in Figure 3.

Arterial Stiffness and Cerebral Small Vessel Disease

Of the studies included in the systematic review of the association between arterial stiffness and cSVD, 47 were eligible for inclusion in the meta-analysis. The analyses for studies assessing arterial stiffness included 37 studies (pooled sample n=27952, 53.0% women) that used arterial stiffness as a continuous predictor and 13 studies (pooled sample n=10140, 53.1% women) that used arterial stiffness as a dichotomous predictor; 4 of these studies presented data for arterial stiffness as both a continuous and a dichotomous predictor variable (Table 3). Both the continuous and dichotomous models demonstrated significant ($P < 0.0001$) heterogeneity assessed as the Q statistic (Continuous: 126.97, Dichotomous: 72.96). In the continuous model, Higgin's I^2 value was 71.6% (95% CI: 60.6-79.6%), and the τ^2 value was 0.024 (95% CI: 0.020-0.22). In the dichotomous model, Higgin's I^2 was 83.6% (95% CI: 73.3-89.9%), and the τ^2 was 0.15 (95% CI: 0.038-0.62). Regardless of whether the arterial stiffness predictor was continuous

Table 2-2. Cerebral autoregulation (CA) studies presenting odds ratios or crude event rates.

Study	Assessment Modalities for CA	cSVD Subtype(s)	Sample (n, % women)	Age (years)	Subject Characteristics	Risk of Bias Score (Max of 9)
Dichotomous Predictors						
Castro et al. 2018[109]	TCD	WMH	46, 58.7%	73.0 ± 12.0	Ischemic Stroke patients	6
Nomura et al. 2018[110]	TCD	WMH, cSVD Score	346, 29.5%	Controls: 71.2 ± 7.8, Cases: 69.9 ± 8.3	Controls vs. Impaired cerebral autoregulation	7
Wu et al. 2022[106]	TCD	Lacunes, WMH, PVS, CMB, cSVD Score	63, 12.7%	56.3 ± 9.9	Small artery occlusion patients	7

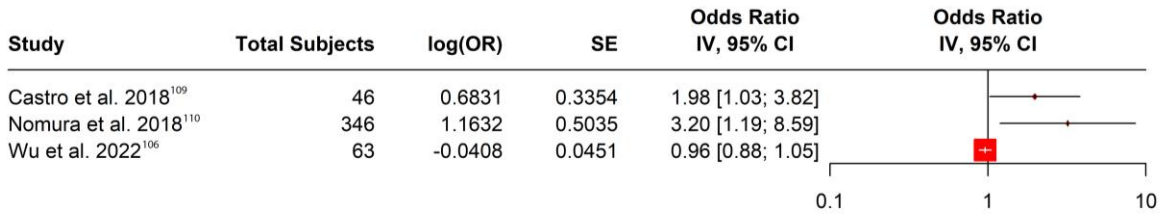


Figure 2-3. Individual study results from studies reporting cerebral autoregulation as a dichotomous exposure measure. Forest plot illustrating the individual effect sizes reported for each study containing dichotomous cerebral autoregulation in relation to cSVD buden. OR, odds ratio; SE, standard error; IV, inverse-variance.

Table 2-3. Arterial stiffness (AS) studies presenting odds ratios or crude event rates.

Study	Assessment Modality for AS	cSVD Subtype(s)	Sample (n, % women)	Age (years)	Subject Characteristics	Risk of Bias Score (Max of 9)
Continuous Predictors						
Amier et al. 2021[118]	MRI; aortic PWV	RSSCI, WMH, PVS, CMB	559, 35.8%	67.9 ± 8.8	Healthy, Cognitive Impairment, CVD	9
Brandts et al. 2009[130]	MRI; aortic PWV	RSSCI, WMH	50, 62.0%	49.2 ± 12.7	Hypertensive	9
Brisset et al. 2013[119]	Carotid DC	RSSCI, WMH	1800, 59.9%	72.5 ± 4.1	Healthy	8
Choi et al. 2013[131]	CAVI	WMH, Lacunes, CMB	484, 35.5%	50 ± 7	Healthy	7
Cooper et al. 2016[120]	cfPWV	RSSCI, CMB	1820, 60.0%	80 ± 5	Healthy	9
Coutinho et al. 2011[172]	cfPWV	WMH	812, 57.6%	58.4 ± 9.7	Healthy with hypertensive family members	9
Ding et al. 2015[37]	Carotid DC	CMB	2512, 58.5%	74.6	Healthy	9
Gustavsson et al. 2015[156]	cfPWV	WMH, CMB	208, 59.1%	71.0 ± 4.8	Healthy	8
Hashimoto et al. 2008[121]	btPWV	RSSCI	351, 71.8%	65 ± 6	Healthy	8
Hashimoto et al. 2018[163]	cfPWV	WMH	286, 60.1%	54 ± 13	Patients referred for hypertension, CVD risk	8
Henskens et al. 2008[132]	cfPWV	WMH, Lacunes, CMB	167, 49.1%	51.8 ± 13.1	Patients referred for hypertension	8
Hughes et al. 2013[164]	cfPWV	WMH	91, 34.1%	86.9 ± 2.8	Healthy elderly	9
Hughes et al. 2018[165]	cfPWV	WMH	19, *	*	Healthy	9
Jochemsen et al. 2015[122]	Carotid DC	WMH, RSSCI	526, 17.0%	59 ± 10	Healthy	8

Kim et al. 2016[133]	baPWV	WMH, Lacunes, CMB, cSVD Score	1282, 42.3%	67.6 ± 12.2	Ischemic or transient stroke patients	6
Laugesen et al. 2013[166]	cfPWV	WMH	178, 48.3%	59 ± 10	Type 2 Diabetes	9
Liu et al. 2020[194]	baPWV	cSVD Score	684, 34.5%	54.0, IQR: 48.0 – 60.0	Healthy	8
Matsumoto et al. 2007[135]	baPWV	Lacunes	476, 42.6%	51.5 ± 7.8	Healthy	6
Mitchell et al. 2011[21]	cfPWV	RSSCI, WMH	668, 56.6%	75.4 ± 4.0	Healthy	9
Ochi et al. 2010[136]	baPWV	Lacunes	500, 61.8%	66.9 ± 8.4	Healthy elderly	8
Ohmine et al. 2008[169]	baPWV	WMH	144, 62.9%	70.3 ± 9.0	Healthy elderly	9
Pase et al. 2016[137]	cfPWV	Lacunes	3207, 53.1%	46 ± 9	Healthy	9
Poels et al. 2012[123]	cfPWV	RSSCI, WMH, CMB	1460, 55.4%	58.2 ± 7.2	Healthy	9
Riba-Llena et al. 2018[138]	cfPWV	WMH, Lacunes, PVS, CMB, cSVD Score	782, 49.6%	62.7 ± 5.4	Healthy	9
Robert et al. 2022[139]	Carotid PWV	WMH, Lacunes, CMB, cSVD Score	272, 57.7%	75.4 ± 6.8	Healthy	8
Rundek et al. 2017[146]	Carotid β-stiffness	Lacunes	1166, 60.0%	71 ± 9	Healthy	9
Saji et al. 2012a[140]	baPWV	WMH, Lacunes	240, 49.6%	69 ± 9	Healthy	8
Saji et al. 2012b[141]	CAVI	WMH, Lacunes	220, 40.4%	69 ± 10	Healthy	8
Shan et al. 2016[142]	MRI; aortic arch PWV	RSSCI, WMH	62, 40.3%	56.8 ± 7.5	Type 2 diabetes patients	8
Shimoyama et al. 2012[157]	CAVI	CMB	105, 32.4%	70.0, IQR: 68.0-76.5	Cerebral infarct or transient ischemic stroke patients	8
Song et al. 2014[158]	baPWV	CMB	1137, 37.7%	65 ± 12	Cerebral infarct or transient ischemic stroke patients	8

Tsao et al. 2013[147]	cfPWV	Lacunes	1587, 55.0%	61 ± 9	Healthy	9
Turk et al. 2016[171]	Carotid PWV	WMH	96, 43.8%	53.8 ± 7.9	Controls vs. WMH-positive patients	5
van Elderen et al. 2010[125]	MRI; aortic PWV	RSSCI, WMH, CMB	86, 43.0%	46.9 ± 11.7	Type 1 diabetes patients	8
van Sloten et al. 2016[143]	cfPWV	RSSCI	2058, 59.0%	79.6 ± 4.6	Healthy	9
Zhai et al. 2018[144]	baPWV	Lacunes, PVS, CMB	953, 62.5%	55.7 ± 9.4	Healthy	9
Zhang et al. 2020[145]	baPWV	WMH, Lacunes, PVS, CMB	904, 55.3%	59.7 ± 3.0	Healthy	7
Dichotomous Predictors						
Bae et al. 2021[153]	baPWV	PVS, CMB	854, 48.2%	68.2 ± 12.5	Ischemic or transient stroke patients	9
Chang et al. 2023[196]	baPWV	cSVD Score	820, 39.9%	68.0, IQR: 59.0 – 78.0	Ischemic Stroke patients	5
Choi et al. 2013[131]	CAVI	WMH, Lacunes, CMB	484, 35.5%	50 ± 7	Healthy	7
Kinjo et al. 2016[134]	baPWV	WMH, Lacunes, CMB	990, 53.6%	53, range: 24-86	Recruited from brain check-ups	8
Liu et al. 2021[152]	CAVI	WMH	1176, 51.3%	67.5 ± 13.2	Healthy	7
Palta et al. 2019[126]	cfPWV	RSSCI, WMH, CMB	3703, 59.3%	75.2 ± 5.0	Healthy	9
Rosano et al. 2013[170]	cfPWV	WMH	303, 56.0%	82.9	Healthy	9
Saji et al. 2012a[140]	baPWV	WMH, Lacunes	240, 49.6%	69 ± 9	Healthy	8
Saji et al. 2012b[141]	CAVI	WMH, Lacunes	220, 40.4%	69 ± 10	Healthy	8
Shimoyama et al. 2012[157]	CAVI	CMB	105, 32.4%	70.0, IQR: 68.0-76.5	Cerebral infarct or transient ischemic stroke patients	8
Tabata et al. 2017[124]	baPWV	RSSCI, CMB	149, 32.9%	70.8 ± 10.0	Coronary artery disease patients	6
Zijlstra et al. 2020[149]	MRI; aortic PWV	Lacunes, CMB	85, 34.1%	75.6 ± 6.9	Healthy	4
Zhou et al. 2023[148]	estimated PWV	Lacunes	1011, 64.5%	64.2 ± 9.1	Healthy	7

(Figure 4) or dichotomized (Figure 5), an increased arterial stiffness was associated with an increased burden of cSVD. Random effects meta-analyses indicated this for both measures (Continuous Predictor Pooled OR, 1.24; 95% CI: 1.15-1.33, P < 0.0001) (Dichotomous Predictor Pooled OR, 1.86; 95% CI: 1.41-2.45, P < 0.0001).

For specific subtypes of cSVD, only subgroup analyses between continuous arterial stiffness and white matter hyperintensities or lacunes had enough studies to be appropriately powered. Eleven studies[119,125,132,156,163-166,169,171,172] investigated white matter hyperintensities. The pooled random effects OR was 1.42 (95% CI: 1.18-1.70; number of studies=11), and the heterogeneity was not significant (I^2=31%, τ^2=0.023, P=0.15, Figure S5). Thirteen studies[21,130,135-137,139-144,146,147] investigated lacunes specifically. For this analysis, the pooled random effects OR was 1.28 (95% CI: 1.10-1.49; number of studies=13) with significant heterogeneity (I^2=70%, τ^2=0.038, P<0.01) (Figure S5).

Within the subtype of white matter hyperintensities, only 4 studies[125,130,138,169] reported measures separately for deep white matter hyperintensities and periventricular white matter hyperintensities. Within the subtype of cerebral microbleeds, only 5 studies[37,123,133,144,158] reported separate effect sizes for strictly lobar cerebral microbleeds and deep cerebral microbleeds. The limited number of studies precluded formal meta-analyses, so the individual results of these studies are presented qualitatively in Figure S6.

Fourteen studies[21,120,123,132,137,138,143,147,156,163-166,172] specifically assessed arterial stiffness with the gold-standard, cfPWV, as a continuous measure. In this secondary analysis, the pooled random effects OR was 1.23 (95% CI: 1.09-1.39, P=0.001; number of studies=14). The model demonstrated significant heterogeneity (I^2=47.5%, τ^2=0.021, P=0.03). This model suggests that

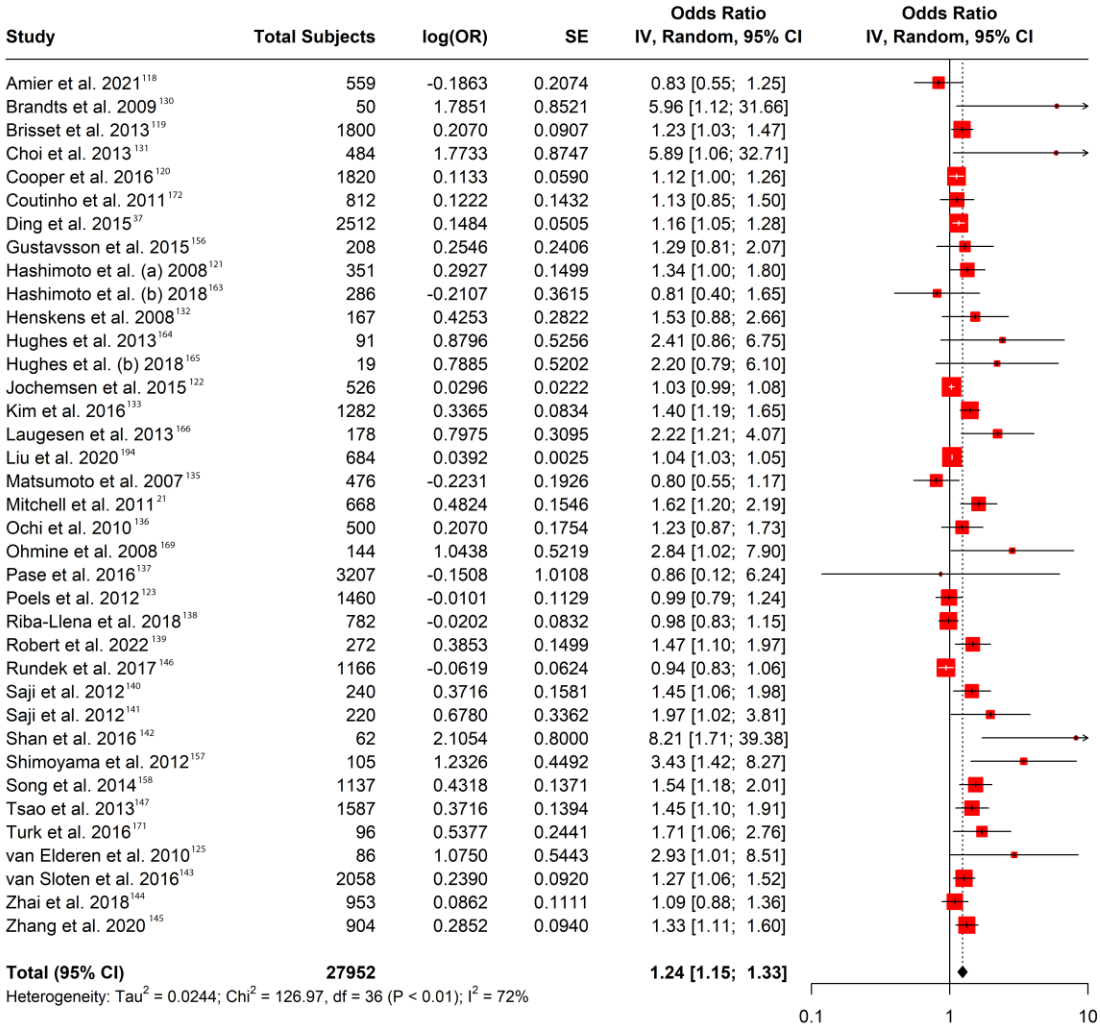


Figure 2-4. Odds ratio results from pooled studies reporting arterial stiffness as a continuous exposure variable. Forest plot illustrating the effect sizes for studies reporting continuous arterial stiffness in relation to cSVD burden. Effect sizes are reported per 1-SD increase. The overall effect favored a greater presence of cSVD as arterial stiffness increases (OR: 1.24, 95% CI: 1.15-1.33). OR, odds ratio; SE, standard error; IV, inverse-variance.

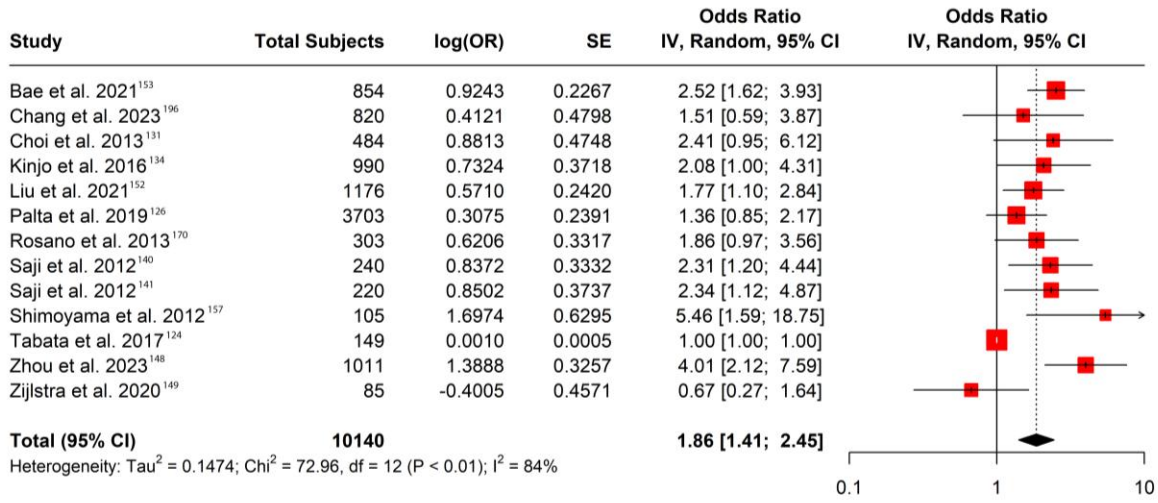


Figure 2-5. Odds ratio results from pooled studies reporting arterial stiffness as a dichotomous exposure variable. Forest plot illustrating the effect sizes for studies reporting dichotomous arterial stiffness in relation to cSVD burden. Effect sizes are reported per 1-SD increase. The overall effect suggested a greater arterial stiffness was associated with a greater presence of cSVD (OR: 1.86, 95% CI: 1.41-2.45). OR, odds ratio; SE, standard error; IV, inverse-variance.

for each standard deviation-increase in the clinically relevant cfPWV, the odds of cSVD increase by 23 percent (Figure S7).

The continuous arterial stiffness measures were analyzed to investigate the differences between central arterial stiffness and conduit (carotid) arterial stiffness. Six studies[37,119,122,139,146,171] reported measures of carotid arterial stiffness, and 18 studies[21,118,120,123,125,130,132,137,138,142,143,147,156,163-166,172] reported measures of central arterial stiffness. Both carotid artery assessments (OR: 1.15, 95% CI: 1.00-1.31; number of studies=6) and central artery assessments (OR: 1.26, 95% CI: 1.10-1.44; number of studies=18) were associated with pooled markers of cSVD. Random-effects analysis of subgroup differences did not reveal significant differences (P=0.34), suggesting that central and conduit arterial stiffness may be associated with cSVD burden (Figure S8).

Analysis of the funnel plots for continuous and dichotomous arterial stiffness measures (Figures S11 and S12) suggested suspicion of evidence of publication bias. Conducting sensitivity analyses using the leave-one-out approach revealed that the significance in the random effects models for continuous and dichotomous arterial stiffness was maintained throughout the analysis. In further sensitivity analysis, numerical differences but not statistical differences were detected when applying fixed-effects analysis to continuous arterial stiffness (OR: 1.04, 95% CI: 1.04-1.05) or to dichotomous arterial stiffness (OR: 1.001, 95% CI: 1.000-1.002).

Secondary Analyses

Meta-analysis of longitudinal studies from the studies included in quantitative primary statistical analysis was not feasible due to a limited number of studies. One study[61] investigated

the longitudinal relationship between dichotomous cerebrovascular reactivity and cSVD, 3 studies[37,122,147] investigated longitudinal relationships between continuous measures of arterial stiffness and cSVD, and 1 study[170] investigated the longitudinal relationship between arterial stiffness as a dichotomous exposure and cSVD. The individual results of these studies are presented qualitatively in Figure S4.

The multivariate meta-regression results are presented in the supplementary materials (Table S4). Only the continuous arterial stiffness category of studies was applicable for appropriately powered analysis.[200,201] Therefore, we conducted multivariate meta-regression with the continuous arterial stiffness model, incorporating the study-level covariates of hypertension, diabetes mellitus, and hyperlipidemia (or medication use targeting these conditions). Twelve studies[125,130,132,137,142-144,146,147,164,165,171] were removed from the original 37 studies on the basis of incomplete demographic information, leaving 25 studies for meta-regression. The random-effects meta-analysis of the remaining studies remained statistically significant (OR, 1.21; 95% CI: 1.12-1.32, $P < 0.0001$; number of studies=25). In the risk factor-adjusted model, the three risk factors (hypertension, diabetes mellitus, and hyperlipidemia) extracted from study-level data, not individual patient data, accounted for 29.4% of the model heterogeneity. Among the 25 studies analyzed, the majority (20 out of 25, 80%) centered around generally healthy participants with different risk factors, while 7 out of the 12 (58%) not included in the meta-regression focused on healthy populations. Meta-regression results for the model adjusted for each factor individually and for all three risk factors are presented in Table S4. Bubble plots for the individual covariates are presented in supplementary materials (Figures S6-S8).

Risk of Bias Assessment and GRADE Rating of Primary Outcomes

Studies included in quantitative meta-analyses for primary outcomes were assessed for risk of bias using the Newcastle-Ottawa scale. The average risk of bias for studies reporting dichotomous cerebrovascular reactivity was 6.6 ± 1.0, for studies reporting continuous arterial stiffness measures was 8.2 ± 1.0, and for studies reporting dichotomous arterial stiffness was 7.3 ± 1.5. Individual study assessment outcomes are reported in Tables 1 and 3. The GRADE assessment of the certainty of evidence for the primary outcomes is presented in Table S5.

Discussion

The present systematic review and meta-analyses represent the most recent and updated work summarizing the relationship between cerebral autoregulation, cerebrovascular reactivity, and arterial stiffness in relation to cSVD burden. The main findings of the present systematic review are: 1) the majority of studies included in the qualitative systematic review found associations between cSVD and cerebrovascular reactivity (80.4% of studies), cerebral autoregulation (78.6% of studies), and arterial stiffness (85.4% of studies); and 2) in quantitative meta-analyses, cSVD was associated with increases in arterial stiffness and borderline associated with impaired cerebrovascular reactivity. While the vast majority of literature, to date, surrounding the risk of cSVD has focused on traditional vascular risk factors, specifically hypertension, their role as dominant factors in the pathogenesis of cSVD has been challenged.[12,202,203] Emerging theories propose complex vascular etiologies, including an impaired cerebrovascular function, as important factors mediating cSVD, alongside or independent of hypertension.[12,18] These relate to the varied complexities of the vascular etiologies proposed for cSVD subtypes, given cerebrovascular regulatory functions are mediated, in part, by local endothelial function[27,28] and arterial stiffness is, in part, dictated by vascular matrix composition and endothelial dysregulation of vascular smooth muscle tone.[17,204,205] Importantly, the collective findings of the present systematic review expand our current understanding of the pathogenesis of cSVD by demonstrating associations of increasing cSVD burden with reductions in cerebrovascular reactivity (37 of 46 studies, or 80.4%), impairments in cerebral autoregulation (11 of 14 studies, or 78.6%), and increases in arterial stiffness (70 of 82 studies, or 85.4%) (Figure 6). In addition, our subsequent meta-analysis of eligible studies comprising 27,952 participants (53.0% women) revealed a significant association between

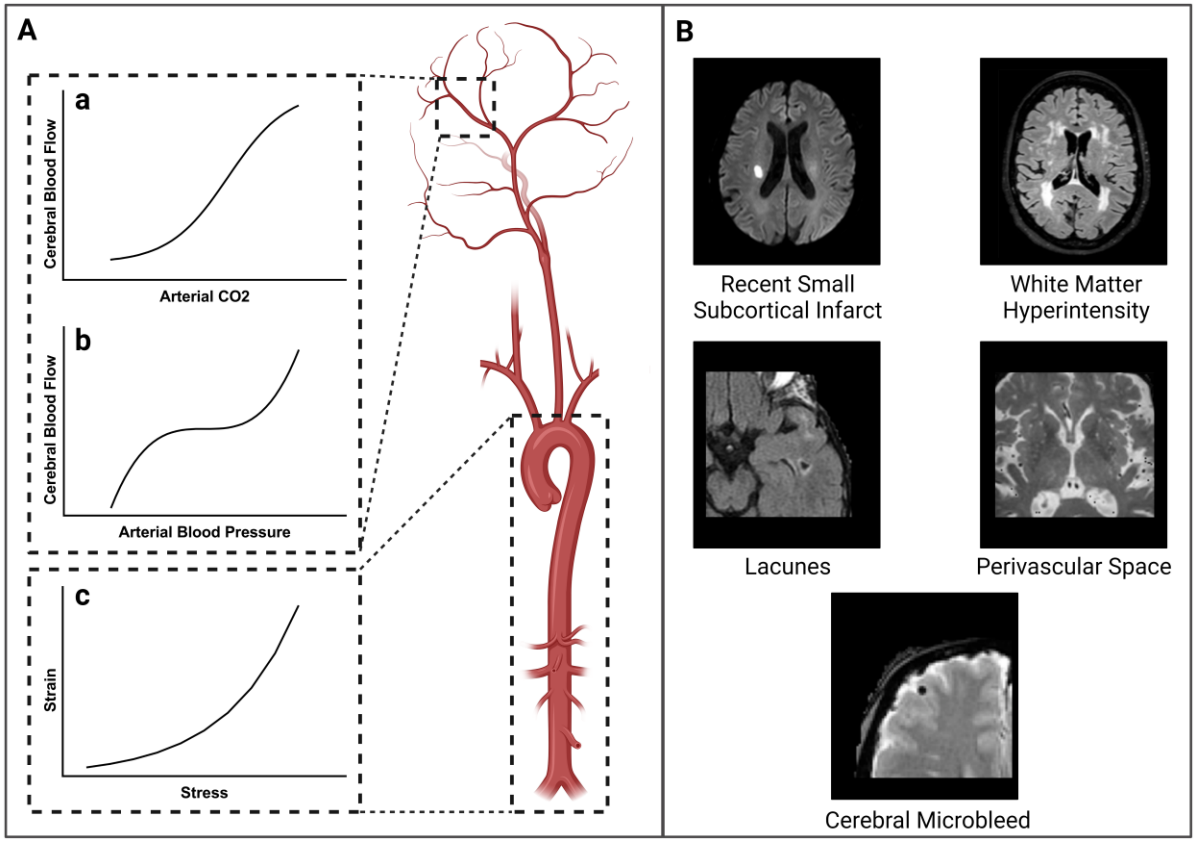


Figure 2-6. Characterization of arterial stiffness and cerebrovascular regulation in relation to cSVD. A: a) Cerebrovascular reactivity is an aspect of the chemo-regulation of cerebral blood flow, where a healthy cerebrovascular system should demonstrate a positive relationship between cerebral blood flow and arterial carbon dioxide content (arterial CO2). b) Cerebral autoregulation is the regulatory process by which the cerebral circulation maintains relatively stable perfusion through a range of arterial blood pressures. c) In-vivo measurements of arterial stiffness capture incremental stress-strain relationships, describing the overall impact that arterial structural and functional components have on arterial elasticity, buffering of pulsatile pressures, etc. **B:** Examples of MRI images depicting the five primary radiological markers of cSVD. Created with BioRender.com. MRI images adapted from Table 1 from Bennett et al.[206] used under CC BY 4.0.

increases in arterial stiffness and cSVD. In a limited number of studies however, meta-analysis only revealed a borderline association between cerebrovascular reactivity and cSVD. However, the heterogeneity and limited data result in uncertainty to the strength of these relationships and highlight the need for longitudinal investigations into vascular stiffness and regulatory function as possible risk factors for cSVD.

Cerebrovascular Reactivity

The present study suggests that reduced cerebrovascular reactivity is associated with an increased cSVD burden. As one of the standard measures of cerebrovascular reactivity first used in 1990 by Ratnatunga and Adiseshiah[207], the BHI has demonstrated predictive capabilities in patients with asymptomatic carotid artery stenosis for the risk of ischemic stroke events.[208] While typically measured in the context of cerebrovascular disease[29,209], cerebrovascular reactivity has been previously shown to relate to cardiac arrythmias[210], cardiorespiratory fitness[211], and all-cause mortality[212], which suggests systemic influences. This may be due to the assessment of cerebrovascular reactivity as an indicator of endothelial function, a known predictor of regional and global cardiovascular dysfunction.[27,28]

While hypertension and other vascular risk factors are often referred to as critical factors in the development and progression of cSVD, more recent evidence suggests that the etiology of cSVD is complex and likely extends beyond arterial blood pressure.[11,12] The hypothesis that the characterization of cSVD may include reductions in cerebrovascular reactivity alongside other risk factors is supported by Molina et al.[51], who reported that in 92 participants, cerebrovascular reactivity and hypertension were both significantly associated with lacunar infarction.

It is worth noting that the limited number of studies available for meta-analysis coupled with different methods of assessing cerebrovascular reactivity could affect the strength of the relationship between cerebrovascular reactivity and cSVD. The wide variability in approaches may have contributed to the considerable heterogeneity (I^2=68%) observed in the meta-analysis of dichotomous cerebrovascular reactivity. Due to the limited number of studies and inconsistencies in determining cut-off values, it was not possible to explore this possible source of heterogeneity further in the present analyses. Adapting a standard approach in future investigations may help establish cerebrovascular reactivity as a functional assessment of the cerebrovasculature for further investigations. Moreover, of the eligible studies for inclusion in the quantitative meta-analysis, we were only able to pool the results from 7 studies out of the 46 included in the systematic review, highlighting a critical need for expansion of the body of evidence.

Cerebral Autoregulation

Cerebral autoregulation is a regulatory mechanism serving to maintain cerebral perfusion throughout a range of physiological variations in perfusion pressure[32]. Of the 14 articles extracted for cerebral autoregulation in the systematic analysis, 11 studies suggested a significant association between impaired cerebral autoregulation and greater cSVD. However, numerous methods exist for determining cerebral autoregulation, limiting formal interpretation across studies. One prominent approach uses frequency domain transformations of arterial blood pressure and cerebral artery blood velocity, calculating gain and phase from frequency data.[32,33] Other approaches incorporate time domain indices, including moving correlation coefficients between blood pressure and cerebral artery blood velocity.[34] Work beginning less than a decade

ago has attempted to establish a standardized approach[213], but methodological convergence is still progressing.[34,214] Present qualitative findings suggest the possibility of cerebral autoregulatory impairments as a target for understanding the pathophysiology of cSVD. However, the limited number of studies eligible for meta-analysis demonstrates an avenue for future work to expand on the contributions of impaired cerebral autoregulation to cSVD.

Arterial Stiffness

Central arterial stiffening, which occurs with healthy aging and in many pathological states, leads to a diminished impedance gradient between the proximal aorta, carotid arteries, and cerebral vasculature.[17-19] This reduces wave reflection and enhances the transmission of pulsatile waves deeper into the cerebral vascular bed.[18,20] Pre-clinical models using transverse aortic constriction surgery[215] or a genetic model of arterial stiffness[216] to increase carotid artery pulse pressure have suggested that enhanced cerebrovascular pulsatility impaired endothelium-dependent vasodilation[216], disrupted blood-brain barrier function, and resulted in cerebral microbleeds.[215] While our qualitative and quantitative syntheses of studies do not have enough longitudinal information to address the specific underlying pathophysiological mechanisms that precede cSVD development, the significant associations between arterial stiffness, cerebrovascular reactivity, and cSVD demonstrated herein suggest possible mechanisms associated with the presence of cSVD.

Both the continuous arterial stiffness meta-analysis and the dichotomous arterial stiffness meta-analysis displayed significant heterogeneity (I^2=72% and 84%, respectively). To address this, we performed a sub-analysis analysis using only the studies that measured cfPWV to support this point, which revealed a heterogeneity numerically less than observed in the primary

analysis. Another possible explanation is the challenge of disentangling arterial stiffness from lifestyle factors and comorbidities associated with arterial stiffness and cSVD. Positive lifestyle activities, such as physical activity, have previously been shown to reduce arterial stiffness.[217,218] It is possible that this may have been a factor in studies such as Amier et al.[118] or Hashimoto et al.[163], where the average cfPWV values of participants were lower than expected for the sample age and blood pressure.[219] However, physical activity assessments were not commonly reported in presently-included studies, and none incorporated laboratory measurements of gold-standard indices of cardiorespiratory fitness (i.e., VO_2 peak or maximal values) that have been shown to be associated with arterial stiffness.[217] We performed meta-regression to investigate further sources of heterogeneity associated with three comorbidities (hypertension, diabetes mellitus, and hypercholesterolemia), demonstrating that including these three study-level covariates explained ~30% of the overall model heterogeneity. However, caution with interpretation is warranted – these covariates were extracted from study-level data, not individual patient data. Therefore, extrapolation of this information is dependent on the study samples included. Of relevance, the majority of the studies included (20 of the 25, 80%) focused on generally healthy participants with varying proportions of risk factors. In contrast, only 7 of the remaining 12 studies (58%) not included in the meta-regression focused on healthy populations.

An additional cause of heterogeneity in the present study is the different pathogenic processes of the individual cSVD subtypes. In subgroup analyses between arterial stiffness, white matter hyperintensities, and lacunes, the heterogeneity was not significant in the white matter hyperintensity analysis (I^2=31%, P=0.15), while the lacune analysis remained considerably heterogeneous (I^2=70%). These findings may be related to a strong dependence of

lacunes on age[144], whereas white matter hyperintensities have been suggested to be associated with arterial stiffness, vascular injury, and blood-brain barrier dysfunction.[4,12,220,221]

Implications

The implications of the present work are that impairments in cerebrovascular regulatory functions and increased arterial stiffness are associated with cSVD burden. Assessing these vascular traits may aid in further understanding the vascular etiologies or pathophysiological progression of cSVD. Despite a prevalence of cSVD that has been suggested to be ≥90% in aging populations[222], the underlying mechanisms of cSVD are still incompletely understood. Previous literature has established the contributions of high blood pressure and hypertension[223,224] to cSVD. However, studies using antihypertensive medications targeting reductions in cSVD have been sparse[8] and demonstrate overall mixed responses[225-228], suggesting that additional contributing factors may interact with or independent of hypertension and other traditional comorbidities. In line with the present study's findings, a recent series of randomized controlled trials, the Lacunar Intervention Trial-1 and Trial-2 (LACI-1 and LACI-2), demonstrated that treatments targeting vascular endothelial function improved cerebrovascular reactivity in cSVD patients[229] and improved outcomes in cSVD patients.[230] Importantly, the results of the present study do not suggest replacing monitoring or treatment of hypertension, but suggest that additional assessments of cerebrovascular autoregulation, cerebrovascular reactivity, and/or arterial stiffness may be useful. When assessed alongside traditional cardiovascular measurements (i.e., blood pressure testing, echocardiography, etc.), incorporating these three measures of vascular structure and function in longitudinal studies or studies focusing on

clinically relevant populations may provide further critical insight into the underlying pathophysiology that contributes to the development or progression of cSVD.

Study Limitations

There were three main limitations in the present study. First, age and hypertension are important cardiovascular risk factors that may contribute to the development or progression of cSVD. These factors also impact the three primary measures incorporated into the present review and meta-analyses.[137,163,231-233] Although blood pressure or hypertension may be a confounding factor, many studies adjusted for this. However, it is worth noting that we did not have any randomized-controlled trials in our meta-analyses, which may partly explain discrepancies regarding the impact of hypertension on cSVD.[41,234] Second, there was significant heterogeneity between studies in terms of study population demographics, methods of assessing the primary predictor variable, and the subtype(s) of cSVD used as the outcome. A key possibility that may have affected all three primary exposures is the presence of large or small artery disease, such as arterial stenosis. Stenotic intracranial and extracranial arteries have been associated with an increased burden of cSVD[235] alongside separate associations of arterial stenosis with increased arterial stiffness[236], impaired cerebrovascular reactivity[237], and impaired cerebral autoregulation.[238] As pointed out in a recent consensus statement[239], current measures of arterial stenosis do not provide deeper information into the mechanisms linking stenosis and cSVD. While outside the scope of the present analyses, the findings presented herein suggest that incorporating measures of cerebrovascular regulation and arterial stiffness as mediators of the link between stenosis and cSVD could further our understanding of the pathophysiology. Finally, we acknowledge that there are statistical limitations in using pooled OR as the primary outcome

in that these pooled measures may come from different methodological designs and result in high levels of heterogeneity.

Conclusions

The present systematic review and meta-analyses demonstrate significant associations between impaired cerebrovascular reactivity, increased arterial stiffness and cSVD. Collectively, the current literature suggests that measurements of cerebrovascular regulation and arterial stiffness may provide feasible means of further investigating the mechanisms involved in the development or progression of cSVD. These findings highlight the utility of vascular assessment in furthering our understanding of the underlying pathophysiology of cSVD.

References

1. Pasi M, Cordonnier C. Clinical Relevance of Cerebral Small Vessel Diseases. Stroke. 2020;51:47-53. doi: 10.1161/STROKEAHA.119.024148
2. Nam KW, Kwon HM, Lee YS, Kim JM, Ko SB. Effects of cerebral small vessel disease on the outcomes in cryptogenic stroke with active cancer. Sci Rep. 2021;11. doi: ARTN 1751010.1038/s41598-021-97154-1
3. Smallwood A, Oulhaj A, Joachim C, Christie S, Sloan C, Smith AD, Esiri M. Cerebral subcortical small vessel disease and its relation to cognition in elderly subjects: a pathological study in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort. Neuropath Appl Neuro. 2012;38:337-343. doi: 10.1111/j.1365-2990.2011.01221.x
4. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurology. 2013;12:822-838. doi: Doi 10.1016/S1474-4422(13)70124-8
5. Wardlaw JM, Benveniste H, Williams A. Cerebral Vascular Dysfunctions Detected in Human Small Vessel Disease and Implications for Preclinical Studies. Annu Rev Physiol. 2022;84:409-434. doi: 10.1146/annurev-physiol-060821-014521
6. Liang Y, Chen YK, Deng M, Mok VCT, Wang DF, Ungvari GS, Chu CW, Kamiya A, Tang WK. Association of Cerebral Small Vessel Disease Burden and Health-Related Quality of Life after Acute Ischemic Stroke. Front Aging Neurosci. 2017;9:372. doi: 10.3389/fnagi.2017.00372

7. Song TJ, Kim J, Song D, Yoo J, Lee HS, Kim YJ, Nam HS, Heo JH, Kim YD. Total Cerebral Small-Vessel Disease Score is Associated with Mortality during Follow-Up after Acute Ischemic Stroke. J Clin Neurol. 2017;13:187-195. doi: 10.3988/jcn.2017.13.2.187
8. Wardlaw JM, Debette S, Jokinen H, De Leeuw FE, Pantoni L, Chabriat H, Staals J, Doubal F, Rudilosso S, Eppinger S, et al. ESO Guideline on covert cerebral small vessel disease. Eur Stroke J. 2021;6:CXI-CLXII. doi: 10.1177/23969873211012132
9. Nam KW, Kwon HM, Jeong HY, Park JH, Kwon H, Jeong SM. Cerebral Small Vessel Disease and Stage 1 Hypertension Defined by the 2017 American College of Cardiology/American Heart Association Guidelines. Hypertension. 2019;73:1210-1216. doi: 10.1161/HYPERTENSIONAHA.119.12830
10. Petrea RE, O'Donnell A, Beiser AS, Habes M, Aparicio H, DeCarli C, Seshadri S, Romero JR. Mid to Late Life Hypertension Trends and Cerebral Small Vessel Disease in the Framingham Heart Study. Hypertension. 2020;76:707-714. doi: 10.1161/HYPERTENSIONAHA.120.15073
11. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol. 2013;12:483-497. doi: 10.1016/S1474-4422(13)70060-7
12. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurology. 2019;18:684-696. doi: 10.1016/S1474-4422(19)30079-1
13. Lammie GA, Brannan F, Slattery J, Warlow C. Nonhypertensive cerebral small-vessel disease - An autopsy study. Stroke. 1997;28:2222-2229. doi: Doi 10.1161/01.Str.28.11.2222

14. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women - The Framingham Heart Study. Hypertension. 2004;43:1239-1245. doi: 10.1161/01.HYP.0000128420.01881.aa

15. Li N, Zhang GW, Zhang JR, Jin D, Li Y, Liu T, Wang RT. Non-alcoholic fatty liver disease is associated with progression of arterial stiffness. Nutr Metab Cardiovas. 2015;25:218-223. doi: 10.1016/j.numecd.2014.10.002

16. Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. Curr Opin Nephrol Hy. 2015;24:47-53. doi: 10.1097/Mnh.0000000000000086

17. Chirinos JA, Segers P, Hughes T, Townsend R. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;74:1237-1263. doi: 10.1016/j.jacc.2019.07.012

18. Mitchell GF. Cerebral small vessel disease: role of aortic stiffness and pulsatile hemodynamics. J Hypertens. 2015;33:2025-2028. doi: 10.1097/HJH.0000000000000717

19. Mitchell GF. Aortic stiffness, pressure and flow pulsatility, and target organ damage. J Appl Physiol. 2018;125:1871-1880. doi: 10.1152/japplphysiol.00108.2018

20. Chirinos JA. Large Artery Stiffness, Microvascular Function, and Cardiovascular Risk. Circ Cardiovasc Imaging. 2016;9. doi: 10.1161/CIRCIMAGING.116.005903

21. Mitchell GF, van Buchem MA, Sigurdsson S, Gotal JD, Jonsdottir MK, Kjartansson O, Garcia M, Aspelund T, Harris TB, Gudnason V, et al. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility - Reykjavik Study. Brain. 2011;134:3398-3407. doi: 10.1093/brain/awr253

22. Zhan ZX, Xu T, Xu Y, Fu FW, Cheng ZC, Xia LF, Wu YC, Xu X, Cao YA, Han Z. Associations between computed tomography markers of cerebral small vessel disease and hemorrhagic transformation after intravenous thrombolysis in acute ischemic stroke patients. Front Neurol. 2023;14. doi: ARTN 114456410.3389/fneur.2023.1144564

23. Timmerman N, Rots ML, van Koeverden ID, Haitjema S, van Laarhoven CJHCM, Vuurens AM, den Ruijter HM, Pasterkamp G, Kappelle LJ, de Kleijn DPV, et al. Cerebral Small Vessel Disease in Standard Pre-operative Imaging Reports Is Independently Associated with Increased Risk of Cardiovascular Death Following Carotid Endarterectomy. Eur J Vasc Endovasc. 2020;59:872-880. doi: 10.1016/j.ejvs.2020.02.004

24. Chen CH, Hsu HC, Cheng YW, Chen YF, Tang SC, Jeng JS. Prominent juxtacortical white matter lesion hallmarks -related intracerebral haemorrhage. Stroke Vasc Neurol. 2022;7:38-46. doi: 10.1136/svn-2021-001020

25. Duering M, Biessels GJ, Brodtmann A, Chen C, Cordonnier C, de Leeuw FE, Debette S, Frayne R, Jouvent E, Rost NS, et al. Neuroimaging standards for research into small vessel disease-advances since 2013. Lancet Neurol. 2023;22:602-618. doi: 10.1016/s1474-4422(23)00131-x

26. Chang BH, Hoaglin DC. Meta-Analysis of Odds Ratios Current Good Practices. Med Care. 2017;55:328-335. doi: Doi 10.1097/Mlr.0000000000000696

27. Lavi S, Gaitini D, Milloul V, Jacob G. Impaired cerebral CO2 vasoreactivity: association with endothelial dysfunction. Am J Physiol Heart Circ Physiol. 2006;291:H1856-1861. doi: 10.1152/ajpheart.00014.2006

28. Ainslie PN, Murrell C, Peebles K, Swart M, Skinner MA, Williams MJ, Taylor RD. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. Exp Physiol. 2007;92:769-777. doi: 10.1113/expphysiol.2006.036814

29. Fierstra J, Sobczyk O, Battisti-Charbonney A, Mandell DM, Poublanc J, Crawley AP, Mikulis DJ, Duffin J, Fisher JA. Measuring cerebrovascular reactivity: what stimulus to use? J Physiol. 2013;591:5809-5821. doi: 10.1113/jphysiol.2013.259150

30. Tan CO. Defining the characteristic relationship between arterial pressure and cerebral flow. J Appl Physiol. 2012;113:1194-1200. doi: 10.1152/japplphysiol.00783.2012

31. Willie CK, Tzeng YC, Fisher JA, Ainslie PN. Integrative regulation of human brain blood flow. J Physiol-London. 2014;592:841-859. doi: 10.1113/jphysiol.2013.268953

32. Al-Kawaz M, Cho SM, Gottesman RF, Suarez JI, Rivera-Lara L. Impact of Cerebral Autoregulation Monitoring in Cerebrovascular Disease: A Systematic Review. Neurocrit Care. 2022;36:1053-1070. doi: 10.1007/s12028-022-01484-5

33. Brassard P, Labrecque L, Smirl JD, Tymko MM, Caldwell HG, Hoiland RL, Lucas SJE, Denault AY, Couture EJ, Ainslie PN. Losing the dogmatic view of cerebral autoregulation. Physiol Rep. 2021;9:e14982. doi: 10.14814/phy2.14982

34. Olsen MH, Riberholt CG, Mehlsen J, Berg RM, Moller K. Reliability and validity of the mean flow index (Mx) for assessing cerebral autoregulation in humans: A systematic review of the methodology. J Cereb Blood Flow Metab. 2022;42:27-38. doi: 10.1177/0271678X211052588

35. Aribisala BS, Morris Z, Eadie E, Thomas A, Gow A, Hernandez MCV, Royle NA, Bastin ME, Starr J, Deary IJ, et al. Blood Pressure, Internal Carotid Artery Flow Parameters, and

Age-Related White Matter Hyperintensities. Hypertension. 2014;63:1011-1018. doi: 10.1161/HYPERTENSIONAHA.113.02735

36. de Havenon A, Wong KH, Elkhetali A, McNally JS, Majersik JJ, Rost NS. Carotid Artery Stiffness Accurately Predicts White Matter Hyperintensity Volume 20 Years Later: A Secondary Analysis of the Atherosclerosis Risk in the Community Study. Am J Neuroradiol. 2019;40:1369-1373. doi: 10.3174/ajnr.A6115

37. Ding J, Mitchell GF, Bots ML, Sigurdsson S, Harris TB, Garcia M, Eiriksdottir G, van Buchem MA, Gudnason V, Launer LJ. Carotid Arterial Stiffness and Risk of Incident Cerebral Microbleeds in Older People The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. Arterioscler Thromb Vasc Biol. 2015;35:1889-1895. doi: 10.1161/ATVBAHA.115.305451

38. Potter GM, Marlborough FJ, Wardlaw JM. Wide Variation in Definition, Detection, and Description of Lacunar Lesions on Imaging. Stroke. 2011;42:359-366. doi: 10.1161/Strokeaha.110.594754

39. Makin SDJ, Cook FAB, Dennis MS, Wardlaw JM. Cerebral Small Vessel Disease and Renal Function: Systematic Review and Meta-Analysis. Cerebrovasc Dis. 2015;39:39-52. doi: 10.1159/000369777

40. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25:603-605. doi: 10.1007/s10654-010-9491-z

41. Tully PJ, Yano Y, Launer LJ, Kario K, Nagai M, Mooijaart SP, Claassen J, Lattanzi S, Vincent AD, Tzourio C, et al. Association Between Blood Pressure Variability and

Cerebral Small-Vessel Disease: A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2020;9:e013841. doi: 10.1161/JAHA.119.013841

42. Hannawi Y, Yanek LR, Kral BG, Vaidya D, Becker LC, Becker DM, Nyquist PA. Arterial Stiffness Relationship With Periventricular and Deep White Matter Hyperintensities in a Middle-Aged Population. Stroke. 2021;52. doi: 10.1161/str.52.suppl_1.P65

43. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.4 (updated August 2023). Cochrane, 2023. Available from www.training.cochrane.org/handbook.

44. Wan X, Wang WQ, Liu JM, Tong TJ. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. Bmc Med Res Methodol. 2014;14. doi: Artn 13510.1186/1471-2288-14-135

45. Symons MJ, Moore DT. Hazard rate ratio and prospective epidemiological studies. J Clin Epidemiol. 2002;55:893-899. doi: Pii S0895-4356(02)00443-2

46. Rao G, Lopez-Jimenez F, Boyd J, D'Amico F, Durant NH, Hlatky MA, Howard G, Kirley K, Masi C, Powell-Wiley TM, et al. Methodological Standards for Meta-Analyses and Qualitative Systematic Reviews of Cardiac Prevention and Treatment Studies: A Scientific Statement From the American Heart Association. Circulation. 2017;136:e172-e194. doi: 10.1161/CIR.0000000000000523

47. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, deBeer H, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64:383-394. doi: 10.1016/j.jclinepi.2010.04.026

48. Hultcrantz M, Rind D, Akl EA, Treweek S, Mustafa RA, Iorio A, Alper BS, Meerpohl JJ, Murad MH, Ansari MT, et al. The GRADE Working Group clarifies the construct of certainty of evidence. J Clin Epidemiol. 2017;87:4-13. doi: 10.1016/j.jclinepi.2017.05.006

49. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Bmj-Brit Med J. 1997;315:629-634. doi: DOI 10.1136/bmj.315.7109.629

50. Sterne JAC, Egger M, Smith GD. Systematic reviews in health care - Investigating and dealing with publication and other biases in meta-analysis. Bmj-Brit Med J. 2001;323:101-105. doi: DOI 10.1136/bmj.323.7304.101

51. Molina C, Sabín JA, Montaner J, Rovira A, Abilleira S, Codina A. Impaired cerebrovascular reactivity as a risk marker for first-ever lacunar infarction: A case-control study. Stroke. 1999;30:2296-2301. doi: 10.1161/01.str.30.11.2296

52. Deplanque D, Lavallee PC, Labreuche J, Gongora-Rivera F, Jaramillo A, Brenner D, Abboud H, Klein IF, Touboul PJ, Vicaut E, et al. Cerebral and extracerebral vasoreactivity in symptomatic lacunar stroke patients: a case-control study. Int J Stroke. 2013;8:413-421. doi: 10.1111/j.1747-4949.2011.00755.x

53. Silvestrini M, Pasqualetti P, Baruffaldi R, Catani S, Tibuzzi F, Altamura C, Bartolini M, Provinciali L, Vernieri F. Markers of lacunar stroke in patients with moderate internal carotid artery stenosis. J Neurol. 2006;253:321-327. doi: 10.1007/s00415-005-0989-3

54. Conijn MM, Hoogduin JM, van der Graaf Y, Hendrikse J, Luijten PR, Geerlings MI. Microbleeds, lacunar infarcts, white matter lesions and cerebrovascular reactivity -- a 7 T study. Neuroimage. 2012;59:950-956. doi: 10.1016/j.neuroimage.2011.08.059

55. Bisschops RH, Klijn CJ, Kappelle LJ, van Huffelen AC, van der Grond J. Association between impaired carbon dioxide reactivity and ischemic lesions in arterial border zone territories in patients with unilateral internal carotid artery occlusion. Arch Neurol. 2003;60:229-233. doi: 10.1001/archneur.60.2.229

56. Moreton FC, Cullen B, Delles C, Santosh C, Gonzalez RL, Dani K, Muir KW. Vasoreactivity in CADASIL: Comparison to structural MRI and neuropsychology. J Cereb Blood Flow Metab. 2018;38:1085-1095. doi: 10.1177/0271678x17710375

57. Blair GW, Thrippleton MJ, Shi YL, Hamilton I, Stringer M, Chappell F, Dickie DA, Andrews P, Marshall I, Doubal FN, et al. Intracranial hemodynamic relationships in patients with cerebral small vessel disease. Neurology. 2020;94:E2258-E2269. doi: 10.1212/WNL.0000000000009483

58. Staszewski J, Skrobowska E, Piusinska-Macoch R, Brodacki B, Stepien A. Cerebral and Extracerebral Vasoreactivity in Patients With Different Clinical Manifestations of Cerebral Small-Vessel Disease: Data From the Significance of Hemodynamic and Hemostatic Factors in the Course of Different Manifestations of Cerebral Small-Vessel Disease Study. J Ultrasound Med. 2019;38:975-987. doi: 10.1002/jum.14782

59. Tawfik MM, Ebrahim A, Hamed S, Haroun M. Transcranial Doppler assessment of patients with cerebral small vessel disease. Egypt J Neurol Psych. 2022;58. doi: ARTN 156

60. Moreton FC, Cullen B, Dickie DA, Gonzalez RL, Santosh C, Delles C, Muir KW. Brain imaging factors associated with progression of subcortical hyperintensities in CADASIL over 2-year follow-up. Eur J Neurol. 2021;28:220-228. doi: 10.1111/ene.14534

61. Liem MK, Lesnik Oberstein SA, Haan J, Boom R, Ferrari MD, Buchem MA, Grond J. Cerebrovascular reactivity is a main determinant of white matter hyperintensity progression in CADASIL. AJNR Am J Neuroradiol. 2009;30:1244-1247. doi: 10.3174/ajnr.A1533

62. Kapoor A, Yew B, Jang JY, Dutt S, Li Y, Alitin JPM, Gaubert A, Ho JK, Blanken AE, Sible IJ, et al. Older adults with perivascular spaces exhibit cerebrovascular reactivity deficits. Neuroimage. 2022;264:119746. doi: 10.1016/j.neuroimage.2022.119746

63. Chan ST, Mercaldo ND, Kwong KK, Hersch SM, Rosas HD. Impaired Cerebrovascular Reactivity in Huntington's Disease. Front Physiol. 2021;12. doi: 10.3389/fphys.2021.663898

64. Libecap TJ, Bauer CE, Zachariou V, Pappas CA, Raslau FD, Liu P, Lu H, Gold BT. Association of Baseline Cerebrovascular Reactivity and Longitudinal Development of Enlarged Perivascular Spaces in the Basal Ganglia. Stroke. 2023. doi: 10.1161/STROKEAHA.123.043882

65. Lee BC, Tsai HH, Huang A, Lo YL, Tsai LK, Chen YF, Wu WC. Arterial Spin Labeling Imaging Assessment of Cerebrovascular Reactivity in Hypertensive Small Vessel Disease. Front Neurol. 2021;12. doi: 10.3389/fneur.2021.640069

66. Beaudin AE, McCreary CR, Mazerolle EL, Gee M, Sharma B, Subotic A, Zwiers AM, Cox E, Nelles K, Charlton A, et al. Cerebrovascular Reactivity Across the Entire Brain in Cerebral Amyloid Angiopathy. Neurology. 2022;98:e1716-e1728. doi: 10.1212/wnl.0000000000200136

67. Switzer AR, Cheema I, McCreary CR, Zwiers A, Charlton A, Alvarez-Veronesi A, Sekhon R, Zerna C, Stafford RB, Frayne R, et al. Cerebrovascular reactivity in cerebral

amyloid angiopathy, Alzheimer disease, and mild cognitive impairment. Neurology. 2020;95:e1333-e1340. doi: 10.1212/wnl.0000000000010201

68. Last D, Alsop DC, Abduljalil AM, Marquis RP, de Bazelaire C, Hu K, Cavallerano J, Novak V. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. Diabetes Care. 2007;30:1193-1199. doi: 10.2337/dc06-2052

69. Hoogeveen ES, Pelzer N, Ghariq E, van Osch MJ, Dahan A, Terwindt GM, Kruit MC. Cerebrovascular reactivity in retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations. J Cereb Blood Flow Metab. 2021;41:831-840. doi: 10.1177/0271678x20929430

70. Kozera GM, Dubaniewicz M, Zdrojewski T, Madej-Dmochowska A, Mielczarek M, Wojczal J, Chwojnicki K, Swierblewska E, Schminke U, Wyrzykowski B, et al. Cerebral vasomotor reactivity and extent of white matter lesions in middle-aged men with arterial hypertension: a pilot study. Am J Hypertens. 2010;23:1198-1203. doi: 10.1038/ajh.2010.152

71. Buterbaugh J, Wynstra C, Provencio N, Combs D, Gilbert M, Parthasarathy S. Cerebrovascular reactivity in young subjects with sleep apnea. Sleep. 2015;38:241-250. doi: 10.5665/sleep.4406

72. Fu JH, Lu CZ, Hong Z, Dong Q, Ding D, Wong KS. Relationship between cerebral vasomotor reactivity and white matter lesions in elderly subjects without large artery occlusive disease. J Neuroimaging. 2006;16:120-125. doi: 10.1111/j.1552-6569.2006.00030.x

73. Marstrand JR, Garde E, Rostrup E, Ring P, Rosenbaum S, Mortensen EL, Larsson HB. Cerebral perfusion and cerebrovascular reactivity are reduced in white matter hyperintensities. Stroke. 2002;33:972-976. doi: 10.1161/01.str.0000012808.81667.4b

74. Uh J, Yezhuvath U, Cheng Y, Lu H. In vivo vascular hallmarks of diffuse leukoaraiosis. J Magn Reson Imaging. 2010;32:184-190. doi: 10.1002/jmri.22209

75. Bettermann K, Slocomb JE, Shivkumar V, Lott ME. Retinal vasoreactivity as a marker for chronic ischemic white matter disease? J Neurol Sci. 2012;322:206-210. doi: 10.1016/j.jns.2012.05.041

76. Sam K, Crawley AP, Poublanc J, Conklin J, Sobczyk O, Mandell DM, Duffin J, Venkatraghavan L, Fisher JA, Black SE, et al. Vascular Dysfunction in Leukoaraiosis. AJNR Am J Neuroradiol. 2016;37:2258-2264. doi: 10.3174/ajnr.A4888

77. Ni L, Zhang B, Yang D, Qin R, Xu H, Ma J, Shao P, Xu Y. Lower Cerebrovascular Reactivity Contributed to White Matter Hyperintensity-Related Cognitive Impairment: A Resting-State Functional MRI Study. J Magn Reson Imaging. 2021;53:703-711. doi: 10.1002/jmri.27376

78. Abi Zeid Daou M, Boyd BD, Donahue MJ, Albert K, Taylor WD. Anterior-posterior gradient differences in lobar and cingulate cortex cerebral blood flow in late-life depression. J Psychiatr Res. 2018;97:1-7. doi: 10.1016/j.jpsychires.2017.11.005

79. Moir ME, Corkery AT, Senese KA, Miller KB, Pearson AG, Loggie NA, Howery AJ, Gaynor-Metzinger SHA, Cody KA, Eisenmenger LB, et al. Age at natural menopause impacts cerebrovascular reactivity and brain structure. Am J Physiol Regul Integr Comp Physiol. 2023;324:R207-r215. doi: 10.1152/ajpregu.00228.2022

80. Lee MJ, Park BY, Cho S, Park H, Chung CS. Cerebrovascular reactivity as a determinant of deep white matter hyperintensities in migraine. Neurology. 2019;92:e342-e350. doi: 10.1212/wnl.0000000000006822

81. Lee MJ, Park BY, Cho S, Kim S, Park H, Kim ST, Chung CS. Cerebrovascular reactivity and deep white matter hyperintensities in migraine: A prospective CO(2) targeting study. J Cereb Blood Flow Metab. 2022;42:1879-1889. doi: 10.1177/0271678x221103006

82. Stringer MS, Blair GW, Shi Y, Hamilton I, Dickie DA, Doubal FN, Marshall IM, Thrippleton MJ, Wardlaw JM. A Comparison of CVR Magnitude and Delay Assessed at 1.5 and 3T in Patients With Cerebral Small Vessel Disease. Front Physiol. 2021;12:644837. doi: 10.3389/fphys.2021.644837

83. Atwi S, Shao H, Crane DE, da Costa L, Aviv RI, Mikulis DJ, Black SE, MacIntosh BJ. BOLD-based cerebrovascular reactivity vascular transfer function isolates amplitude and timing responses to better characterize cerebral small vessel disease. NMR Biomed. 2019;32:e4064. doi: 10.1002/nbm.4064

84. Bakker SL, de Leeuw FE, de Groot JC, Hofman A, Koudstaal PJ, Breteler MM. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology. 1999;52:578-583. doi: 10.1212/wnl.52.3.578

85. Rane S, Koh N, Boord P, Madhyastha T, Askren MK, Jayadev S, Cholerton B, Larson E, Grabowski TJ. Quantitative cerebrovascular pathology in a community-based cohort of older adults. Neurobiol Aging. 2018;65:77-85. doi: 10.1016/j.neurobiolaging.2018.01.006

86. Sur S, Lin Z, Li Y, Yasar S, Rosenberg P, Moghekar A, Hou X, Kalyani R, Hazel K, Pottanat G, et al. Association of cerebrovascular reactivity and Alzheimer pathologic

markers with cognitive performance. Neurology. 2020;95:e962-e972. doi: 10.1212/wnl.0000000000010133

87. Lee ST, Jung KH, Lee YS. Decreased vasomotor reactivity in Alzheimer's disease. J Clin Neurol. 2007;3:18-23. doi: 10.3988/jcn.2007.3.1.18

88. Sam K, Conklin J, Holmes KR, Sobczyk O, Poublanc J, Crawley AP, Mandell DM, Venkatraghavan L, Duffin J, Fisher JA, et al. Impaired dynamic cerebrovascular response to hypercapnia predicts development of white matter hyperintensities. NeuroImage-Clin. 2016;11:796-801. doi: 10.1016/j.nicl.2016.05.008

89. Sam K, Crawley AP, Conklin J, Poublanc J, Sobczyk O, Mandell DM, Venkatraghavan L, Duffin J, Fisher JA, Black SE, et al. Development of White Matter Hyperintensity Is Preceded by Reduced Cerebrovascular Reactivity. Ann Neurol. 2016;80:277-285. doi: 10.1002/ana.24712

90. Smoliński Ł, Litwin T, Kruk K, Skowrońska M, Kurkowska-Jastrzębska I, Członkowska A. Cerebrovascular reactivity and disease activity in relapsing-remitting multiple sclerosis. Adv Clin Exp Med. 2020;29:183-188. doi: 10.17219/acem/114762

91. Bian Y, Wang JC, Sun F, Sun ZY, Lin YJ, Liu Y, Zhao B, Liu L, Luo XG. Assessment of cerebrovascular reserve impairment using the breath-holding index in patients with leukoaraiosis. Neural Regen Res. 2019;14:1412-1418. doi: 10.4103/1673-5374.251332

92. Isozaki M, Kataoka H, Fukushima K, Ishibashi-Ueda H, Yamada N, Iida H, Iihara K. Silent ischemic lesion laterality in asymptomatic internal carotid artery stenosis relates to reduced cerebral vasoreactivity. Surg Neurol Int. 2017;8:6. doi: 10.4103/2152-7806.198733

93. Palaiodimou L, Papagiannopoulou G, Bakola E, Papadopoulou M, Kokotis P, Moschovos C, Vrettou AR, Kapsia E, Petras D, Anastasakis A, et al. Impaired cerebral autoregulation in Fabry disease: A case-control study. J Neuroimaging. 2023;33:582-589. doi: 10.1111/jon.13111

94. Rudilosso S, Stringer MS, Thrippleton M, Chappell F, Blair GW, Garcia DJ, Doubal F, Hamilton I, Janssen E, Kopczak A, et al. Blood-brain barrier leakage hotspots collocating with brain lesions due to sporadic and monogenic small vessel disease. J Cereb Blood Flow Metab. 2023;43:1490-1502. doi: 10.1177/0271678x231173444

95. Saka E, Atay LO, Akdemir UO, Yetim E, Balci E, Arsava EM, Topcuoglu MA. Cerebral vasomotor reactivity across the continuum of subjective cognitive impairment, amnestic mild cognitive impairment and probable Alzheimer's dementia: A transcranial Doppler and PET/MRI study. J Cereb Blood Flow Metab. 2023;43:129-137. doi: 10.1177/0271678X221124656

96. Staszewski J, Debiec A, Skrobowska E, Stepien A. Cerebral Vasoreactivity Changes Over Time in Patients With Different Clinical Manifestations of Cerebral Small Vessel Disease. Front Aging Neurosci. 2021;13. doi: 10.3389/fnagi.2021.727832

97. Smeeing DP, Hendrikse J, Petersen ET, Donahue MJ, de Vis JB. Arterial Spin Labeling and Blood Oxygen Level-Dependent MRI Cerebrovascular Reactivity in Cerebrovascular Disease: A Systematic Review and Meta-Analysis. Cerebrovasc Dis. 2016;42:288-307. doi: 10.1159/000446081

98. Zhao MY, Woodward A, Fan AP, Chen KT, Yu Y, Chen DY, Moseley ME, Zaharchuk G. Reproducibility of cerebrovascular reactivity measurements: A systematic review of

neuroimaging techniques(). J Cereb Blood Flow Metab. 2022;42:700-717. doi: 10.1177/0271678X211056702

99. Yezhuvath US, Lewis-Amezcua K, Varghese R, Xiao GH, Lu HZ. On the assessment of cerebrovascular reactivity using hypercapnia BOLD MRI. NMR Biomed. 2009;22:779-786. doi: 10.1002/nbm.1392

100. Schneider PA, Rossman ME, Bernstein EF, Torem S, Ringelstein EB, Otis SM. Effect of internal carotid artery occlusion on intracranial hemodynamics. Transcranial Doppler evaluation and clinical correlation. Stroke. 1988;19:589-593. doi: 10.1161/01.str.19.5.589

101. Apruzzese A, Silvestrini M, Floris R, Vernieri F, Bozzao A, Hagberg G, Caltagirone C, Masala S, Simonetti C. Cerebral hemodynamics in asymptomatic patients with internal carotid artery occlusion: A dynamic susceptibility contrast MR and transcranial Doppler study. Am J Neuroradiol. 2001;22:1062-1067.

102. Guo ZN, Xing YQ, Wang S, Ma HY, Liu J, Yang Y. Characteristics of dynamic cerebral autoregulation in cerebral small vessel disease: Diffuse and sustained. Sci Rep. 2015;5. doi: 10.1038/srep15269

103. Immink RV, van Montfrans GA, Stam J, Karemaker JM, Diamant M, van Lieshout JJ. Dynamic cerebral autoregulation in acute lacunar and middle cerebral artery territory ischemic stroke. Stroke. 2005;36:2595-2600. doi: 10.1161/01.STR.0000189624.06836.03

104. Meyer JS, Shimazu K, Fukuuchi Y, Ouchi T, Okamoto S, Koto A, Ericsson AD. Impaired Neurogenic Cerebrovascular Control and Dysautoregulation After Stroke. Stroke. 1973;4:169-186. doi: 10.1161/01.STR.4.2.169

105. Xiong L, Tian G, Lin W, Wang W, Wang L, Leung T, Mok V, Liu J, Chen X, Wong KS. Is Dynamic Cerebral Autoregulation Bilaterally Impaired after Unilateral Acute Ischemic

Stroke? J Stroke Cerebrovasc Dis. 2017;26:1081-1087. doi: 10.1016/j.jstrokecerebrovasdis.2016.12.024

106. Wu XH, Zhou YX, Qi WW, Shen YX, Lei ZH, Xiao K, Zhang PD, Liu J, Ren LJ. Clinical factors associated with cerebral autoregulation in ischemic stroke related to small artery occlusion. BMC Neurol. 2022;22. doi: 10.1186/s12883-022-02854-4

107. Liu Z, Ma H, Guo ZN, Wang L, Qu Y, Fan L, Liu X, Liu J, Zhu Y, Yang Y. Impaired dynamic cerebral autoregulation is associated with the severity of neuroimaging features of cerebral small vessel disease. CNS Neurosci Ther. 2022;28:298-306. doi: 10.1111/cns.13778

108. Reinhard M, Lorenz L, Sommerlade L, Allignol A, Urbach H, Weiller C, Egger K. Impaired dynamic cerebral autoregulation in patients with cerebral amyloid angiopathy. Brain Res. 2019;1717:60-65. doi: 10.1016/j.brainres.2019.04.014

109. Castro P, Azevedo E, Rocha I, Sorond F, Serrador JM. Chronic kidney disease and poor outcomes in ischemic stroke: is impaired cerebral autoregulation the missing link? BMC Neurol. 2018;18. doi: 10.1186/s12883-018-1025-4

110. Nomura Y, Faegle R, Hori D, Al-Qamari A, Nemeth AJ, Gottesman R, Yenokyan G, Brown C, Hogue CW. Cerebral Small Vessel, But Not Large Vessel Disease, Is Associated With Impaired Cerebral Autoregulation During Cardiopulmonary Bypass: A Retrospective Cohort Study. Anesth Analg. 2018;127:1314-1322. doi: 10.1213/ANE.0000000000003384

111. Brickman AM, Guzman VA, Gonzalez-Castellon M, Razlighi Q, Gu Y, Narkhede A, Janicki S, Ichise M, Stern Y, Manly JJ, et al. Cerebral autoregulation, beta amyloid, and

white matter hyperintensities are interrelated. Neurosci Lett. 2015;592:54-58. doi: 10.1016/j.neulet.2015.03.005

112. Liu J, Tseng BY, Khan MA, Tarumi T, Hill C, Mirshams N, Hodics TM, Hynan LS, Zhang R. Individual variability of cerebral autoregulation, posterior cerebral circulation and white matter hyperintensity. J Physiol-London. 2016;594:3141-3155. doi: 10.1113/JP271068

113. Muller M, Osterreich M, Lakatos L, von Hessling A. Cerebral macro- and microcirculatory blood flow dynamics in successfully treated chronic hypertensive patients with and without white mater lesions. Sci Rep. 2020;10. doi: 10.1038/s41598-020-66317-x

114. Purkayastha S, Fadar O, Mehregan A, Salat DH, Moscufo N, Meier DS, Guttmann CRG, Fisher NDL, Lipsitz LA, Sorond FA. Impaired cerebrovascular hemodynamics are associated with cerebral white matter damage. J Cereb Blood Flow Metab. 2014;34:228-234. doi: 10.1038/jcbfm.2013.180

115. He L, Guo ZN, Qu Y, Wang RT, Zhang P, Yang Y, Jin H. Effect of dynamic cerebral autoregulation on the association between deep medullary vein changes and cerebral small vessel disease. Front Physiol. 2023;14. doi: ARTN 103787110.3389/fphys.2023.1037871

116. Panerai RB, Rennie JM, Kelsall AWR, Evans DH. Frequency-domain analysis of cerebral autoregulation from spontaneous fluctuations in arterial blood pressure. Med Biol Eng Comput. 1998;36:315-322. doi: Doi 10.1007/Bf02522477

117. Liu XY, Czosnyka M, Donnelly J, Budohoski KP, Varsos GV, Nasr N, Brady KM, Reinhard M, Hutchinson PJ, Smielewski P. Comparison of frequency and time domain

methods of assessment of cerebral autoregulation in traumatic brain injury. J Cereb Blood Flow Metab. 2015;35:248-256. doi: 10.1038/jcbfm.2014.192

118. Amier RP, Marcks N, Hooghiemstra AM, Nijveldt R, van Buchem MA, de Roos A, Biessels GJ, Kappelle LJ, van Oostenbrugge RJ, van der Geest RJ, et al. Hypertensive Exposure Markers by MRI in Relation to Cerebral Small Vessel Disease and Cognitive Impairment. JACC Cardiovasc Imaging. 2021;14:176-185. doi: 10.1016/j.jcmg.2020.06.040

119. Brisset M, Boutouyrie P, Pico F, Zhu YC, Zureik M, Schilling S, Dufouil C, Mazoyer B, Laurent S, Tzourio C, et al. Large-vessel correlates of cerebral small-vessel disease. Neurology. 2013;80:662-669. doi: 10.1212/WNL.0b013e318281ccc2

120. Cooper LL, Woodard T, Sigurdsson S, van Buchem MA, Torjesen AA, Inker LA, Aspelund T, Eiriksdottir G, Harris TB, Gudnason V, et al. Cerebrovascular Damage Mediates Relations Between Aortic Stiffness and Memory. Hypertension. 2016;67:176-182. doi: 10.1161/HYPERTENSIONAHA.115.06398

121. Hashimoto J, Aikawa T, Imai Y. Large Artery Stiffening as a Link Between Cerebral Lacunar Infarction and Renal Albuminuria. Am J Hypertens. 2008;21:1304-1309. doi: 10.1038/ajh.2008.291

122. Jochemsen HM, Muller M, Bots ML, Scheltens P, Vincken KL, Mali WP, van der Graaf Y, Geerlings MI. Arterial stiffness and progression of structural brain changes: The SMART-MR study. Neurology. 2015;84:448-455. doi: 10.1212/wnl.0000000000001201

123. Poels MMF, Zaccai K, Verwoert GC, Vernooij MW, Hofman A, van der Lugt A, Witteman JCM, Breteler MMB, Mattace-Raso FUS, Ikram MA. Arterial Stiffness and

Cerebral Small Vessel Disease The Rotterdam Scan Study. Stroke. 2012;43:2637-+. doi: 10.1161/STROKEAHA.111.642264

124. Tabata N, Sueta D, Yamashita T, Utsunomiya D, Arima Y, Yamamoto E, Tsujita K, Kojima S, Kaikita K, Hokimoto S. Relationship between asymptomatic intra-cranial lesions and brachial-ankle pulse wave velocity in coronary artery disease patients without stroke. Hypertens Res. 2017;40:392-398. doi: 10.1038/hr.2016.159

125. van Elderen SGC, Brandts A, Westenberg JJM, van der Grond J, Tamsma JT, van Buchem MA, Romijn JA, Kroft LJM, Smit JWA, de Roos A. Aortic stiffness is associated with cardiac function and cerebral small vessel disease in patients with type 1 diabetes mellitus: assessment by magnetic resonance imaging. Eur Radiol. 2010;20:1132-1138. doi: 10.1007/s00330-009-1655-4

126. Palta P, Sharrett AR, Wei JK, Meyer ML, Kucharska-Newton A, Power MC, Deal JA, Jack CR, Knopman D, Wright J, et al. Central Arterial Stiffness Is Associated With Structural Brain Damage and Poorer Cognitive Performance: The ARIC Study. J Am Heart Assoc. 2019;8. doi: 10.1161/JAHA.118.011045

127. Inkeri J, Tynjala A, Forsblom C, Liebkind R, Tatlisumak T, Thorn LM, Groop PH, Shams S, Putaala J, Martola J, et al. Carotid intima-media thickness and arterial stiffness in relation to cerebral small vessel disease in neurologically asymptomatic individuals with type 1 diabetes. Acta Diabetol. 2021;58:929-937. doi: 10.1007/s00592-021-01678-x

128. Tuijl RJ, Ruigrok YM, Geurts LJ, Schaaf IC, Biessels GJ, Rinkel GJE, Velthuis BK, Zwanenburg JJM. Does the Internal Carotid Artery Attenuate Blood-Flow Pulsatility in Small Vessel Disease? A 7 T 4D-Flow MRI Study. J Magn Reson Imaging. 2022;56:527-535. doi: 10.1002/jmri.28062

129. Webb AJS, Lawson A, Wartolowska K, Mazzucco S, Rothwell PM. Aortic Stiffness, Pulse Pressure, and Cerebral Pulsatility Progress Despite Best Medical Management: The OXVASC Cohort. Stroke. 2022;53:1310-1317. doi: 10.1161/STROKEAHA.121.035560

130. Brandts A, van Elderen SGC, Westenberg JJM, van der Grond J, van Buchem MA, Huisman MV, Kroft LJM, Tamsma JT, de Roos A. Association of Aortic Arch Pulse Wave Velocity with Left Ventricular Mass and Lacunar Brain Infarcts in Hypertensive Patients: Assessment with MR Imaging. Radiology. 2009;253:681-688. doi: 10.1148/radiol.2533082264

131. Choi SY, Park HE, Seo H, Kim M, Cho SH, Oh BH. Arterial Stiffness Using Cardio-Ankle Vascular Index Reflects Cerebral Small Vessel Disease in Healthy Young and Middle Aged Subjects. J Atheroscler Thromb. 2013;20:178-185. doi: 10.5551/jat.14753

132. Henskens LHG, Kroon AA, van Oostenbrugge RJ, Gronenschild E, Fuss-Lejeune M, Hofman PAM, Lodder J, de Leeuw PW. Increased Aortic Pulse Wave Velocity Is Associated With Silent Cerebral Small-Vessel Disease in Hypertensive Patients. Hypertension. 2008;52:1120-U1172. doi: 10.1161/HYPERTENSIONAHA.108.119024

133. Kim YB, Park KY, Chung PW, Kim JM, Moon HS, Youn YC. Brachial-ankle pulse wave velocity is associated with both acute and chronic cerebral small vessel disease. Atherosclerosis. 2016;245:54-59. doi: 10.1016/j.atherosclerosis.2015.12.006

134. Kinjo Y, Ishida A, Kinjo K, Ohya Y. A high normal ankle-brachial index combined with a high pulse wave velocity is associated with cerebral microbleeds. J Hypertens. 2016;34:1586-1593. doi: 10.1097/HJH.0000000000000993

135. Matsumoto M, Inoue K, Moriki A. Associations of brachial-ankle pulse wave velocity and carotid atherosclerotic lesions with silent cerebral lesions. Hypertens Res. 2007;30:767-773. doi: 10.1291/hypres.30.767

136. Ochi N, Kohara K, Tabara Y, Nagai T, Kido T, Uetani E, Ochi M, Igase M, Miki T. Association of Central Systolic Blood Pressure With Intracerebral Small Vessel Disease in Japanese. Am J Hypertens. 2010;23:889-894. doi: 10.1038/ajh.2010.60

137. Pase MP, Himali JJ, Mitchell GF, Beiser A, Maillard P, Tsao C, Larson MG, DeCarli C, Vasan RS, Seshadri S. Association of Aortic Stiffness With Cognition and Brain Aging in Young and Middle-Aged Adults The Framingham Third Generation Cohort Study. Hypertension. 2016;67:513-519. doi: 10.1161/HYPERTENSIONAHA.115.06610

138. Riba-Llena I, Jimenez-Balado J, Castane X, Girona A, Lopez-Rueda A, Mundet X, Jarca CI, Alvarez-Sabin J, Montaner J, Delgado P. Arterial Stiffness Is Associated With Basal Ganglia Enlarged Perivascular Spaces and Cerebral Small Vessel Disease Load. Stroke. 2018;49:1279-+. doi: 10.1161/STROKEAHA.118.020163

139. Robert C, Ling LH, Tan ESJ, Gyanwali B, Venketasubramanian N, Lim SL, Gong LL, Berboso JL, Richards AM, Chen CSP, et al. Effects of Carotid Artery Stiffness on Cerebral Small-Vessel Disease and Cognition. J Am Heart Assoc. 2022;11. doi: 10.1161/JAHA.122.027295

140. Saji N, Kimura K, Shimizu H, Kita Y. Association between Silent Brain Infarct and Arterial Stiffness Indicated by Brachial-ankle Pulse Wave Velocity. Intern Med. 2012;51:1003-1008. doi: 10.2169/internalmedicine.51.6852

141. Saji N, Kimura K, Shimizu H, Kita Y. Silent brain infarct is independently associated with arterial stiffness indicated by cardio-ankle vascular index (CAVI). Hypertens Res. 2012;35:756-760. doi: 10.1038/hr.2012.20

142. Shan Y, Lin J, Xu PJ, Zeng MS, Lin HD, Yan HM. Association of Aortic Compliance and Brachial Endothelial Function with Cerebral Small Vessel Disease in Type 2 Diabetes Mellitus Patients: Assessment with High-Resolution MRI. Biomed Res Int. 2016;2016. doi: 10.1155/2016/1609317

143. van Sloten TT, Mitchell GF, Sigurdsson S, van Buchem MA, Jonsson PV, Garcia ME, Harris TB, Henry RM, Levey AS, Stehouwer CD, et al. Associations between arterial stiffness, depressive symptoms and cerebral small vessel disease: cross-sectional findings from the AGES-Reykjavik Study. J Psychiatry Neurosci. 2016;41:162-168. doi: 10.1503/jpn.140334

144. Zhai FF, Ye YC, Chen SY, Ding FM, Han F, Yang XL, Wang Q, Zhou LX, Ni J, Yao M, et al. Arterial Stiffness and Cerebral Small Vessel Disease. Front Neurol. 2018;9. doi: 10.3389/fneur.2018.00723

145. Zhang KX, Jiang YF, Wang YZ, Suo C, Xu KL, Zhu Z, Zhu CK, Zhao GM, Jin L, Ye WM, et al. Associations of Arterial Stiffness and Carotid Atherosclerosis with Cerebral Small Vessel Disease in a Rural Community-Based Population. J Atheroscler Thromb. 2020;27:922-933. doi: 10.5551/jat.52530

146. Rundek T, Della-Morte D, Gardener H, Dong CH, Markert MS, Gutierrez J, Roberts E, Elkind MSV, DeCarli C, Sacco RL, et al. Relationship between carotid arterial properties and cerebral white matter hyperintensities. Neurology. 2017;88:2036-2042. doi: 10.1212/WNL.0000000000003951

147. Tsao CW, Seshadri S, Beiser AS, Westwood AJ, DeCarli C, Au R, Himali JJ, Hamburg NM, Vita JA, Levy D, et al. Relations of arterial stiffness and endothelial function to brain aging in the community. Neurology. 2013;81:984-991. doi: 10.1212/WNL.0b013e3182a43e1c

148. Zhou Y, Zhang Y, Xu G, Shang X. Association between estimated pulse wave velocity and silent lacunar infarct in a Korean population. Front Cardiovasc Med. 2023;10:1070997. doi: 10.3389/fcvm.2023.1070997

149. Zijlstra LE, Trompet S, Jukema JW, Kroft LJM, de Bresser J, van Osch MJP, Hammer S, Witjes MN, van Buren M, Mooijaart SP. Association of cardiovascular structure and function with cerebrovascular changes and cognitive function in older patients with end-stage renal disease. Aging-US. 2020;12:1496-1511. doi: 10.18632/aging.102696

150. Ao DH, Zhai FF, Han F, Zhou LX, Ni J, Yao M, Zhang DD, Li ML, Fan XH, Jin ZY, et al. Large Vessel Disease Modifies the Relationship Between Kidney Injury and Cerebral Small Vessel Disease. Front Neurol. 2018;9. doi: 10.3389/fneur.2018.00498

151. Kim DH, Kim J, Kim JM, Lee AY. Increased brachial-ankle pulse wave velocity is independently associated with risk of cerebral ischemic small vessel disease in elderly hypertensive patients. Clin Neurol Neurosurg. 2008;110:599-604. doi: 10.1016/j.clineuro.2008.03.009

152. Liu WJ, Chen ZS, Ortega D, Liu XB, Huang XQ, Wang LL, Chen L, Sun J, Hatsukami TS, Yuan C, et al. Arterial elasticity, endothelial function and intracranial vascular health: A multimodal MRI study. J Cereb Blood Flow Metab. 2021;41:1390-1397. doi: 10.1177/0271678X20956950

153. Bae JH, Kim JM, Park KY, Han SH. Association between arterial stiffness and the presence of cerebral small vessel disease markers. Brain Behav. 2021;11. doi: 10.1002/brb3.1935

154. Bown CW, Khan OA, Liu D, Remedios SW, Pechman KR, Terry JG, Nair S, Davis LT, Landman BA, Gifford KA, et al. Enlarged perivascular space burden associations with arterial stiffness and cognition. Neurobiol Aging. 2023;124:85-97. doi: 10.1016/j.neurobiolaging.2022.10.014

155. Thomas O, Cain J, Nasralla M, Jackson A. Aortic Pulsatility Propagates Intracranially and Correlates with Dilated Perivascular Spaces and Small Vessel Compliance. J Stroke Cerebrovasc Dis. 2019;28:1252-1260. doi: 10.1016/j.jstrokecerebrovasdis.2019.01.020

156. Gustavsson AM, Stomrud E, Abul-Kasim K, Minthon L, Nilsson PM, Hansson O, Nägga K. Cerebral Microbleeds and White Matter Hyperintensities in Cognitively Healthy Elderly: A Cross-Sectional Cohort Study Evaluating the Effect of Arterial Stiffness. Cerebrovasc Dis Extra. 2015;5:41-51. doi: 10.1159/000377710

157. Shimoyama T, Iguchi Y, Kimura K, Mitsumura H, Sengoku R, Kono Y, Morita M, Mochio S. Stroke patients with cerebral microbleeds on MRI scans have arteriolosclerosis as well as systemic atherosclerosis. Hypertens Res. 2012;35:975-979. doi: 10.1038/hr.2012.84

158. Song TJ, Kim J, Kim YD, Nam HS, Lee HS, Nam CM, Heo JH. The distribution of cerebral microbleeds determines their association with arterial stiffness in non-cardioembolic acute stroke patients. Eur J Neurol. 2014;21:463-469. doi: 10.1111/ene.12332

159. Allison EY, Al-Khazraji BK. Association of Arterial Stiffness Index and Brain Structure in the UK Biobank: A 10-Year Retrospective Analysis. Aging Dis. 2023. doi: 10.14336/Ad.2023.0419

160. Alqarni A, Wen W, Lam BCP, Crawford JD, Sachdev PS, Jiang JY. Hormonal factors moderate the associations between vascular risk factors and white matter hyperintensities. Brain Imaging Behav. 2023;17:172-184. doi: 10.1007/s11682-022-00751-5

161. Azahar NM, Yano Y, Kadota A, Shiino A, Miyagawa N, Kondo K, Moniruzzaman M, Torii S, Segawa H, Hisamatsu T, et al. Associations of Arterial Stiffness and Atherosclerotic Burden with Brain Structural Changes among Japanese Men. J Hypertens. 2023;41:E327-E327.

162. Haidegger M, Lindenbeck S, Hofer E, Rodler C, Zweiker R, Perl S, Pirpamer L, Kneihsl M, Fandler-Hofler S, Gattringer T, et al. Arterial stiffness and its influence on cerebral morphology and cognitive function. Ther Adv Neurol Diso. 2023;16. doi: Artn 175628642311807151 0.1177/17562864231180715

163. Hashimoto J, Westerhof BE, Ito S. Carotid Flow Augmentation, Arterial Aging, and Cerebral White Matter Hyperintensities: Comparison With Pressure Augmentation. Arterioscler Thromb Vasc Biol. 2018;38:2843-2853. doi: 10.1161/ATVBAHA.118.311873

164. Hughes TM, Kuller LH, Barinas-Mitchell EJM, Mackey RH, McDade EM, Klunk WE, Aizenstein HJ, Cohen AD, Snitz BE, Mathis CA, et al. Pulse wave velocity is associated with beta-amyloid deposition in the brains of very elderly adults. Neurology. 2013;81:1711-1718. doi: 10.1212/01.wnl.0000435301.64776.37

165. Hughes TM, Wagenknecht LE, Craft S, Mintz A, Heiss G, Palta P, Wong D, Zhou Y, Knopman D, Mosley TH, et al. Arterial stiffness and dementia pathology Atherosclerosis Risk in Communities (ARIC)-PET Study. Neurology. 2018;90:E1248-+. doi: 10.1212/WNL.0000000000005259

166. Laugesen E, Hoyem P, Stausbol-Gron B, Mikkelsen A, Thrysoe S, Erlandsen M, Christiansen JS, Knudsen ST, Hansen KW, Kim WY, et al. Carotid-Femoral Pulse Wave Velocity Is Associated With Cerebral White Matter Lesions in Type 2 Diabetes. Diabetes Care. 2013;36:722-728. doi: 10.2337/dc12-0942

167. Liu H, Liu JB, Zhao HW, Wang HY. Association of brain white matter lesions with arterial stiffness assessed by cardio-ankle vascular index. The Beijing Vascular Disease Evaluation STudy (BEST). Brain Imaging Behav. 2021;15:1025-1032. doi: 10.1007/s11682-020-00309-3

168. McBride CA, Russom Z, Achenbach E, Bernstein IM, Dumas JA. Cardiovascular profiles associated with white matter hyperintensities in healthy young women. Front Physiol. 2023;13. doi: ARTN 97989910.3389/fphys.2022.979899

169. Ohmine T, Miwa Y, Yao H, Yuzuriha T, Takashima Y, Uchino A, Takahashi-Yanaga F, Morimoto S, Maehara Y, Sasaguri T. Association between arterial stiffness and cerebral white matter lesions in community-dwelling elderly subjects. Hypertens Res. 2008;31:75-81. doi: 10.1291/hypres.31.75

170. Rosano C, Watson N, Chang YF, Newman AB, Aizenstein HJ, Du Y, Venkatraman V, Harris TB, Barinas-Mitchell E, Sutton-Tyrrell K. Aortic Pulse Wave Velocity Predicts Focal White Matter Hyperintensities in a Biracial Cohort of Older Adults. Hypertension. 2013;61:160-+. doi: 10.1161/HYPERTENSIONAHA.112.198069

171. Turk M, Zaletel M, Pretnar-Oblak J. Ratio between carotid artery stiffness and blood flow - a new ultrasound index of ischemic leukoaraiosis. Clin Interv Aging. 2016;11:65-71. doi: 10.2147/CIA.S94163

172. Coutinho T, Turner ST, Kullo IJ. Aortic Pulse Wave Velocity Is Associated With Measures of Subclinical Target Organ Damage. JACC Cardiovasc Imaging. 2011;4:754-761. doi: 10.1016/j.jcmg.2011.04.011

173. Balestrini CS, Al-Khazraji BK, Suskin N, Shoemaker JK. Does vascular stiffness predict white matter hyperintensity burden in ischemic heart disease with preserved ejection fraction? Am J Physiol Heart Circ Physiol. 2020;318:H1401-H1409. doi: 10.1152/ajpheart.00057.2020

174. Bown CW, Khan OA, Moore EE, Liu DD, Pechman KR, Cambronero FE, Terry JG, Nair S, Davis LT, Gifford KA, et al. Elevated Aortic Pulse Wave Velocity Relates to Longitudinal Gray and White Matter Changes. Arterioscler Thromb Vasc Biol. 2021;41:3015-3024. doi: 10.1161/ATVBAHA.121.316477

175. Brutto OHD, Mera RM, Costa AF, Recalde BY, Rumbea DA, Sedler MJ. Arterial stiffness and progression of white matter hyperintensities of presumed vascular origin in community-dwelling older adults of Amerindian ancestry: The Atahualpa Project Cohort. Clin Neurol Neurosurg. 2022;221. doi: 10.1016/j.clineuro.2022.107411

176. Caughey MC, Qiao Y, Meyer ML, Palta P, Matsushita K, Tanaka H, Wasserman BA, Heiss G. Relationship Between Central Artery Stiffness, Brain Arterial Dilation, and White Matter Hyperintensities in Older Adults: The ARIC Study-Brief Report. Arterioscler Thromb Vasc Biol. 2021;41:2109-2116. doi: 10.1161/ATVBAHA.120.315692

177. Cheng CY, Cheng HM, Chen SP, Chung CP, Lin YY, Hu HH, Chen CH, Wang SJ. White matter hyperintensities in migraine: Clinical significance and central pulsatile hemodynamic correlates. Cephalalgia. 2018;38:1225-1236. doi: 10.1177/0333102417728751

178. Coffin C, Suerken CK, Bateman JR, Whitlow CT, Williams BJ, Espeland MA, Sachs BC, Cleveland M, Yang M, Rogers S, et al. Vascular and microstructural markers of cognitive pathology. Alzheimers Dement-Diagn Assess Dis Monit. 2022;14. doi: 10.1002/dad2.12332

179. Elyas S, Adingupu D, Aizawa K, Casanova F, Gooding K, Fulford J, Mawson D, Gates PE, Shore AC, Strain D. Cerebral small vessel disease, systemic vascular characteristics and potential therapeutic targets. Aging-US. 2021;13:22030-22039.

180. Funck KL, Laugesen E, Hoyem P, Stausbol-Gron B, Kim WY, Ostergaard L, Grauballe D, Hansen TK, Buhl CS, Poulsen PL. Arterial stiffness and progression of cerebral white matter hyperintensities in patients with type 2 diabetes and matched controls: a 5-year cohort study. Diabetol Metab Syndr. 2021;13. doi: 10.1186/s13098-021-00691-y

181. Haidar MA, van Buchem MA, Sigurdsson S, Gotal JD, Gudnason V, Launer LJ, Mitchell GF. Wave Reflection at the Origin of a First-Generation Branch Artery and Target Organ Protection The AGES-Reykjavik Study. Hypertension. 2021;77:1169-1177. doi: 10.1161/HYPERTENSIONAHA.120.16696

182. Hajdarevic DS, Pavlovic AM, Smajlovic D, Podgorac A, Jovanovic Z, Medjedovic TS, Sternic NC. Carotid artery wall stiffness is increased in patients with small vessel disease: A case-control study. Srp Ark Celok Lek. 2016;144:6-9. doi: 10.2298/SARH1602006S

183. Hannawi Y, Vaidya D, Yanek LR, Johansen MC, Kral BG, Becker LC, Becker DM, Nyquist PA. Association of Vascular Properties With the Brain White Matter Hyperintensity in Middle-Aged Population. J Am Heart Assoc. 2022;11. doi: 10.1161/JAHA.121.024606

184. King KS, Chen KX, Hulsey KM, McColl RW, Weiner MF, Nakonezny PA, Peshock RM. White Matter Hyperintensities: Use of Aortic Arch Pulse Wave Velocity to Predict Volume Independent of Other Cardiovascular Risk Factors. Radiology. 2013;267:709-717. doi: 10.1148/radiol.13121598

185. Kroner ESJ, van der Grond J, Westenberg JJM, van der Wall EE, Siebelink HMJ, Lamb HJ. Morphological and functional carotid vessel wall properties in relation to cerebral white matter lesions in myocardial infarction patients. Neth Heart J. 2015;23:314-320. doi: 10.1007/s12471-015-0693-6

186. Kuo HK, Chen CY, Liu HM, Yen CJ, Chang KJ, Chang CC, Yu YH, Lin LY, Hwang JJ. Metabolic risks, white matter hyperintensities, and arterial stiffness in high-functioning healthy adults. Int J Cardiol. 2010;143:184-191. doi: 10.1016/j.ijcard.2009.02.005

187. Maillard P, Mitchell GF, Himali JJ, Beiser A, Fletcher E, Tsao CW, Pase MP, Satizabal CL, Vasan RS, Seshadri S, et al. Aortic Stiffness, Increased White Matter Free Water, and Altered Microstructural Integrity A Continuum of Injury. Stroke. 2017;48:1567-+. doi: 10.1161/STROKEAHA.116.016321

188. Sala M, van den Berg-Huysmans A, van der Grond J, Huisman M, Brandts A, Westenberg JJ, de Roos A. Aortic Arch Stiffness Is Associated With Incipient Brain Injury in Patients With Hypertension. Am J Hypertens. 2016;29:705-712. doi: 10.1093/ajh/hpv161

189. Suri S, Chiesa ST, Zsoldos E, Mackay CE, Filippini N, Griffanti L, Mahmood A, Singh-Manoux A, Shipley MJ, Brunner EJ, et al. Associations between arterial stiffening and brain structure, perfusion, and cognition in the Whitehall II Imaging Sub-study: A retrospective cohort study. PLos Med. 2020;17. doi: 10.1371/journal.pmed.1003467

190. Tap L, van Opbroek A, Niessen WJ, Smits M, Mattace-Raso FUS. Aortic stiffness and brain integrity in elderly patients with cognitive and functional complaints. Clin Interv Aging. 2018;13:2161-2167. doi: 10.2147/CIA.S181437

191. Tsao CW, Himali JJ, Beiser AS, Larson MG, DeCarli C, Vasan RS, Mitchell GF, Seshadri S. Association of arterial stiffness with progression of subclinical brain and cognitive disease. Neurology. 2016;86:619-626. doi: 10.1212/WNL.0000000000002368

192. Werhane ML, Thomas KR, Bangen KJ, Weigand AJ, Edmonds EC, Nation DA, Sundermann EE, Bondi MW, Delano-Wood L. Arterial Stiffening Moderates the Relationship Between Type-2 Diabetes Mellitus and White Matter Hyperintensity Burden in Older Adults With Mild Cognitive Impairment. Front Aging Neurosci. 2021;13. doi: 10.3389/fnagi.2021.716638

193. Del Brutto OH, Mera RM, Penaherrera R, Penaherrera E, Zambrano M, Costa AF. Arterial stiffness and total cerebral small vessel disease score in community-dwelling older adults: Results from the Atahualpa Project. Vasc Med. 2019;24:6-11. doi: 10.1177/1358863X18806583

194. Liu TT, Liu YY, Wang SH, Du XL, Zheng ZF, Wang N, Hou XY, Shen C, Chen J, Liu XP. Brachial-Ankle Pulse Wave Velocity is Related to the Total Cerebral Small-Vessel Disease Score in an Apparently Healthy Asymptomatic Population. J Stroke Cerebrovasc Dis. 2020;29. doi: 10.1016/j.jstrokecerebrovasdis.2020.105221

195. Huang X, Kang X, Xue J, Kang C, Lv H, Li Z. Evaluation of carotid artery elasticity changes in patients with cerebral small vessel disease. Int J Clin Exp Med. 2015;8:18825-18830.

196. Chang YM, Lee TL, Su HC, Chien CY, Lin TY, Lin SH, Chen CH, Sung PS. The Association between Ankle-Brachial Index/Pulse Wave Velocity and Cerebral Large and Small Vessel Diseases in Stroke Patients. Diagnostics. 2023;13. doi: ARTN 145510.3390/diagnostics13081455

197. Cheng YLJ, Imperatore G, Geiss LS, Wang J, Saydah SH, Cowie CC, Gregg EW. Secular Changes in the Age-Specific Prevalence of Diabetes Among US Adults: 1988-2010. Diabetes Care. 2013;36:2690-2696. doi: 10.2337/dc12-2074

198. Fang J, Gillespie C, Ayala C, Loustalot F. Prevalence of Self-Reported Hypertension and Antihypertensive Medication Use Among Adults Aged >= 18 Years - United States, 2011-2015. Mmwr-Morbid Mortal W. 2018;67:219-224. doi: DOI 10.15585/mmwr.mm6707a4

199. Sterne JAC, Sutton AJ, Ioannidis JPA, Terrin N, Jones DR, Lau J, Carpenter J, Rucker G, Harbord RM, Schmid CH, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. Bmj-Brit Med J. 2011;343. doi: ARTN d400210.1136/bmj.d4002

200. Thompson SG, Higgins JPT. How should meta-regression analyses be undertaken and interpreted? Stat Med. 2002;21:1559-1573. doi: 10.1002/sim.1187

201. van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. Stat Med. 2002;21:589-624. doi: DOI 10.1002/sim.1040

202. van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MMB. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences - Rotterdam Scan Study. Stroke. 2008;39:2712-2719. doi: 10.1161/Strokeaha.107.513176

203. Goldstein ED, Wolcott Z, Garg G, Navarro K, Delic A, Yaghi S, Sederholm B, Prabhakaran S, Wong KH, McLean K, et al. Effect of Antihypertensives by Class on Cerebral Small Vessel Disease: A Post Hoc Analysis of SPRINT-MIND. Stroke. 2022;53:2435-2440. doi: 10.1161/Strokeaha.121.037997

204. Saphirstein RJ, Morgan KG. The Contribution of Vascular Smooth Muscle to Aortic Stiffness Across Length Scales. Microcirculation. 2014;21:201-207. doi: 10.1111/micc.12101

205. Van den Bergh G, Opdebeeck B, D'Haese PC, Verhulst A. The Vicious Cycle of Arterial Stiffness and Arterial Media Calcification. Trends Mol Med. 2019;25:1133-1146. doi: 10.1016/j.molmed.2019.08.006

206. Bennett J, van Dinther M, Voorter P, Backes W, Barnes J, Barkhof F, Captur G, Hughes AD, Sudre C, Treibel TA. Assessment of Microvascular Disease in Heart and Brain by MRI: Application in Heart Failure with Preserved Ejection Fraction and Cerebral Small Vessel Disease. Medicina (Kaunas). 2023;59. doi: 10.3390/medicina59091596

207. Ratnatunga C, Adiseshiah M. Increase in middle cerebral artery velocity on breath holding: a simplified test of cerebral perfusion reserve. Eur J Vasc Surg. 1990;4:519-523. doi: 10.1016/s0950-821x(05)80795-9

208. Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli F, Troisi E, Caltagirone C. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid

artery stenosis. Jama-J Am Med Assoc. 2000;283:2122-2127. doi: DOI 10.1001/jama.283.16.2122

209. Sleight E, Stringer MS, Marshall I, Wardlaw JM, Thrippleton MJ. Cerebrovascular Reactivity Measurement Using Magnetic Resonance Imaging: A Systematic Review. Front Physiol. 2021;12. doi: ARTN 643468

210. Junejo RT, Lip GYH, Fisher JP. Cerebrovascular Dysfunction in Atrial Fibrillation. Front Physiol. 2020;11:1066. doi: 10.3389/fphys.2020.01066

211. Tarumi T, Gonzales MM, Fallow B, Nualnim N, Lee J, Pyron M, Tanaka H, Haley AP. Cerebral/Peripheral Vascular Reactivity and Neurocognition in Middle-Age Athletes. Med Sci Sports Exerc. 2015;47:2595-2603. doi: 10.1249/MSS.0000000000000717

212. Portegies ML, de Bruijn RF, Hofman A, Koudstaal PJ, Ikram MA. Cerebral vasomotor reactivity and risk of mortality: the Rotterdam Study. Stroke. 2014;45:42-47. doi: 10.1161/STROKEAHA.113.002348

213. Claassen JAHR, Meel-van den Abeelen ASS, Simpson DM, Panerai RB, Ne ICAR. Transfer function analysis of dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation Research Network. J Cereb Blood Flow Metab. 2016;36:665-680. doi: 10.1177/0271678x15626425

214. Panerai RB, Brassard P, Burma JS, Castro P, Claassen JAHR, van Lieshout JJ, Liu J, Lucas SJ, Minhas JS, Mitsis GD, et al. Transfer function analysis of dynamic cerebral autoregulation: a CARNet white paper 2022 update. J Cereb Blood Flow Metab. 2023;43:3-25. doi: 10.1177/0271678x221119760

215. de Montgolfier O, Pincon A, Pouliot P, Gillis MA, Bishop J, Sled JG, Villeneuve L, Ferland G, Levy BI, Lesage F, et al. High Systolic Blood Pressure Induces Cerebral

Microvascular Endothelial Dysfunction, Neurovascular Unit Damage, and Cognitive Decline in Mice. Hypertension. 2019;73:217-228. doi: 10.1161/HYPERTENSIONAHA.118.12048

216. Walker AE, Henson GD, Reihl KD, Morgan RG, Dobson PS, Nielson EI, Ling J, Mecham RP, Li DY, Lesniewski LA, et al. Greater impairments in cerebral artery compared with skeletal muscle feed artery endothelial function in a mouse model of increased large artery stiffness. J Physiol-London. 2015;593:1931-1943. doi: 10.1113/jphysiol.2014.285338

217. Boreham CA, Ferreira I, Twisk JW, Gallagher AM, Savage MJ, Murray LJ. Cardiorespiratory fitness, physical activity, and arterial stiffness - The Northern Ireland Young Hearts Project. Hypertension. 2004;44:721-726. doi: 10.1161/01.HYP.0000144293.40699.9a

218. Gando Y, Yamamoto K, Murakami H, Ohmori Y, Kawakami R, Sanada K, Higuchi M, Tabata I, Miyachi M. Longer Time Spent in Light Physical Activity Is Associated With Reduced Arterial Stiffness in Older Adults. Hypertension. 2010;56:540-546. doi: 10.1161/Hypertensionaha.110.156331

219. Reference Values for Arterial Stiffness C. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. Eur Heart J. 2010;31:2338-2350. doi: 10.1093/eurheartj/ehq165

220. Rajani RM, Quick S, Ruigrok SR, Graham D, Harris SE, Verhaaren BFJ, Fornage M, Seshadri S, Atanur SS, Dominiczak AF, et al. Reversal of endothelial dysfunction reduces white matter vulnerability in cerebral small vessel disease in rats. Sci Transl Med. 2018;10. doi: ARTN eaam950710.1126/scitranslmed.aam9507

221. Wardlaw JM, Makin SJ, Hernandez MCV, Armitage PA, Heye AK, Chappell FM, Munoz-Maniega S, Sakka E, Shuler K, Dennis MS, et al. Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia: evidence from a cohort study. Alzheimers Dement. 2017;13:634-643. doi: 10.1016/j.jalz.2016.09.006

222. Han F, Zhai FF, Wang Q, Zhou LX, Ni J, Yao M, Li ML, Zhang SY, Cui LY, Jin ZY, et al. Prevalence and Risk Factors of Cerebral Small Vessel Disease in a Chinese Population Based Sample. J Stroke. 2018;20:239-+. doi: 10.5853/jos.2017.02110

223. Khan U, Porteous L, Hassan A, Markus HS. Risk factor profile of cerebral small vessel disease and its subtypes. J Neurol Neurosurg Psychiatry. 2007;78:702-706. doi: 10.1136/jnnp.2006.103549

224. Wang Z, Chen Q, Chen JJ, Yang N, Zheng K. Risk factors of cerebral small vessel disease A systematic review and meta-analysis. Medicine. 2021;100. doi: ARTN e2822910.1097/MD.0000000000028229

225. Yusuf S, Diener HC, Sacco RL, Cotton D, Ounpuu S, Lawton WA, Palesch Y, Martin RH, Albers GW, Bath P, et al. Telmisartan to prevent recurrent stroke and cardiovascular events. N Engl J Med. 2008;359:1225-1237. doi: 10.1056/NEJMoa0804593

226. Weber R, Weimar C, Blatchford J, Hermansson K, Wanke I, Moller-Hartmann C, Gizewski ER, Forsting M, Demchuk AM, Sacco RL, et al. Telmisartan on top of antihypertensive treatment does not prevent progression of cerebral white matter lesions in the prevention regimen for effectively avoiding second strokes (PRoFESS) MRI substudy. Stroke. 2012;43:2336-2342. doi: 10.1161/STROKEAHA.111.648576

227. Rashid T, Li K, Toledo JB, Nasrallah I, Pajewski NM, Dolui S, Detre J, Wolk DA, Liu H, Heckbert SR, et al. Association of Intensive vs Standard Blood Pressure Control With

Regional Changes in Cerebral Small Vessel Disease Biomarkers: Post Hoc Secondary Analysis of the SPRINT MIND Randomized Clinical Trial. JAMA Netw Open. 2023;6:e231055. doi: 10.1001/jamanetworkopen.2023.1055

228. Dufouil C, Chalmers J, Coskun O, Besancon V, Bousser MG, Guillon P, MacMahon S, Mazoyer B, Neal B, Woodward M, et al. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke: the PROGRESS (Perindopril Protection Against Recurrent Stroke Study) Magnetic Resonance Imaging Substudy. Circulation. 2005;112:1644-1650. doi: 10.1161/CIRCULATIONAHA.104.501163

229. Blair GW, Janssen E, Stringer MS, Thrippleton MJ, Chappell F, Shi YL, Hamilton I, Flaherty K, Appleton JP, Doubal FN, et al. Effects of Cilostazol and Isosorbide Mononitrate on Cerebral Hemodynamics in the LACI-1 Randomized Controlled Trial. Stroke. 2022;53:29-33. doi: 10.1161/Strokeaha.121.034866

230. Wardlaw JM, Woodhouse LJ, Mhlanga II, Oatey K, Heye AK, Bamford J, Cvoro V, Doubal FN, England T, Hassan A, et al. Isosorbide Mononitrate and Cilostazol Treatment in Patients With Symptomatic Cerebral Small Vessel Disease The Lacunar Intervention Trial-2 (LACI-2) Randomized Clinical Trial. JAMA Neurol. 2023;80:682-692. doi: 10.1001/jamaneurol.2023.1526

231. Muscari A, Conte C, Esposti DD, Faccioli L, Falcone R, Kolce B, Marabini L, Puddu GM, Rosticci M, Spinardi L, et al. Risk Factors for Lacunar Strokes with Visible Cerebral Lesions on Computed Tomography Scan. J Stroke Cerebrovasc Dis. 2016;25:1381-1388. doi: 10.1016/j.jstrokecerebrovasdis.2016.01.050

232. Laurent S, Boutouyrie P. Arterial stiffness and stroke in hypertension - Therapeutic implications for stroke prevention. CNS Drugs. 2005;19:1-11. doi: 10.2165/00023210-200519010-00001

233. Wartolowska KA, Webb AJS. Blood Pressure Determinants of Cerebral White Matter Hyperintensities and Microstructural Injury: UK Biobank Cohort Study. Hypertension. 2021;78:532-539. doi: 10.1161/HYPERTENSIONAHA.121.17403

234. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B, Sever PS, Poulter NR. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. Lancet. 2010;375:895-905. doi: Doi 10.1016/S0140-6736(10)60308-X

235. Zhai FF, Yan S, Li ML, Han F, Wang Q, Zhou LX, Ni J, Yao M, Zhang SY, Cui LY, et al. Intracranial Arterial Dolichoectasia and Stenosis Risk Factors and Relation to Cerebral Small Vessel Disease. Stroke. 2018;49:1135-+. doi: 10.1161/Strokeaha.117.020130

236. Han F, Zhai FF, Li ML, Zhou LX, Ni J, Yao M, Jin ZY, Cui LY, Zhang SY, Zhu YC. Arterial Stiffness is Associated with Intracranial Arterial Stenosis other than Dolichoectasia in the General Population. J Atheroscler Thromb. 2021;28:283-292. doi: 10.5551/jat.55863

237. Furst H, Hartl WH, Janssen I. Patterns of Cerebrovascular Reactivity in Patients with Unilateral Asymptomatic Carotid-Artery Stenosis. Stroke. 1994;25:1193-1200. doi: Doi 10.1161/01.Str.25.6.1193

238. Chen J, Liu J, Xu WH, Xu R, Hou B, Cui LY, Gao S. Impaired Dynamic Cerebral Autoregulation and Cerebrovascular Reactivity in Middle Cerebral Artery Stenosis. PLoS One. 2014;9. doi: ARTN e8823210.1371/journal.pone.0088232

239. Donahue MJ, Achten E, Cogswell PM, De Leeuw FE, Derdeyn CP, Dijkhuizen RM, Fan AP, Ghaznawi R, Heit JJ, Ikram MA, et al. Consensus statement on current and emerging methods for the diagnosis and evaluation of cerebrovascular disease. J Cereb Blood Flow Metab. 2018;38:1391-1417. doi: 10.1177/0271678x17721830

Chapter 3 - Cerebral Macro- and Microvascular Regulation in Cancer Survivorship

Abstract

Background: Approximately 46% of cancer survivors report cognitive impairments across many types of cancer, which has been associated with anti-cancer therapy and often results in reduced quality of life. Pre-clinical studies suggest that determinants of brain health in chronic disease states, such as cancer survivorship, are likely to involve cerebrovascular dysfunction. We aimed to determine cerebrovascular hemodynamic function (cerebrovascular reactivity to carbon dioxide, cerebrovascular pulsatility) at the macrovascular and microvascular levels in survivors of cancer. We also conducted exploratory assessments of cognitive function in a subset of the cohort. **Methods:** Participants were recruited locally and through the Stormont-Vail Healthcare Network. Testing included resting measurements of cerebral hemodynamics to determine resting blood velocities, microvascular oxygen saturation and concentrations of hemoglobin, and cerebrovascular pulsatility. Then, cerebral blood velocity in the large arteries (middle cerebral artery or MCA) and oxygenated hemoglobin in microcirculation (HbO_2; prefrontal cortex region), blood pressure, and end-tidal CO_2 were collected during a rebreathing task. MAP was used to calculate indices of cerebrovascular conductance. Cerebrovascular reactivity (CVR) was calculated as the change in cerebral blood velocity, HbO_2, or vascular conductance over the change in end-tidal CO_2. Cognitive tests were also administered, which included Dimensional Change Card Sort, Picture Sequence Memory Form A, and Pattern Comparison Processing Speed from the National Institute of Health Toolbox. **Results:** Twenty-four women were recruited (10 healthy controls [HC], 14 survivors of cancer [CS]). Cancer types included

melanoma, lymphoma, and breast cancer. There were no differences in age, body-mass index, or blood pressure resting measurements between groups. At baseline, there were no significant differences in resting MCA mean velocity between groups (HC: 61.4 ± 34.4 cm/s vs. CS: 58.7 ± 29.7 cm/s; p>0.9) or in resting HbO_2 (HC: 25.6 ± 3.0 μM vs. CS: 24.7 ± 7.5 μM; p=0.69). However, there was a significantly lower microcirculation pulsatility index in HC (HC: 2.1 ± 0.5 μM vs. CS: 2.7 ± 0.7 μM; p=0.040). In the absolute CVR measurements, there were no observed differences between HC and CS in CVR_{MCA} (HC: 0.87 ± 0.37 cm/s/mmHg vs. CS: 0.80 ± 0.50 cm/s/mmHg; p>0.70) or CVR_{HbO2} (HC: 0.22 ± 0.27 μM/mmHg vs. CS: 0.11 ± 0.13 μM/mmHg; p=0.37). Adjusting for changes in MAP by using vascular conductance measures revealed a significantly lower CVR_{HbO2} in CS (HC: 0.90 ± 1.06 μM/mmHg/mmHg*10 vs. CS: -0.07 ± 0.22 μM/mmHg/mmHg*10; p=0.040). There were no differences between HC and CS in the Dimensional Card Change Sort test (p=0.51), the Picture Sequence Memory Form A (p=0.64), or the Pattern Comparison Processing Speed (p=0.32). **Conclusions:** Survivors of cancer had a greater pulsatility and reduced vascular conductance reactivity in the microcirculation of the brain, highlighting the injury present in a region critical for maintaining cerebral functions. These findings highlight the importance of monitoring cerebrovascular function, especially in the cerebral small vessels, in cancer survivors.

Introduction

For the first time in the United States, over 2 million people are expected to be diagnosed with cancer – roughly equivalent to one new diagnosis every 15 seconds.[1,2] Improvements in cancer detection and treatment have significantly reduced cancer mortality over prior decades, resulting in an estimated 20.3 million cancer survivors in the U.S. by 2026.[3] This medical progress has led to awareness of an emerging concern: many cancer survivors experience long-term complications, such as heightened risk for cardiovascular disease or cerebrovascular disease.[4,5]

Numerous studies have provided evidence that anti-cancer therapies, namely chemotherapy, are primary contributing factors. From the early studies of cardiotoxicity in doxorubicin[6] to contemporary investigations into immunotherapy-related myocarditis[7], many of these studies have focused on the timeframe from the beginning of treatment to within a year of the end of treatment. With modern survival rates increasing, attention has shifted to long-term fatal and non-fatal events. A key example is cognitive impairment, which is reportedly experienced by up to 1 in 3 breast cancer survivors.[8] This cognitive impairment, or "chemo-fog", remains a poorly understood mediator of reduced quality of life in cancer survivors[9,10] and a gap in the literature highlighted by the National Cancer Institute.[11]

One of the leading hypotheses for mechanisms underlying chemo-fog is that neuroinflammation and oxidative stress induced by chemotherapy impair cerebrovascular function. The diminished cerebrovascular regulation results in a mismatch between neuronal metabolism and cerebral blood flow, leading to cognitive decline.[12,13] Similar hypotheses have been investigated and supported in general population samples[14], multiple sclerosis[15], cerebral

amyloid angiopathy[16], and several other chronic conditions including aging.[17] However, this concept has had limited attention in cancer survivorship.

One study from Downs et al.[18] investigated cerebrovascular reactivity to carbon dioxide (a standard marker of cerebrovascular regulatory function[19]) and found that breast cancer survivors had a reduced cerebrovascular reactivity compared to cancer-free controls. The authors suggested that this may have contributed to the lower cognitive scores also observed in the breast cancer survivors. However, the study only assessed blood velocity in the middle cerebral artery (MCA). While the large arteries and pial vessels contribute substantially to the vascular resistance of the brain, the microvasculature remains the site of metabolic exchange between blood and the cerebral parenchyma.[20] Assessment of microvascular responses may provide information beyond that of the large vessel reactivity.

Therefore, the aim of the present study was to compare large and small vessel reactivity between cancer survivors and cancer-free controls. We additionally tested a select battery of cognitive function assessments between groups. We hypothesized that cancer survivors would have reduced large and small vessel reactivity, and reduced cognitive function. Furthermore, we hypothesized that the reductions in vascular reactivity would be correlated with cognitive impairment.

Methods

All procedures in this study were conducted in accordance with the Declaration of Helsinki and received institutional ethical approval from Kansas State University and Stormont Vail Health (IRB #11181). The authors declare that all supporting data are available upon reasonable request.

Study Cohort

Males and female cancer survivors (CS) and cancer-free controls (CF) were eligible for participation in the study. Participants were recruited from June 2022 through August 2024 from the Manhattan, Kansas area and surrounding regions through social media campaigns, campus media announcements, and recruitment through the Stormont Vail Health network. Eligibility criteria for CS were: 1) 21 years of age or older, 2) primary non-CNS cancer diagnosis within the previous 15 years, and 3) completion of primary treatment (surgery, radiation, and/or chemotherapy). For CF, criteria were: 1) 21 years of age or older, and 2) no prior diagnoses of any form of cancer. Exclusion criteria for both groups were: 1) younger than 21 years of age, 2) current or previous cerebrovascular, neurodegenerative, and/or pulmonary disease, and 3) unable to provide written and verbal informed consent.

Experimental Design

Experiments were performed in Manhattan, Kansas (altitude of 327 meters above sea level) in a controlled clinical environment (Stormont Vail Health Manhattan Campus) or in a controlled laboratory setting (Lafene Health Center, Kansas State University). Temperature, humidity, and ambient pressure did not differ between locations during testing. Participants were

instructed to refrain from caffeine and strenuous exercise prior to the experimental visit, but to maintain any daily medication and supplement use according to their normal schedule. At the beginning of each visit, informed consent, anthropometric measures, health history information, and medical record release documents were collected. Brachial blood pressure (OMRON Intellisense Pro; OMRON Healthcare, Inc., IL, USA) was assessed as the average of 2 measurements performed after ~10 minutes of quiet rest. Visits lasted 60-90 minutes in total, and started between 7:00am and 6:00pm with no difference in morning or afternoon visit allocation between CF and CS groups.

Cerebral Hemodynamics

All equipment herein was calibrated and checked for quality according to manufacturer recommendations before experiments. Equipment checks and operations were performed throughout all experiments by the same investigators (B.S., C.A.).

Transcranial Doppler Ultrasound

Transcranial doppler (TCD) ultrasonography was used for collection of cerebral hemodynamics. Participants were seated in an upright position while the TCD operator (B.S.) positioned a convex probe connected to a traditional ultrasonography system (General Electric HealthCare Technologies, Inc., Chicago, IL, USA). B-mode and color flow scans were performed to identify the middle cerebral artery (MCA) at a depth of 40-65 mm through the temporal window. Pulse-wave doppler scans assessed minimum, maximum, and mean MCA velocities with an insonation angle less than 15 degrees.[21]

Near-infrared Spectroscopy

Non-invasive measurements of cerebrovascular hemodynamics will be performed serially by cerebral near-infrared spectroscopy (NIRS) using a commercially available frequency-domain system (OxiplexTS, ISS Inc., Champaign, IL, USA). In brief, NIRS devices emit near-infrared light into biological tissue which is absorbed primarily by heme; in cerebral tissue, heme is primarily found circulating as hemoglobin in red blood cells.[22] The optical properties of hemoglobin vary if the hemoglobin is bound to oxygen, allowing NIRS to differentiate between oxygenated and deoxygenated hemoglobin (HbO_2 and HHb, respectively).[23] The absolute concentrations of these species, along with their sum (total-Hb) and the oxygen-saturation percent (Tissue Oxygenation Index, or TOI%), provide non-invasive indices of cerebral microvascular hemodynamics.[24,25] For the present work, we used HbO_2 and total-Hb as our primary measurements in line with previous literature.[24-26]

Assessment of Microvascular Pulsatility

In a subsample of the present study, we determined the pulsatility in the microcirculation using the NIRS. Cerebral microcirculatory data collected via NIRS during a resting period preceding cerebrovascular reactivity testing (described in the following section) was exported at 10Hz and imported into LabChart 8 (ADInstruments Inc., CO, USA). Built-in software algorithms for peak detection were used to identify cardiac cycles, using 2 standard deviations as a threshold criteria to exclude peaks resulting from noise during the refractory window. Within each cycle, the maximum and minimum total-Hb values were used to calculate the amplitude (the maximum minus the minimum). These amplitudes were averaged over ≥10 cycles, resulting in a NIRS-derived pulsatility index (PI) in the microcirculation. Consistent with prior work[26], the

total-Hb signal was selected to represent the local cerebral blood volume (assuming hematocrit remains constant during the resting period).[27]

Cerebrovascular Reactivity

To assess MCA and cerebral microcirculation responsiveness to changes in blood gases, a modified rebreathing approach was adopted.[28] Participants breathed room air for a 2 minute baseline period before switching to rebreathing from a 6-liter anesthesia bag pre-filled with 4 liters of room air until pre-determined cardiorespiratory endpoints were achieved. End-tidal partial pressures of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) were collected breath-by-breath with a metabolic cart (Ultima CPX, MGC Diagnostics Corporation, MN, USA) or cardiorespiratory monitor (CardioCap 5, General Electric HealthCare, IL, USA). The final 60 seconds of baseline and the last 10 seconds of rebreathing data were used for analyses. Due to simultaneous changes in both $P_{ET}O_2$ and $P_{ET}CO_2$ that occur during rebreathing, we calculated a previously described stimulus index (SI)[29], the ratio of $P_{ET}CO_2$ over $P_{ET}O_2$, as the blood gas stimulus for cerebrovascular responses. To account for changes in blood pressure, vascular conductance indices were calculated as the hemodynamic variable divided by mean arterial pressure (MAP). MAP was calculated as one-third of the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP) added to DBP. Cerebrovascular reactivity (CVR) was determined as 1) the absolute or relative change in MCA velocity or microcirculatory HbO_2 over the change in SI, and 2) as the absolute or relative change in MCA vascular conductance or in microcirculatory vascular conductance over the change in SI. Equations are provided below:

$$CVR_{X\text{-}Abs} = (X_{end} - X_{bsl}) / (SI_{end} - SI_{bsl}) \quad [1]$$

$$CVR_{X\text{-}Rel} = [(X_{end} - X_{bsl}) / (X_{bsl})*100] / (SI_{end} - SI_{bsl}) \quad [2]$$

$$CVR_{X.VC\text{-}Abs} = (VC_{X\text{-}end} - VC_{X\text{-}bsl}) / (SI_{end} - SI_{bsl}) \quad [3]$$

$$CVR_{X.VC\text{-}Rel} = [(VC_{X\text{-}end} - VC_{X\text{-}bsl}) / (VC_{X\text{-}bsl}) * 100] / (SI_{end} - SI_{bsl}) \quad [4]$$

where X represents either MCA velocity or HbO_2, the subscript *end* indicates the measurement during the last 10 seconds of rebreathing, the subscript *bsl* indicates the measurement taken during baseline, and VC_X is the vascular conductance measure for either MCA velocity or HbO_2 obtained by dividing the hemodynamic variable by MAP.

Cognitive Assessments

Participants were comfortably seated at a table in the testing site for cognitive testing. These tests were performed on an iPad (10^{th} generation, Apple Inc., Cupertino, CA, USA) after entering participant age and education level. Tests included Dimensional Change Card Sort, Picture Sequence Memory Form A, and Pattern Comparison Processing Speed from the National Institute of Health Toolbox version 3.10.1.3. Analyses of the cognitive tests were performed by researchers blinded to participant cancer history (T.B., A.S., O.E., S.S.). Demographic-corrected T-scores for the tests are reported by adjusting raw test scores for age, sex and race/ethnicity.

Statistical Analyses

Statistical analyses were performed in GraphPad Prism 10.3.0 (Dotmatics, Boston, MA, USA) or SigmaPlot 13.0 (Systat Software Inc., Chicago, IL, USA). Normality of data was assessed with Shapiro-Wilks tests. Outliers were determined with the ROUT method set at a

standard threshold (Q=1%). Comparisons between groups were assessed as independent t-tests, Mann-Whitney tests if the normality assumption was violated, or t-test with Welch's correction if variances were unequal. Associations between cognitive test outcomes and NIRS-derived microcirculation measures were assessed using the Spearman r coefficient and corresponding p-value. The microcirculatory outcomes were selected for correlational analyses as the cerebral microcirculation is the site of the neurovascular unit, linking cognition to vascular function.[30-32] Statistical significance was set *a priori* at $p<0.05$. Data in results are presented as means ± standard deviations unless otherwise noted.

Results

Demographics for the overall study cohort are reported in Table 3-1. Healthy controls (HC) were well matched to survivors of cancer (CS) for age, height, weight, body mass index, systolic and diastolic blood pressure (all $p>0.05$). For all cerebrovascular assessments, all HC and CS participants were women. Due to technical challenges present in cerebrovascular and cognitive testing, some participants had to be excluded from certain tests.

Cerebrovascular Reactivity and Pulsatility

At baseline, there were no significant differences in resting MCA mean velocity between groups (HC: 61.4 ± 34.4 cm/s vs. CS: 58.7 ± 29.7 cm/s; $p>0.9$) or in resting HbO_2 (HC: 25.6 ± 3.0 μM vs. CS: 24.7 ± 7.5 μM; $p=0.69$). However, as shown in Figure 3-1, there was a significantly lower microcirculation PI in HC (HC: 2.1 ± 0.5 μM vs. CS: 2.7 ± 0.7 μM; $p=0.04$). For cardiopulmonary measures, there were no differences in MAP ($p=0.28$) or SI ($p=0.25$). The change in SI from baseline to end-test was not different between groups (HC: 0.13 ± 0.11 mmHg vs. 0.14 ± 0.11 mmHg; $p>0.9$); however, there was a significantly greater change in MAP from baseline to end-test in CS (HC: 1.1 ± 5.6 mmHg vs. CS: 8.1 ± 8.4 mmHg; $p=0.04$).

In the absolute CVR measurements, there were no observed differences between HC and CS in $CVR_{MCA\text{-}Abs}$ (HC: 0.87 ± 0.37 cm/s/mmHg vs. CS: 0.80 ± 0.50 cm/s/mmHg; $p>0.7$) or $CVR_{HbO2\text{-}Abs}$ (HC: 0.22 ± 0.27 μM/mmHg vs. CS: 0.11 ± 0.13 μM/mmHg; $p=0.37$). The same was true for relative CVR measurements, both in $CVR_{MCA\text{-}Rel}$ (HC: 1.95 ± 1.35 %/mmHg vs. CS: 1.47 ± 0.93 %/mmHg; $p=0.39$) and in $CVR_{HbO2\text{-}Rel}$ (HC: 1.05 ± 1.28 %/mmHg vs. CS: 0.50 ± 0.50 %/mmHg; $p=0.48$). Results are presented in Figure 3-2.

Table 3-1. Study cohort demographics and comparisons.

	Healthy Controls	Cancer Survivors	P-value for Comparison
Sample Size (n)	10	14	--
Age (yrs)	43.8 ± 16.2	52.9 ± 15.3	0.18
Sex, female (%)	100	100	1
BMI (kg/m2)	25.7 ± 7.5	29.1 ± 5.9	0.23
SBP (mmHg)	127.6 ± 10.5	126.9 ± 12.4	0.9
DBP (mmHg)	80.6 ± 5.4	84.8 ± 7.4	0.15
MAP (mmHg)	96.2 ± 6.0	98.8 ± 8.7	0.44
PP (mmHg)	47.0 ± 9.6	42.1 ± 7.5	0.19
HR (bpm)	73.6 ± 9.7	76.6 ± 9.5	0.47

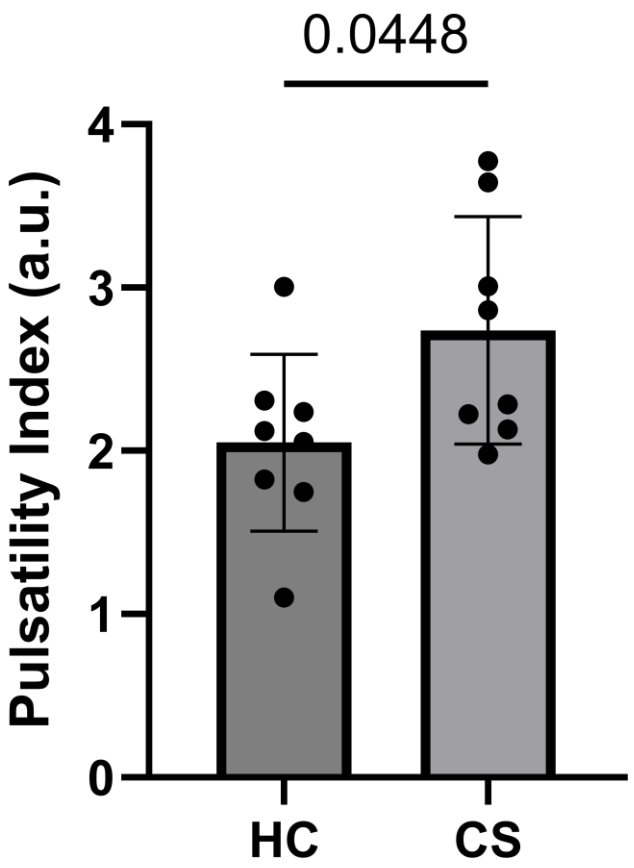


Figure 3-1. A comparison of microvascular pulsatility between cancer-free, healthy controls (HC) and survivors of cancer (CS). The pulsatility index was derived from near-infrared spectroscopy assessments of the prefrontal cortex microcirculation during a resting baseline.

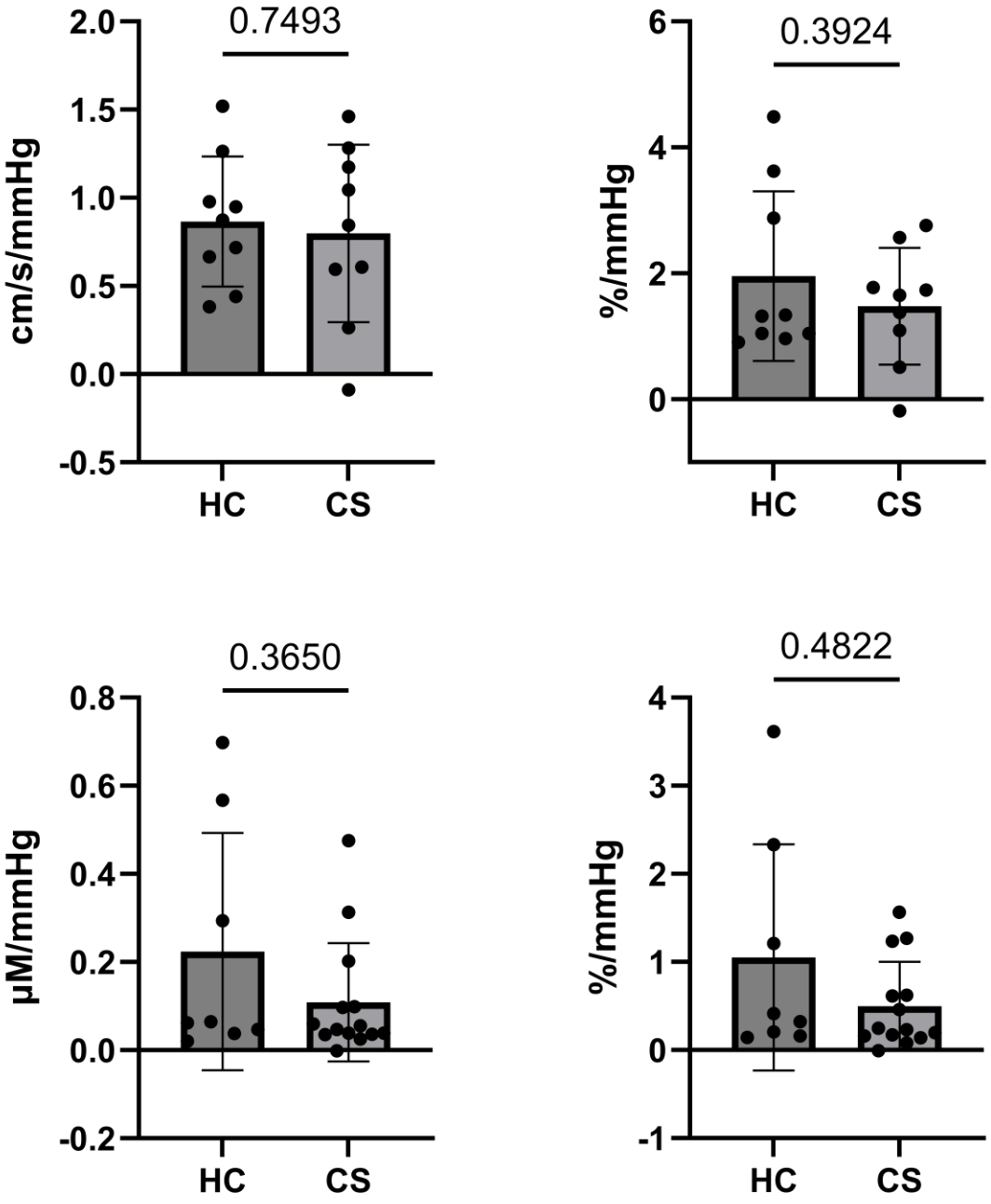


Figure 3-2. (*Top-Left*) Absolute middle cerebral artery cerebrovascular reactivity in response to a rebreathing protocol. (*Top-Right*) Relative middle cerebral artery cerebrovascular reactivity in response to a rebreathing protocol. (*Bottom-Left*) Absolute microcirculatory cerebrovascular reactivity in response to a rebreathing protocol. (*Bottom-Right*) Relative microcirculatory cerebrovascular reactivity in response to a rebreathing protocol. See Methods for details of calculation. HC, healthy controls; CS, cancer survivors.

In contrast, adjusting for changes in MAP by using vascular conductance measures revealed a significantly lower $CVR_{HbO2.VC\text{-}Abs}$ in CS (HC: 0.90 ± 1.06 μM/mmHg/mmHg*10 vs. CS: -0.07 ± 0.22 μM/mmHg/mmHg*10; p=0.04). This was consistent with the relative measure, $CVR_{HbO2.VC\text{-}Rel}$ (HC: 0.39 ± 0.49 %/mmHg vs. CS: -0.02 ± 0.11 %/mmHg; p=0.03). However, there was a lack of significant difference between groups MCA vascular conductance CVR in both absolute and relative terms (both p>0.1). Results for vascular conductance CVR outcomes are presented in Figure 3-3.

Cognitive Assessments

There were no differences between HC and CS in the Dimensional Card Change Sort test (HC: 56.4 ± 21.1 vs. CS: 45.1 ± 10.9; p=0.51), the Picture Sequence Memory Form A (HC: 61.6 ± 16.4 vs. CS: 60.4 ± 7.5; p=0.64), or the Pattern Comparison Processing Speed (HC: 53.6 ± 13.7 vs. CS: 48.2 ± 9.6; p=0.32).

In correlational analyses, greater $CVR_{HbO2\text{-}Abs}$ (Spearman r: 0.66, p<0.05) and greater $CVR_{HbO2\text{-}Rel}$ (Spearman r: 0.65, p<0.05) were associated with higher Picture Sequence Memory Form A scores. However, this correlation was not observed in the vascular conductance CVR measures (both p>0.05). There was also a borderline but non-significant association between microcirculatory PI and the Picture Sequence Memory Form A scores (p=0.05). None of the CVR measures were correlated with either Dimension Card Change Sort test or Pattern Comparison Processing Speed test performance (all p>0.05). Test scores and the correlation matrix are presented in Figure 3-4.

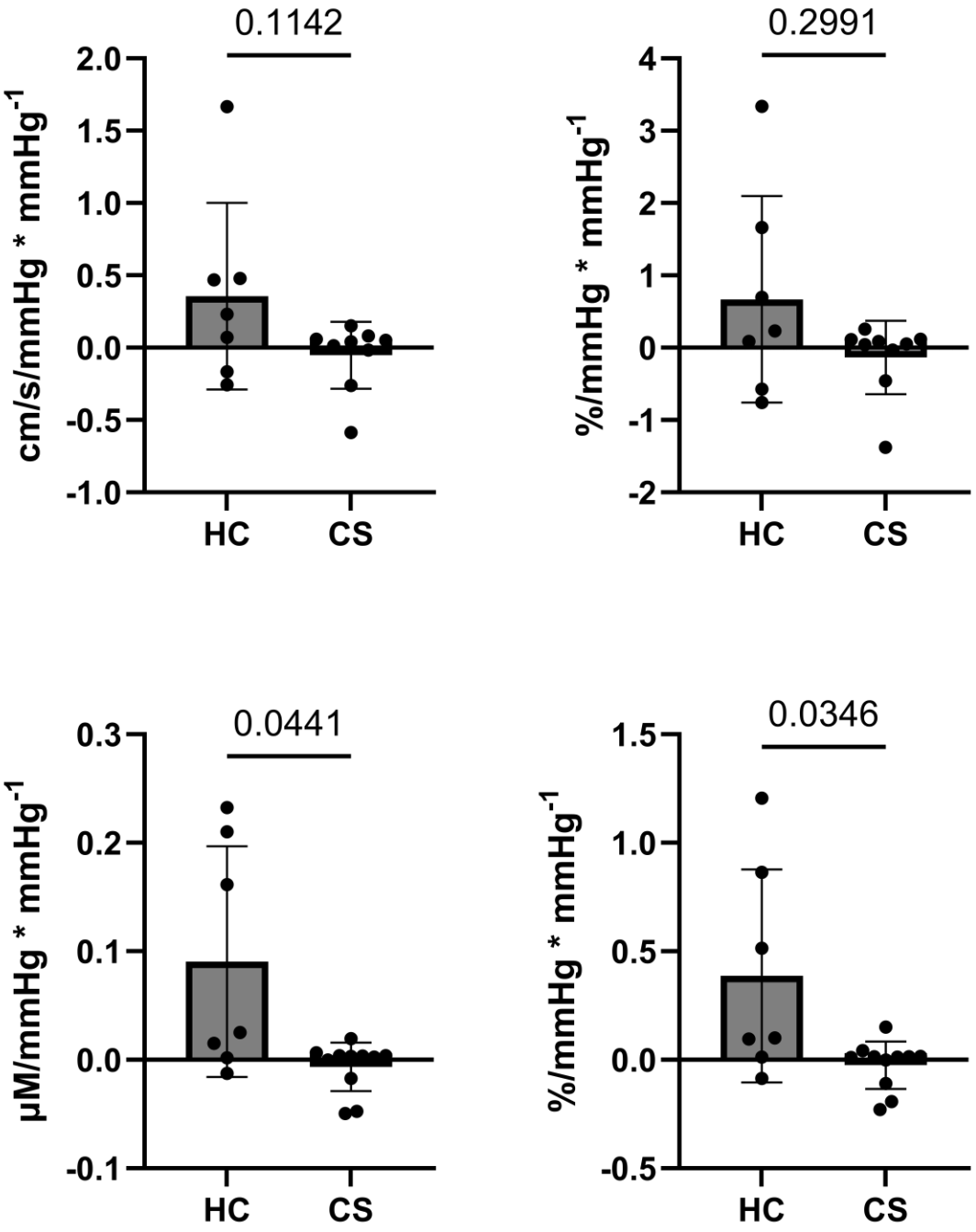


Figure 3-3. (*Top-Left*) Absolute middle cerebral artery vascular conductance in response to a rebreathing protocol. (*Top-Right*) Relative middle cerebral artery vascular conductance in response to a rebreathing protocol. (*Bottom-Left*) Absolute microcirculatory vascular conductance in response to a rebreathing protocol. (*Bottom-Right*) Relative microcirculatory vascular conductance in response to a rebreathing protocol. See Methods for details of calculation. HC, healthy controls; CS, cancer survivors.

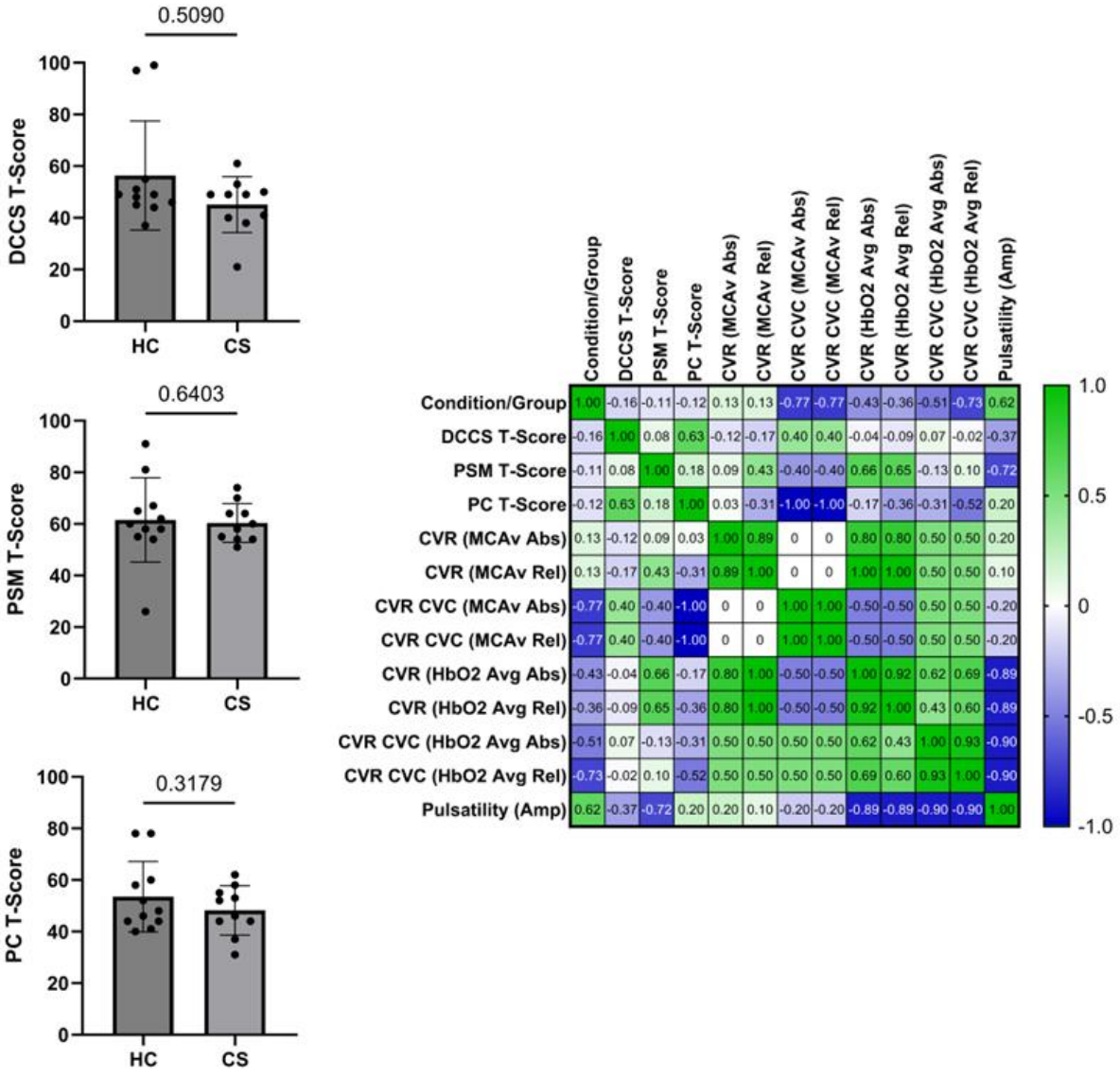


Figure 3-4. (*Left*) Comparisons between cancer-free, health controls (HC) and survivors of cancer (CS) for three cognitive assessments. DCCS, Dimensional Card Change Sort; PSM, Picture Sequence Memory Form A; PC, Pattern Comparison Processing Speed. Raw scores from the tests were adjusted for participant demographics and reported as T-scores. (*Right*) Correlation matrix for cognitive assessments and cerebral hemodynamics. CVR, Cerebrovascular reactivity; CVC, Cerebrovascular conductance; HbO_2, oxygenated hemoglobin.

Discussion

The main findings of this study demonstrate a reduced vascular conductance CVR response and a greater pulsatility in the cerebral microcirculation of survivors of cancer, which were suggested to be correlated with cognitive performance on a memory task. However, in contrast to our initial hypothesis, we found no significant differences in large artery (MCA) CVR or reduced cognitive performance in survivors of cancer compared to cancer-free controls. Overall, these findings support a primarily cerebral microvascular dysfunction that may persist throughout cancer survivorship, potentially relating to cognitive impairment over time.

Cerebrovascular Reactivity and Pulsatility

One of the key findings in the present study was that the microcirculatory vascular conductance reactivity was lower by ~110% in CS compared to HC counterparts, suggesting an almost completely reversed direction of the expected hemodynamic response in some participants.[33,34] Interestingly, this significantly impaired response was only observed when considering changes in MAP over the course of the stimulus. A similar phenomenon was described by Miller et al.[35] in a study comparing CVR in the MCA between young and old healthy, physically active individuals. In that work, the authors suggest that this may indicate an age-related reliance on changes in perfusion pressure to augment cerebral blood flow as a compensatory mechanism for reduced cerebral microcirculatory function. We extended on those ideas by non-invasively measuring the large and small cerebral arteries simultaneously, and we found that the perfusion pressure dependent (i.e., vascular conductance) differences between groups were significant only in the microvasculature. It should be noted that the ages were not different between groups in this study; however, cancer and many anti-cancer treatments may

accelerate biological aging that result in survivors presenting with phenotypes and physiological responses characteristic of an older individual.[36-38] Thus, insight into cerebrovascular regulation in both aging and cancer survivorship may lead to overlapping understanding of the underlying pathology.

It is also likely that other factors may play a role; for example, prior work has suggested an increased chemoreflex response to hypoxia[39,40] and a reduced CO_2 threshold[41] in older adults. This sympathetic hyperexcitation would drive MAP increases to a greater extent, and excessive sympathetic activity has been well documented in cancer survivors.[42-44] The role of sympathetic activity in CVR to hypercapnia is a topic of debate[45,46], but more recent literature provides strong evidence for a role that may vary between health and disease.[47]

We also found a significantly greater pulsatility in the cerebral microcirculation of CS, with an increase of ~30% relative to HC. Generally, conduit arteries between the heart and brain act to dampen the pulsatile energy generated by the left ventricle during contraction in order to prevent transmission of excess force into the vulnerable cerebral microvascular beds.[48,49] Increased microvascular pulsatility has been linked to impaired endothelium-dependent vasodilation[50], blood-brain barrier breakdown[51], and development of cerebral small vessel disease.[52] The findings from the present study (impaired CVR, microvascular pulsatility) - and prior work from our laboratory demonstrating increased arterial stiffness[53] - provide strong evidence supporting the likelihood of small vessel disease, given that these physiological processes and functions are associated with the disease in the general population.[54]

Cognitive Function in Cancer Survivorship

Impairments in cognitive function have been demonstrated throughout the continuum of cancer survivorship, from before treatment[55] to beyond 20 years from the end of treatment.[56] Our present findings did not suggest any substantial differences in cognitive ability across the domains tested. While this was an opposing finding to our hypothesis, it was also not entirely unexpected given that prevalence rates for detectable cancer- or treatment-related cognitive impairment vary widely, and do not often exceed 40% when using short, standardized cognitive batteries.[8] A substantial portion of patients and survivors report cognitive complaints, but challenges in methodology and the need for specific tools designed to encompass the multi-faceted and heterogeneous forms of cancer-related cognitive impairment may warrant novel approaches.[57] In a similar vein, incorporation of physiological measures alongside neurocognitive outcomes may reveal insight that extends beyond only using one or the other. Our correlative findings between microcirculatory pulsatility, reactivity, and memory-specific deficits may suggest that survivors who present with cerebrovascular dysfunction may be more predisposed to a certain cognitive phenotype; further work is needed to fill critical gaps in present knowledge.[58]

Limitations

Our study is not without limitations. Our primary limitation was sample size – due to the nature of the study design, larger sample sizes could not be included in the present work. This limits the ability to stratify by duration of cancer survivorship or types of treatment received, key targets for future work. Secondly, our use of a rebreathing test that elicited both hypercapnia and hypoxia prevents isolation of physiological responses specific to CO_2 or oxygen. However, this

method has been previously established[29] and aids in interpreting the two changing stimuli simultaneously. Third, we used transcranial doppler ultrasonography to assess cerebral blood velocity in the MCA, which does not account for changes in the cross-sectional area of the vessel. The concept that MCA cross-sectional area remains constant during short rebreathing assessments has been challenged.[59] It is important to note that in that study, substantially smaller increases in cross-sectional area were observed in older adults (~5% vs. ~12% in younger adults). Thus, in the present study where the average age of the cohort was ~50 years old, vasodilation may have had less of an impact.

Conclusions

Our study demonstrates that cerebral microvascular conductance responses to a rebreathing challenge are impaired in survivors of cancer. Additionally, survivors of cancer demonstrated greater cerebral microvascular pulsatility than cancer-free controls at rest. Future work should examine this effect across a variety of cancer types and chemotherapy regimens to investigate the differential impacts that these may have. Furthermore, the implications of this cerebrovascular dysfunction on cognitive outcomes should be explored with a wider array of neurocognitive assessments.

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74:12-49. doi: 10.3322/caac.21820
2. Dizon DS, Kamal AH. Cancer statistics 2024: All hands on deck. CA Cancer J Clin. 2024;74:8-9. doi: 10.3322/caac.21824
3. Mayer DK, Nasso SF, Earp JA. Defining cancer survivors, their needs, and perspectives on survivorship health care in the USA. Lancet Oncol. 2017;18:e11-e18. doi: 10.1016/S1470-2045(16)30573-3
4. Hooning MJ, Botma A, Aleman BM, Baaijens MH, Bartelink H, Klijn JG, Taylor CW, van Leeuwen FE. Long-term risk of cardiovascular disease in 10-year survivors of breast cancer. J Natl Cancer Inst. 2007;99:365-375. doi: 10.1093/jnci/djk064
5. Strongman H, Gadd S, Matthews A, Mansfield KE, Stanway S, Lyon AR, Dos-Santos-Silva I, Smeeth L, Bhaskaran K. Medium and long-term risks of specific cardiovascular diseases in survivors of 20 adult cancers: a population-based cohort study using multiple linked UK electronic health records databases. Lancet. 2019;394:1041-1054. doi: 10.1016/S0140-6736(19)31674-5
6. Gottdiener JS, Mathisen DJ, Borer JS, Bonow RO, Myers CE, Barr LH, Schwartz DE, Bacharach SL, Green MV, Rosenberg SA. Doxorubicin cardiotoxicity: assessment of late left ventricular dysfunction by radionuclide cineangiography. Ann Intern Med. 1981;94:430-435. doi: 10.7326/0003-4819-94-4-430
7. Brumberger ZL, Branch ME, Klein MW, Seals A, Shapiro MD, Vasu S. Cardiotoxicity risk factors with immune checkpoint inhibitors. Cardiooncology. 2022;8:3. doi: 10.1186/s40959-022-00130-5

8. Whittaker AL, George RP, O'Malley L. Prevalence of cognitive impairment following chemotherapy treatment for breast cancer: a systematic review and meta-analysis. Sci Rep. 2022;12:2135. doi: 10.1038/s41598-022-05682-1

9. Vardy J, Dhillon H. The fog hasn't lifted on "chemobrain" yet: ongoing uncertainty regarding the effects of chemotherapy and breast cancer on cognition. Breast Cancer Res Tr. 2010;123:35-37. doi: 10.1007/s10549-009-0719-0

10. Von Ah D, Crouch AD, Monahan PO, Stump TE, Unverzagt FW, Storey S, Cohee AA, Cella D, Champion VL. Association of cognitive impairment and breast cancer survivorship on quality of life in younger breast cancer survivors. J Cancer Surviv. 2022;16:812-822. doi: 10.1007/s11764-021-01075-x

11. Mohamed M, Ahmed M, Williams AM, Gilmore N, Lin PJ, Yilmaz S, Jensen-Battaglia M, Mustian K, Janelsins M, Mohile S. A scoping review evaluating physical and cognitive functional outcomes in cancer survivors treated with chemotherapy: charting progress since the 2018 NCI think tank on cancer and aging phenotypes. J Cancer Surviv. 2024;18:1089-1130. doi: 10.1007/s11764-024-01589-0

12. Toth P, Tarantini S, Csiszar A, Ungvari Z. Functional vascular contributions to cognitive impairment and dementia: mechanisms and consequences of cerebral autoregulatory dysfunction, endothelial impairment, and neurovascular uncoupling in aging. Am J Physiol Heart Circ Physiol. 2017;312:H1-H20. doi: 10.1152/ajpheart.00581.2016

13. de Montgolfier O, Thorin-Trescases N, Thorin E. Pathological Continuum From the Rise in Pulse Pressure to Impaired Neurovascular Coupling and Cognitive Decline. Am J Hypertens. 2020;33:375-390. doi: 10.1093/ajh/hpaa001

14. Wolters FJ, de Bruijn RF, Hofman A, Koudstaal PJ, Ikram MA, Heart Brain Connection Collaborative Research G. Cerebral Vasoreactivity, Apolipoprotein E, and the Risk of Dementia: A Population-Based Study. Arterioscler Thromb Vasc Biol. 2016;36:204-210. doi: 10.1161/ATVBAHA.115.306768

15. Marshall O, Lu H, Brisset JC, Xu F, Liu P, Herbert J, Grossman RI, Ge Y. Impaired cerebrovascular reactivity in multiple sclerosis. JAMA Neurol. 2014;71:1275-1281. doi: 10.1001/jamaneurol.2014.1668

16. Beaudin AE, McCreary CR, Mazerolle EL, Gee M, Sharma B, Subotic A, Zwiers AM, Cox E, Nelles K, Charlton A, et al. Cerebrovascular Reactivity Across the Entire Brain in Cerebral Amyloid Angiopathy. Neurology. 2022;98:e1716-e1728. doi: 10.1212/WNL.0000000000200136

17. Hutton DA, Cavalier AN, Clayton ZS. Cerebrovascular reactivity: a new frontier for measuring cognitive health in models of accelerated ageing? J Physiol. 2020;598:3323-3325. doi: 10.1113/JP279949

18. Downs TL, Whiteside EJ, Foot G, Mills DE, Bliss ES. Differences in total cognition and cerebrovascular function in female breast cancer survivors and cancer-free women. Breast. 2023;69:358-365. doi: 10.1016/j.breast.2023.03.018

19. Sobczyk O, Crawley AP, Poublanc J, Sam K, Mandell DM, Mikulis DJ, Duffin J, Fisher JA. Identifying Significant Changes in Cerebrovascular Reactivity to Carbon Dioxide. AJNR Am J Neuroradiol. 2016;37:818-824. doi: 10.3174/ajnr.A4679

20. Faraci FM. Protecting against vascular disease in brain. Am J Physiol Heart Circ Physiol. 2011;300:H1566-1582. doi: 10.1152/ajpheart.01310.2010

21. Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, et al. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. J Neurosci Methods. 2011;196:221-237. doi: 10.1016/j.jneumeth.2011.01.011

22. Hallacoglu B, Sassaroli A, Wysocki M, Guerrero-Berroa E, Schnaider Beeri M, Haroutunian V, Shaul M, Rosenberg IH, Troen AM, Fantini S. Absolute measurement of cerebral optical coefficients, hemoglobin concentration and oxygen saturation in old and young adults with near-infrared spectroscopy. J Biomed Opt. 2012;17:081406-081401. doi: 10.1117/1.JBO.17.8.081406

23. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. J Appl Physiol (1985). 2019;126:1360-1376. doi: 10.1152/japplphysiol.00166.2018

24. De Blasi RA, Almenrader N, Aurisicchio P, Ferrari M. Comparison of two methods of measuring forearm oxygen consumption (VO2) by near infrared spectroscopy. J Biomed Opt. 1997;2:171-175. doi: 10.1117/12.269893

25. Davis ML, Barstow TJ. Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy. Respir Physiol Neurobiol. 2013;186:180-187. doi: 10.1016/j.resp.2013.01.012

26. Strangman GE, Zhang Q, Marshall-Goebel K, Mulder E, Stevens B, Clark JB, Bershad EM. Increased cerebral blood volume pulsatility during head-down tilt with elevated carbon dioxide: the SPACECOT Study. J Appl Physiol (1985). 2017;123:62-70. doi: 10.1152/japplphysiol.00947.2016

27. Firbank M, Elwell CE, Cooper CE, Delpy DT. Experimental and theoretical comparison of NIR spectroscopy measurements of cerebral hemoglobin changes. J Appl Physiol (1985). 1998;85:1915-1921. doi: 10.1152/jappl.1998.85.5.1915

28. Read DJ, Leigh J. Blood-brain tissue Pco2 relationships and ventilation during rebreathing. J Appl Physiol. 1967;23:53-70. doi: 10.1152/jappl.1967.23.1.53

29. Bruce CD, Steinback CD, Chauhan UV, Pfoh JR, Abrosimova M, Vanden Berg ER, Skow RJ, Davenport MH, Day TA. Quantifying cerebrovascular reactivity in anterior and posterior cerebral circulations during voluntary breath holding. Exp Physiol. 2016;101:1517-1527. doi: 10.1113/EP085764

30. Schaeffer S, Iadecola C. Revisiting the neurovascular unit. Nat Neurosci. 2021;24:1198-1209. doi: 10.1038/s41593-021-00904-7

31. Levit A, Hachinski V, Whitehead SN. Neurovascular unit dysregulation, white matter disease, and executive dysfunction: the shared triad of vascular cognitive impairment and Alzheimer disease. Geroscience. 2020;42:445-465. doi: 10.1007/s11357-020-00164-6

32. Yang Q, Wei X, Deng B, Chang Z, Jin D, Huang Y, Zhang JH, Yenari MA, Jin K, Wang Q. Cerebral small vessel disease alters neurovascular unit regulation of microcirculation integrity involved in vascular cognitive impairment. Neurobiol Dis. 2022;170:105750. doi: 10.1016/j.nbd.2022.105750

33. Ainslie PN, Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. Am J Physiol Regul Integr Comp Physiol. 2009;296:R1473-1495. doi: 10.1152/ajpregu.91008.2008

34. Lee JK, Kibler KK, Benni PB, Easley RB, Czosnyka M, Smielewski P, Koehler RC, Shaffner DH, Brady KM. Cerebrovascular reactivity measured by near-infrared spectroscopy. Stroke. 2009;40:1820-1826. doi: 10.1161/STROKEAHA.108.536094

35. Miller KB, Howery AJ, Rivera-Rivera LA, Johnson SC, Rowley HA, Wieben O, Barnes JN. Age-Related Reductions in Cerebrovascular Reactivity Using 4D Flow MRI. Front Aging Neurosci. 2019;11:281. doi: 10.3389/fnagi.2019.00281

36. Nguyen KL, Alrezk R, Mansourian PG, Naeim A, Rettig MB, Lee CC. The Crossroads of Geriatric Cardiology and Cardio-Oncology. Curr Geriatr Rep. 2015;4:327-337. doi: 10.1007/s13670-015-0147-4

37. Hurria A, Jones L, Muss HB. Cancer Treatment as an Accelerated Aging Process: Assessment, Biomarkers, and Interventions. Am Soc Clin Oncol Educ Book. 2016;35:e516-522. doi: 10.1200/EDBK_156160

38. Ioffe D, Bhatia-Patel SC, Gandhi S, Hamad EA, Dotan E. Cardiovascular Concerns, Cancer Treatment, and Biological and Chronological Aging in Cancer: JACC Family Series. JACC CardioOncol. 2024;6:143-158. doi: 10.1016/j.jaccao.2024.02.001

39. Lhuissier FJ, Canoui-Poitrine F, Richalet JP. Ageing and cardiorespiratory response to hypoxia. J Physiol. 2012;590:5461-5474. doi: 10.1113/jphysiol.2012.238527

40. Paleczny B, Niewinski P, Rydlewska A, Piepoli MF, Borodulin-Nadzieja L, Jankowska EA, Ponikowska B. Age-related reflex responses from peripheral and central chemoreceptors in healthy men. Clin Auton Res. 2014;24:285-296. doi: 10.1007/s10286-014-0263-9

41. Garcia-Rio F, Villamor A, Gomez-Mendieta A, Lores V, Rojo B, Ramirez T, Villamor J. The progressive effects of ageing on chemosensitivity in healthy subjects. Respir Med. 2007;101:2192-2198. doi: 10.1016/j.rmed.2007.04.015

42. Lakoski SG, Jones LW, Krone RJ, Stein PK, Scott JM. Autonomic dysfunction in early breast cancer: Incidence, clinical importance, and underlying mechanisms. Am Heart J. 2015;170:231-241. doi: 10.1016/j.ahj.2015.05.014

43. Coumbe BGT, Groarke JD. Cardiovascular Autonomic Dysfunction in Patients with Cancer. Curr Cardiol Rep. 2018;20:69. doi: 10.1007/s11886-018-1010-y

44. Yoon SY, Oh J. Cardiovascular Autonomic Dysfunction Before and After Chemotherapy in Cancer Patients. J Clin Neurol. 2024;20:551-562. doi: 10.3988/jcn.2024.0221

45. Peebles KC, Ball OG, MacRae BA, Horsman HM, Tzeng YC. Sympathetic regulation of the human cerebrovascular response to carbon dioxide. J Appl Physiol (1985). 2012;113:700-706. doi: 10.1152/japplphysiol.00614.2012

46. Brassard P, Tymko MM, Ainslie PN. Sympathetic control of the brain circulation: Appreciating the complexities to better understand the controversy. Auton Neurosci. 2017;207:37-47. doi: 10.1016/j.autneu.2017.05.003

47. Koep JL, Taylor CE, Coombes JS, Bond B, Ainslie PN, Bailey TG. Autonomic control of cerebral blood flow: fundamental comparisons between peripheral and cerebrovascular circulations in humans. J Physiol. 2022;600:15-39. doi: 10.1113/JP281058

48. Singer J, Trollor JN, Baune BT, Sachdev PS, Smith E. Arterial stiffness, the brain and cognition: a systematic review. Ageing Res Rev. 2014;15:16-27. doi: 10.1016/j.arr.2014.02.002

49. Fico BG, Miller KB, Rivera-Rivera LA, Corkery AT, Pearson AG, Eisenmann NA, Howery AJ, Rowley HA, Johnson KM, Johnson SC, et al. The Impact of Aging on the Association Between Aortic Stiffness and Cerebral Pulsatility Index. Front Cardiovasc Med. 2022;9:821151. doi: 10.3389/fcvm.2022.821151

50. Raignault A, Bolduc V, Lesage F, Thorin E. Pulse pressure-dependent cerebrovascular eNOS regulation in mice. J Cereb Blood Flow Metab. 2017;37:413-424. doi: 10.1177/0271678X16629155

51. Garcia-Polite F, Martorell J, Del Rey-Puech P, Melgar-Lesmes P, O'Brien CC, Roquer J, Ois A, Principe A, Edelman ER, Balcells M. Pulsatility and high shear stress deteriorate barrier phenotype in brain microvascular endothelium. J Cereb Blood Flow Metab. 2017;37:2614-2625. doi: 10.1177/0271678X16672482

52. Shi Y, Thrippleton MJ, Blair GW, Dickie DA, Marshall I, Hamilton I, Doubal FN, Chappell F, Wardlaw JM. Small vessel disease is associated with altered cerebrovascular pulsatility but not resting cerebral blood flow. J Cereb Blood Flow Metab. 2020;40:85-99. doi: 10.1177/0271678X18803956

53. Parr SK, Liang J, Schadler KL, Gilchrist SC, Steele CC, Ade CJ. Anticancer Therapy-Related Increases in Arterial Stiffness: A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2020;9:e015598. doi: 10.1161/JAHA.119.015598

54. Scheuermann BC, Parr SK, Schulze KM, Kunkel ON, Turpin VG, Liang J, Ade CJ. Associations of Cerebrovascular Regulation and Arterial Stiffness With Cerebral Small Vessel Disease: A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2023;12:e032616. doi: 10.1161/JAHA.123.032616

55. Pendergrass JC, Targum SD, Harrison JE. Cognitive Impairment Associated with Cancer: A Brief Review. Innov Clin Neurosci. 2018;15:36-44.

56. Koppelmans V, Breteler MM, Boogerd W, Seynaeve C, Gundy C, Schagen SB. Neuropsychological performance in survivors of breast cancer more than 20 years after adjuvant chemotherapy. J Clin Oncol. 2012;30:1080-1086. doi: 10.1200/JCO.2011.37.0189

57. Ahles TA, Hurria A. New Challenges in Psycho-Oncology Research IV: Cognition and cancer: Conceptual and methodological issues and future directions. Psychooncology. 2018;27:3-9. doi: 10.1002/pon.4564

58. Nekhlyudov L, Campbell GB, Schmitz KH, Brooks GA, Kumar AJ, Ganz PA, Von Ah D. Cancer-related impairments and functional limitations among long-term cancer survivors: Gaps and opportunities for clinical practice. Cancer. 2022;128:222-229. doi: 10.1002/cncr.33913

59. Coverdale NS, Badrov MB, Shoemaker JK. Impact of age on cerebrovascular dilation versus reactivity to hypercapnia. J Cereb Blood Flow Metab. 2017;37:344-355. doi: 10.1177/0271678X15626156

Chapter 4 - The Effects of Breast Cancer and Immune Checkpoint Inhibition on Cerebral Small Vessel Disease Burden: A Mendelian Randomization Analysis

Abstract

Importance: Breast cancer survivorship is rapidly increasing in the U.S., largely due to novel anti-cancer treatments such as immune checkpoint inhibitors (ICIs). However, robust evidence investigating adverse effects of ICIs is lacking despite the reported variety of neurotoxicities. Understanding the effects of ICIs on cerebrovascular injury, specifically cerebral small vessel disease (CSVD), may inform monitoring and management strategies. **Objective:** To use genetic instruments for estimating the causal effects of breast cancer or exposure to ICIs on CSVD burden. **Design:** This was a genetic association study using two-sample and drug-target Mendelian randomization methods. Analyses were performed between December 2024 and March 2025. **Setting:** Genetic summary statistics were used, primarily representing a European population. **Participants:** Genetic data for primary analyses were predominantly extracted from the Breast Cancer Association Consortium, UK Biobank, Alzheimer's Disease Neuroimaging Initiative or stroke consortium meta-analysis. **Exposures:** Exposures were: 1) genetically predicted breast cancer, stratified into any breast cancer, estrogen-receptor positive or negative, or triple-negative subtypes; 2) a genetic instrument designed and validated to proxy the effects of ICIs. **Main Outcomes and Measures:** Beta coefficients (β) or odds ratios (OR) and 95% confidence intervals (CI) for four CSVD markers: white matter hyperintensity volume (WMH), perivascular space burden (PVS), cerebral microbleeds (CMB), and small vessel stroke (SVS).

Results: Summary data were available for WMH (n=18381), PVS (n=40095; 9339 cases), CMB (n=25862; 3556 cases) and SVS (n=1614080; 13620 cases). Breast cancer - regardless of type - was not associated with any CSVD marker (all p>0.30). However, the genetically-proxied effect of ICIs was associated with a greater WMH volume (β 0.045, 95%CI:[0.003-0.087], p=0.034) and a greater risk of PVS (OR 1.02, 95%CI:[1.00-1.03], p=0.023). **Conclusions and Relevance:** Our findings suggest that breast cancer does not have a direct effect on CSVD, but exposure to ICIs may increase CVSD burden – particularly WMH and PVS.

Introduction

Advances in cancer screening and innovative treatments have dramatically improved survival rates for breast cancer, with the number of survivors in the United States projected to exceed 26 million by 2040.[1] One such innovation in treatment is the addition of immune checkpoint inhibitors (ICIs), which have emerged in the last decade as a promising approach to reduce breast cancer-associated mortality, especially in advanced stage breast cancer.[2] Evidence from clinical trials have demonstrated improved pathologic complete response and overall survival with ICIs compared to chemotherapy alone—successes highlighted in the KEYNOTE-522 (NCT03036488)[3] and KEYNOTE-756 (NCT03725059)[4] clinical trials—underscoring their potential role in shaping the future of breast cancer care. Despite the improvement in cancer-specific outcomes, ICIs are associated with acute and late-developing adverse events - including neurologic and cerebrovascular injury - that can carry severe consequences.[5-7] As recently highlighted[8], we are entering "a new era of immunotherapy survivorship", with ICI-related long-term complications that are becoming increasingly relevant to clinical care.

The increased risk of cerebrovascular morbidity and mortality in long-term survivorship[9,10] is potentially due to common pathophysiological mechanisms between cerebrovascular disease and cancer - such as oxidative stress and inflammation[11,12] – along with the acute and chronic toxicity associated with some cancer treatments, including ICIs.[13] These complications are suggestive of cerebral small vessel disease (CSVD)[14,15], a pathology that primarily develops in regions of vascular endothelial dysfunction[16,17] and is detected on magnetic resonance imaging (MRI).[18] Notably, evidence suggests that both endothelial dysfunction[19-21] and markers of CSVD, such as cerebral microbleeds (CMB)[22], are associated with conventional

chemotherapy. The burden of CSVD after treatment with ICIs remains critically underexplored despite the severity of these cerebral adverse events.[23]

There are obstacles to advancing our knowledge of ICI-related long-term toxicity. As pointed out in a recent *JAMA Oncology* Viewpoint, reporting of adverse events beyond 90 days in ICI clinical trials is incredibly rare[8], restricting the ability to investigate this phenomenon. The few cancer registries reporting ICI-related adverse events are limited by selection bias, among other biases.[24] An innovative approach to circumvent these obstacles is the application of Mendelian randomization (MR), a study design that uses genetic variants as instrumental variables to examine the causal association between an exposure and an outcome.[25,26] This unique methodology also assesses long-term consequences of an exposure[27], thereby addressing a critically understudied window of time in "immunotherapy survivorship". Taking advantage of MR study design, we aimed to identify the effects of breast cancer and ICIs on MRI-derived measures of CSVD. We hypothesized that immune checkpoint inhibition, but not breast cancer, would be associated with an increased burden of CSVD.

Methods

Overview of Mendelian Randomization

Mendelian randomization (MR) begins with identifying genetic variants associated with a selected exposure trait; these variants are used as a genetic instrumental variable. Using genetic summary statistics for an outcome trait, the association between the selected exposure and the target outcome can be assessed. If the core assumptions of MR are met, then this approach results in an estimate of the causal relationship under investigation.[28] There are three core assumptions: 1) relevance – the instrumental variable is associated with the exposure of interest; 2) independence – the instrumental variable is not related to any confounders, and 3) exclusion restriction – the instrumental variable is not related to the outcome measure.[29] Further details are provided in the Supplementary Methods (Appendix B).

This study performed two separate sets of two-sample MR analyses, shown in the study design overview (Figure 1). In both analysis stages, exposure and outcome information was derived from genome-wide association studies (GWAS). The first stage of analysis focuses on the direct effects of breast cancer – and breast cancer subtypes – on markers of CSVD. Exposure data for breast cancer and breast cancer subtypes were obtained from the Breast Cancer Association Consortium.[30] The primary outcomes were individual imaging markers of CSVD (described further below) assessed from MRI-scans and analyzed in a manner consistent with international guidelines.[18] In the second stage of analysis, genetic variants were used to create an instrument to proxy the effects of ICIs. This exposure was assessed in two-sample MR analyses with the same outcomes (CSVD markers) as in the first stage of analysis.

All methods and reporting conducted herein were performed to be consistent with the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian

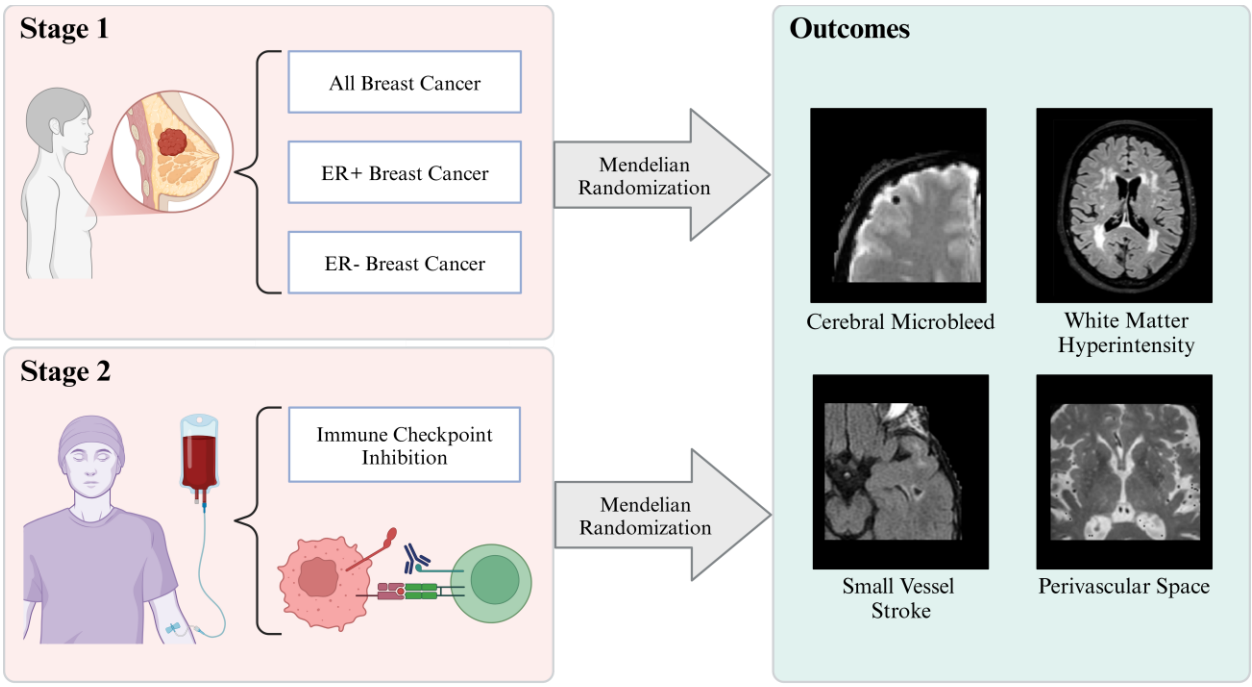


Figure 4-1. An overview of the study design. In stage 1, we used two-sample Mendelian Randomization (MR) to assess the causal associations of all breast cancer types, estrogen receptor positive (ER+) breast cancer, and estrogen receptor negative (ER-) breast cancer with cerebral small vessel disease (CSVD) imaging markers. In stage 2, we identified a genetic instrument designed to mimic the effects of immune checkpoint inhibition, and used this as the exposure for the same outcomes as stage 1. Figure adapted in part from Scheuermann et al.[78]

Randomization (STROBE-MR) recommendations (Appendix B).[31] All analyses used publicly available GWAS summary statistics from studies that obtained ethical approval and participant informed consent; therefore, approval by local institutional research boards were not required.

Exposure Instrumental Variables

Breast Cancer and Subtypes

GWAS summary level data for breast cancer, estrogen-receptor positive (ER+) breast cancer, and estrogen-receptor negative (ER-) breast cancer were derived from a Breast Cancer Association Consortium[30] study. Data for triple-negative (TN) breast cancer was retrieved from a separate meta-analysis of Breast Cancer Association Consortium studies.[32] Study characteristics for the published GWAS are provided in Table 4-1. Extracted single nucleotide polymorphism (SNP) variants met standard threshold criteria (significant p-value < $5x10^{-8}$, window size = 10,000 kb) to ensure that SNPs were significantly associated with the exposure (i.e., breast cancer and subtypes) and not in linkage disequilibrium with one another ($r^2<0.001$). The linkage disequilibrium reference used was the 1000 Genomes reference panel.[33]

Genetic Instrument for PD-1/PD-L1 Signaling Inhibition.

To determine a genetic instrument reflecting the effects of ICIs, we first identified drug targets with supported pharmacological action using the DrugBank database.[34] Cis-variants (i.e., variants within ±100kb of target gene region) significantly associated with the expression of these target genes in whole blood were extracted from the Genotype Tissue Expression (GTEx v10) database[35] and combined.[36] In contrast to the breast cancer analyses, variants were allowed

Table 4-1. Characteristics of the genome-wide association studies (GWAS) used in the present investigation.

Extracted Variable	Source GWAS	Predominant Ethnicity	Sample Size	Adjustments in Model [a]
Exposures				
Any Breast Cancer, ER+/ER- Breast Cancer	PMID: 29059683	European	106,776 [c]	Study site, first 10 PCs.
Triple-Negative Breast Cancer	PMID: 32424353	European	106,377	Age, first 10 PCs.
PDCD1, *CD274* eQTL[b]	PMID: 32913098	European	943	Sex, PCs, PEER factors [d], sequencing platform, sequencing protocol.
Primary Outcomes				
White Matter Hyperintensity	PMID: 32358547	European	18,381	Age, sex, genotyping array, UK Biobank site, first 10 PCs, MRI head motion.
Perivascular Spaces	PMID: 37069360	European	40,095	Age, sex, intracranial volume, study site, PCs.
Cerebral Microbleeds	PMID: 32913026	European	25,862	Age, sex, PCs.
Small Vessel Stroke	PMID: 36180795	European	1,614,080	Age, sex, PCs, study-specific covariates.
Secondary Outcomes [e]				
PD-1, PD-L1 Protein Expression	PMID: 29875488	European	3301	Age, sex, duration between blood draw and processing, first 3 PCs.
Leukocyte Count	PMID: 29892013	European	459,327	Age, age squared, sex, assessment center, genotyping array.
Neutrophil, Lymphoctye, Monocyte Counts	PMID: 32888493	European	562,132	Age, age squared, sex, first 10 PCs, study-specific covariates (i.e., study site).
Interleukin-8 Levels	PMID: 33067605	European	21,758	PCs, study-specific covariates.

[a] PCs, principal components used to adjust for population stratification.
[b] eQTL, expression quantitative trait loci.
[c] Only the oncoarray-based summary statistics were used in the present study.
[d] PEER, probabilistic estimation of expression residuals. A method used to identify a set of covariates based on sample size.
[e] Secondary outcomes were those used in Mendelian randomization analyses aimed at providing biological evidence supporting the validity of the immune checkpoint inhibition genetic instrument.

to be in weak linkage disequilibrium (r^2<0.4) to increase statistical power of analyses.[36-38] Further details on variant selection are provided in Supplementary Methods (Appendix B).

Validation of Genetic Instruments.

For both drug targets and breast cancer, instrument strength for each SNP was assessed using the F-statistic, calculated as ($\beta_{exposure}^2/SE_{exposure}^2$) where SE is standard error.[39] The overall F-statistic for the genetic instrument was taken as the average of all individual F-statistic values. An F-value>10 indicates sufficient instrument strength.[40]

For the genetic instrument designed to proxy the effects of ICIs, additional validation steps were conducted. First, the genetic instrument was assessed in two-sample MR to determine the effect on protein expression of programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) to account for physiological states that may result in differences between predictors of gene and protein expression.[37,41] Second, biological validation was conducted to establish consistent effects of the genetic instrument compared to the clinical effects of ICIs.[37] This was conducted via two-sample MR, estimating the effect of immune checkpoint signaling inhibition on immune cell counts and circulating inflammatory markers, which have been reported before and after ICI exposure in a previous study.[42]

Primary Outcome Variables

Primary outcomes in the present study for CSVD were white matter hyperintensity (WMH) volume, perivascular spaces burden (PVS), cerebral microbleeds (CMB), and small vessel strokes (SVS). WMH data was obtained from a previously published GWAS[43] conducted using the UK Biobank cohort, which has been previously described elsewhere.[44,45] Genetic

associations with PVS were obtained from a study combining 18 population-based cohorts.[46] Data for CMB was extracted from an Alzheimer's Disease Neuroimaging Initiative study incorporating 11 population-based studies and 3 case-control or control-only stroke cohorts.[47] Finally, SVS summary GWAS statistics were derived from a meta-analysis of >1 million individuals of European descent.[48] Cohort characteristics are presented in Table 4-1, and detailed information CSVD marker assessment methods can be found in the respective publications.[43,46-48]

Phenome-Wide Associations of Immune Checkpoint Inhibition

We used phenome-wide GWAS results available through a web-based platform, PheWeb[49], to identify potential phenotypes associated with immune checkpoint inhibition . Specifically, this step of analyses was conducted to identify potential confounding phenotypes that influence the associations between ICI effects and CSVD.[50] Phenotypes were extracted from the FinnGen study, a genomics initiative that has analyzed over 500,000 Finnish biobank samples and correlated genetic variation with health data to understand disease mechanisms and predispositions.[51] Significant phenotypes were identified at $p<2.0x10^{-5}$ to account for the 2469 phenotypes listed in PheWeb.

Statistical Analyses

Our primary outcome for MR analyses was the inverse-variance weighted method, which is often considered as the primary approach for two-sample MR.[25] We additionally assessed all models in sensitivity analyses using MR-Egger, weighted-median, and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) methods. The MR-Egger approach tests for a causal effect but

additionally is used to detect horizontal pleiotropy.[52] The weighted-median method provides consistent results even when up to 50% of the information comes from invalid SNPs.[53] The MR-PRESSO method detects, and corrects for, potential outlier SNPs.[54] MR-PRESSO models were run with 1000 bootstraps.

Model heterogeneity was assessed with the MR-Egger Q statistic.[53,55,56] Horizontal pleiotropy was tested with the MR-Egger intercept, where a value significantly different from zero indicates potential horizontal pleiotropy.[52] Finally, scatter plots were used to visualize the agreement and consistency between the various methods applied.[55]

All analyses were performed in R Studio (v2024.12.0), and figures were created using GraphPad Prism (v10.4.0). MR estimates are presented as beta and 95% confidence intervals (95% CI) for continuous outcomes (WMH), or odds ratio (OR) and 95% CI for binary outcomes (PVS, CMB, SVS). These estimates are given per additional copy of the effect allele, and represent the inverse-variance weighted results unless otherwise stated. Statistical significance was set *a priori* at a 2-sided $p<0.05$. Results were considered robust when statistical significance was achieved in ≥2 methods of assessment.

Results

Effect of Breast Cancer on Cerebral Small Vessel Disease

Instrument strength was sufficient with an average F-statistic value of 78.6 (range 29.8-686.4) for breast cancer, 81.2 (range 30.3-787.2) for ER+, 49.3 (range 31.8-92.1) for ER- exposures, and 67.8 (range 30.3-217.8) for TN breast cancer. None of the methods applied in MR analyses demonstrated a significant relationship between breast cancer and markers of CSVD (all $p>0.3$; Figure 4-2). There was evidence of heterogeneity in tests for WMH, CMB and SVS (Q $p<0.05$); however, adjusting for outliers with MR-PRESSO did not substantially alter results in any case (all $p>0.3$). There was no evidence of horizontal pleiotropy. Results were similar for ER+, ER- and TN subtypes (Appendix Table B-1). Visual inspection of scatter plots indicated agreement across the methods applied (Appendix Figures B-1 through B-4).

Effect of Immune Checkpoint Inhibition on Cerebral Small Vessel Disease

Validation of the Genetic Instrument

The genetic instrument for immune checkpoint signaling inhibition comprised 17 variants in cis-regions for the identified gene targets, PD-1 and PD-L1 (Appendix Table B-2), with an average F-statistic of 33.5 (range 13.5-115.4). Results from MR analyses indicated that the instrument was associated with both PD-1 protein expression (β 0.23, 95% CI: [0.12-0.34], $p=2.4\times10^{-5}$) and PD-L1 protein expression (β 0.34, 95% CI: [0.23-0.45], $p=6.6\times10^{-10}$), mirroring the effects of pembrolizumab observed in patients previously.[57] Subsequent analyses performed for changes in immune cell type distribution and inflammatory biomarkers provided further evidence for the validity of the genetic instrument when compared to observations from a recent trial in responders and non-responders to immunotherapy.[42] Specifically, both the genetic

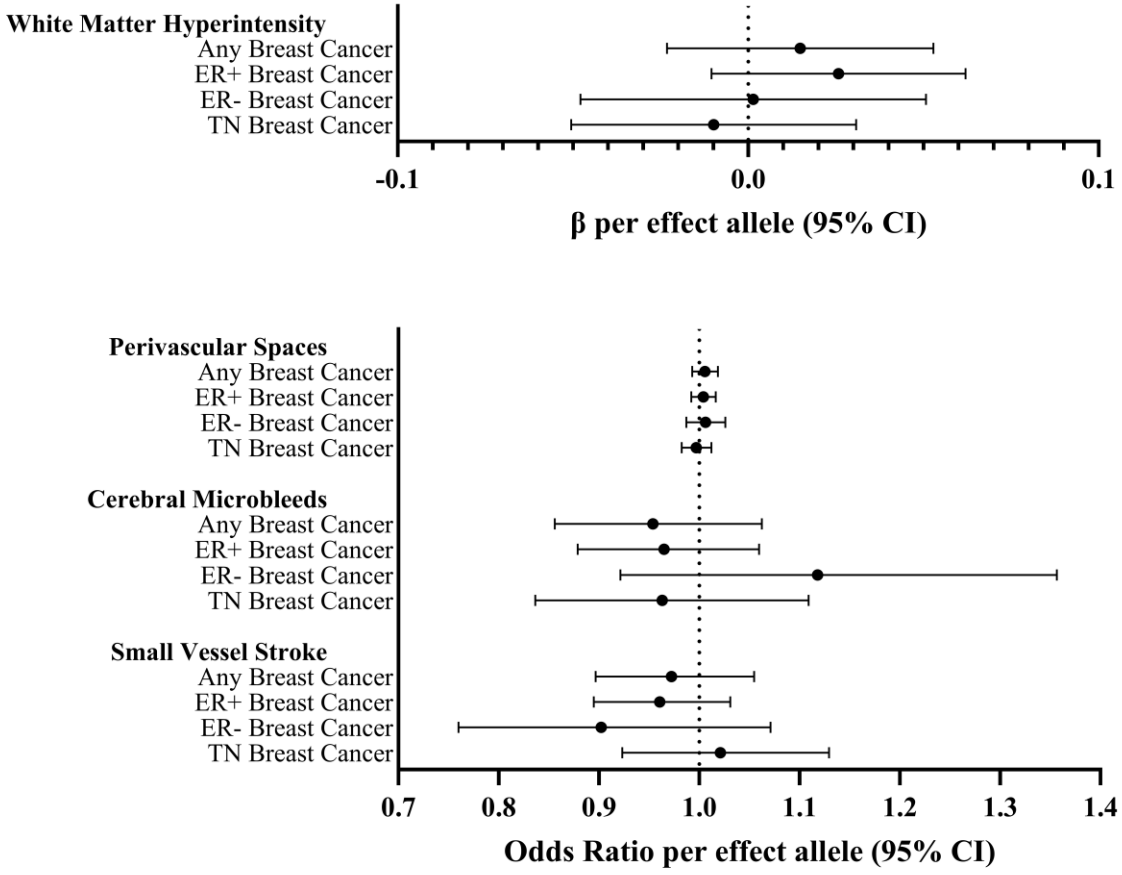


Figure 4-2. Primary inverse-variance weighted (IVW) results of the Mendelian randomization analyses for the effect of any breast cancer type, estrogen-receptor positive (ER+) breast cancer, estrogen-receptor negative (ER-) breast cancer, and triple-negative (TN) breast cancer on the four included markers of cerebral small vessel disease. 95% CI indicates that the plotted error bars show the 95% confidence interval.

instrument and observational data indicated increased lymphocytes and interleukin-8 levels, decreased leukocyte and neutrophils, and no change in monocytes. Evidence supporting biological validity of the genetic instrument is shown in Figure 4-3.

Effect on CSVD Outcomes.

The genetic associations of the effects of ICIs with specific CSVD markers are shown in Figure 4-4. Notably, ICI effects were significantly associated with greater WMH volume (β 0.045, 95% CI: [0.003-0.087], p=0.034) and higher risk for more severe PVS burden (OR 1.02, 95% CI: [1.00-1.03], p=0.023). Both were considered robust, with significant associations supported by at least one additional method (Appendix Table B-3). ICI effects were not associated with CMBs or the risk of SVS (p>0.20). MR-PRESSO detected potential outliers for the SVS model (outlier test p=0.025), however, adjusting for outliers did not substantially alter results (outlier-corrected p=0.37). Scatter plots indicated generally consistent results across the MR methods applied (Appendix Figure B-5).

Phenome-Wide Associations of Immune Checkpoint Inhibition

Of the genetic variants included in the instrument for ICI effects, 5 unique variants were associated with 9 unique phenotypes (Appendix Table B-4). The phenotypes were primarily categorized into diseases of the digestive system (i.e., inflammatory bowel disease, noninfective enteritis and colitis, ulcerative colitis) or the respiratory disease (i.e., chronic lower respiratory diseases, asthma/COPD). Notably, one variant (rs73399174) was associated with the broad phenotype of autoimmune disease ($p=1.9x10^{-5}$).

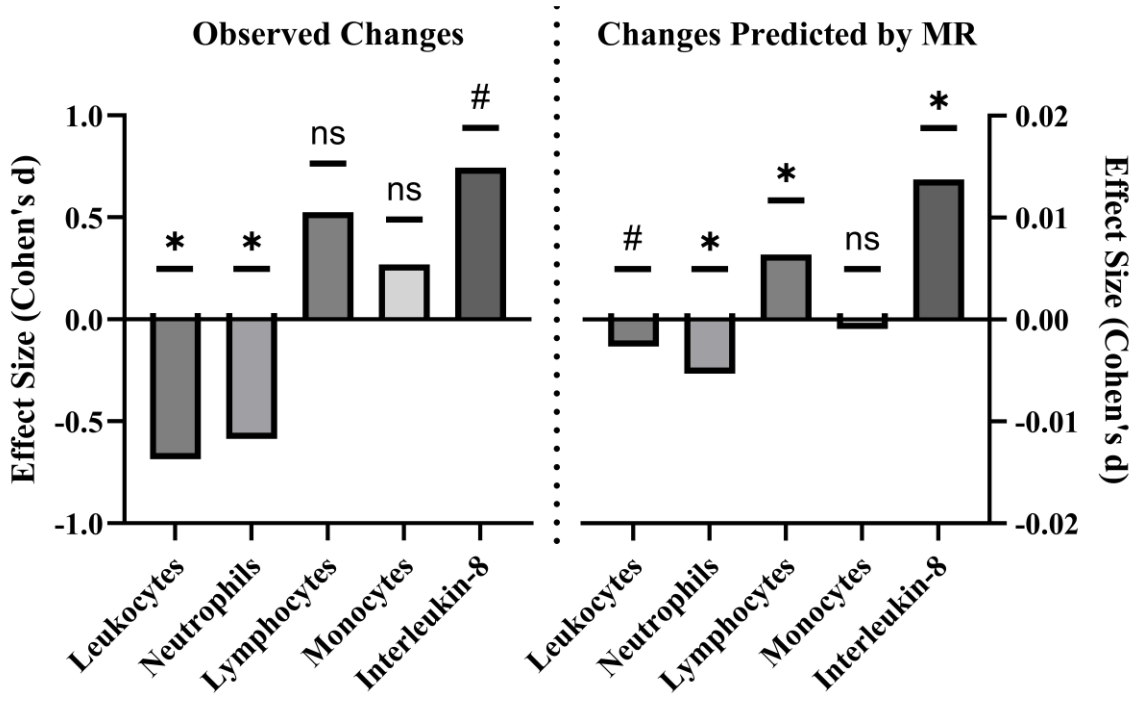
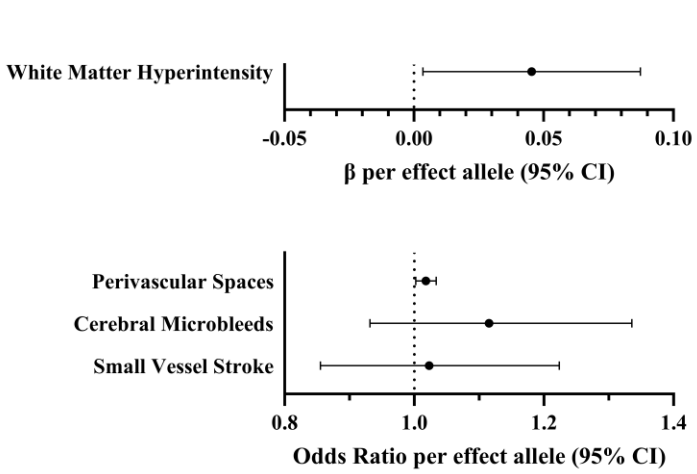


Figure 4-3. The effect of immune checkpoint inhibition on immune and inflammatory circulating markers, compared between clinical data observed in a recent study[42] and from Mendelian randomization analyses conducted with the genetic instrument designed in the present study. Data reported by Juliá et al.[42] were estimated from figures using PlotDigitizer (https://plotdigitizer.com/). Mendelian randomization results shown were conducted using the inverse-variance weighted (IVW) method. *p<0.05, #p<0.10.



	β	*95% CI*	*P-value*
White Matter Hyperintensity	0.045	(0.003-0.087)	**0.034**
	OR	***95% CI***	***P-value***
Perivascular Spaces	1.02	(1.00-1.03)	**0.023**
Cerebral Microbleeds	1.12	(0.93-1.34)	0.23
Small Vessel Stroke	1.02	(0.86-1.22)	0.80

Figure 4-4. Primary inverse-variance weighted (IVW) results of drug-target Mendelian randomization analyses for immune checkpoint inhibition on imaging markers of cerebral small vessel disease (CSVD). 95% CI indicates that the plotted error bars show the 95% confidence interval. Bolded p-values indicate statistically significant results ($p<0.05$).

Discussion

In the present study, we first used genetic analyses to provide causal estimates of the associations between breast cancer (and breast cancer subtypes) with CSVD. We subsequently identified genetic variants to design a genetic instrument mimicking the effects of immune checkpoint signaling inhibition (i.e., the effects of ICIs), and provided evidence supporting the biological validity of the instrument. This instrument was used in genetic analyses to assess the association between ICI effects and markers of CSVD. Consistent with our hypothesis, our present findings suggested: 1) breast cancer itself is not associated with a greater CSVD burden, but 2) exposure to pharmacological immune checkpoint signaling inhibition is associated with CSVD.

Collectively, these findings may advance our understanding of cerebrovascular health – and its associated consequences – in breast cancer survivors. The presence of pre-treatment, breast cancer-associated decrements in brain health remain controversial. Several studies have reported cognitive deficits in treatment-naïve patients with breast cancer[58], which has been suggested to attribute to proinflammatory cytokines or cognitive-emotional stress associated with the cancer diagnosis.[59] The risk of stroke in survivors of cancer is also significantly higher around the time of cancer diagnosis, potentially due to a cancer-induced hypercoagulable state.[60] However, assessments of CSVD burden in treatment-naïve breast cancer are rare in the literature. One study found that survivors of breast cancer treated with chemotherapy - but not survivors without prior chemotherapy - had impaired white matter integrity compared to cancer-free controls.[61] Conversely, a longitudinal study more recently suggested that patients with breast cancer with or without systemic treatment both demonstrated greater WMH volume compared to cancer-free controls.[62] The present findings are consistent with the former report, suggesting that

breast cancer is not associated with direct injury to cerebral white matter. Given the paucity of studies investigating CSVD in survivors of any cancer, further studies are warranted.

Detrimental effects of different anti-cancer therapies on cerebral structure and function are more consistently observed in both the human[63] and pre-clinical research[64]; however, few studies have investigated the newer class of ICIs in the context of cancer survivorship. One of the largest studies in patients who received ICIs suggested a 4-fold increased risk of ischemic stroke[65], but this study did not separate stroke outcomes into subtypes that would specifically examine CSVD-related outcomes. Another study[66] assessed survivors of cancer diagnosed with ICI-induced encephalitis, and found that more than half of those patients with focal encephalitis had abnormal MRI T2/FLAIR hyperintensity (a marker used to determine WMH volume[18]) but again, did not directly assess CSVD burden. Remaining evidence largely comes from case reports[7], which suggest clinical symptoms and cerebrovascular events that are consistent with the known sequelae of CSVD.[67] Interestingly, administration of pembrolizumab has shown promising results in human JC polyoma virus-induced progressive multifocal leukoencephalopathy (PML).[68,69] Why our present findings contrast with observations in PML is unknown; however, it is worth noting that treatment with ICIs in PML patients may lead to immune reconstitution inflammatory syndrome (IRIS), which is associated with worsening leukoencephalopathy and severe immune infiltration in the brain.[70] While some degree of immune-inflammation is necessary to clear the human JC polyoma virus from cerebral tissue, this may explain the adverse cerebrovascular and neurological effects observed in survivors of cancer.

Phenome-wide analyses generally did not indicate confounding phenotypes that may influence the associations between ICI effects and CSVD burden. While asthma has been

reported as related to WMH volume and PVS burden[71], the nature of these relationships remain unclear. Conversely, the association between variant rs73399174 and broad autoimmune disease requires further investigation. It is plausible that autoimmune responses and associated cytokine release[72] may in part underly ICI-related toxicity.

Strengths and Limitations

Our study has strengths to consider beyond the strengths traditionally discussed in the context of MR analyses.[28,29] Specifically, investigations into long-term ICI-associated toxicities are hampered by the sparse nature of available data from prospective or retrospective studies.[8] The key notable strength of our study is the design and validation of a genetic instrument to investigate the effect of immune checkpoint signaling inhibition on cerebrovascular injury (specifically CSVD), which may inform future investigations.

There are a few limitations in our present study. First, the GWAS summary statistics used in this study were primarily conducted in individuals of European descent. Therefore, generalizability to other ethnicities may be limited. Second, the genetic data used to examine breast cancer and subtype exposures did not allow for specific investigations of the effects of breast tumor grade or other tumor characteristics. Third, and related to our second limitation, it should be noted that breast cancer predominantly affects women – this likely leads to an imbalanced distribution of sex in our breast cancer exposure variables versus our CSVD outcome variables. As pointed out in a recent commentary[73], this limitation may bias results towards the null due to unknown sex differences in genetic variants associated with CSVD outcomes. Fourth, the gene targets of ICIs selected for this study were specified using the on-target effects derived from DrugBank. However, there are side effects of these drugs and other anti-cancer therapies, such as inflammation and oxidative stress, that could directly impact cerebral tissue[74-76] or

predicate damage to regulatory functions that result in cerebral damage.[77,78] Finally, despite our sensitivity analyses, it remains possible that there is heterogeneity or pleiotropy not accounted for in our results.

Conclusions

The results from the present two-sample MR study, using two separate sets of exposures, suggest that breast cancer does not have a causal effect on CSVD but that ICIs used for some subtypes of breast cancer (and many other cancers) may increase CSVD burden. This work implicates changes in markers of CSVD after ICI exposure as a critical target for future investigation, especially as the clinical use of these antibodies expands rapidly in cancer and other conditions.

References

1. Emery J, Butow P, Lai-Kwon J, Nekhlyudov L, Rynderman M, Jefford M. Management of common clinical problems experienced by survivors of cancer. Lancet. Apr 16 2022;399(10334):1537-1550. doi:10.1016/S0140-6736(22)00242-2
2. Caswell-Jin JL, Plevritis SK, Tian L, et al. Change in Survival in Metastatic Breast Cancer with Treatment Advances: Meta-Analysis and Systematic Review. JNCI Cancer Spectr. Nov 2018;2(4):pky062. doi:10.1093/jncics/pky062
3. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. Feb 27 2020;382(9):810-821. doi:10.1056/NEJMoa1910549
4. Cardoso F, O'Shaughnessy J, Liu Z, et al. Pembrolizumab and chemotherapy in high-risk, early-stage, ER(+)/HER2(-) breast cancer: a randomized phase 3 trial. Nat Med. Feb 2025;31(2):442-448. doi:10.1038/s41591-024-03415-7
5. Feng S, Coward J, McCaffrey E, Coucher J, Kalokerinos P, O'Byrne K. Pembrolizumab-Induced Encephalopathy: A Review of Neurological Toxicities with Immune Checkpoint Inhibitors. J Thorac Oncol. Nov 2017;12(11):1626-1635. doi:10.1016/j.jtho.2017.08.007
6. Feng J, Ross L, Ontaneda D. Pembrolizumab-Induced CNS Vasculitis: Neurologic Adverse Events Due to Checkpoint Inhibitors. Neurol Clin Pract. Feb 2021;11(1):e30-e32. doi:10.1212/CPJ.0000000000000768
7. Erritzoe-Jervild M, Moller SN, Kruuse C, Stenor C. Immune checkpoint inhibitor-related CNS vasculitis - A systematic review and report of 6 cases. J Stroke Cerebrovasc Dis. Feb 19 2025;34(5):108265. doi:10.1016/j.jstrokecerebrovasdis.2025.108265

8. Cronin C, O'Sullivan A, Naidoo J. Long-Term Toxicity of Immune Checkpoint Inhibitors-Time to Widen the Lens. JAMA Oncol. Feb 27 2025;doi:10.1001/jamaoncol.2024.6809

9. Sturgeon KM, Deng L, Bluethmann SM, et al. A population-based study of cardiovascular disease mortality risk in US cancer patients. Eur Heart J. Dec 21 2019;40(48):3889-3897. doi:10.1093/eurheartj/ehz766

10. Florido R, Daya NR, Ndumele CE, et al. Cardiovascular Disease Risk Among Cancer Survivors: The Atherosclerosis Risk In Communities (ARIC) Study. J Am Coll Cardiol. Jul 5 2022;80(1):22-32. doi:10.1016/j.jacc.2022.04.042

11. Koene RJ, Prizment AE, Blaes A, Konety SH. Shared Risk Factors in Cardiovascular Disease and Cancer. Circulation. Mar 15 2016;133(11):1104-14. doi:10.1161/CIRCULATIONAHA.115.020406

12. Gao H, Chen Z, Yao Y, He Y, Hu X. Common biological processes and mutual crosstalk mechanisms between cardiovascular disease and cancer. Front Oncol. 2024;14:1453090. doi:10.3389/fonc.2024.1453090

13. Lenneman CG, Sawyer DB. Cardio-Oncology: An Update on Cardiotoxicity of Cancer-Related Treatment. Circ Res. Mar 18 2016;118(6):1008-20. doi:10.1161/CIRCRESAHA.115.303633

14. de Ruiter MB, Reneman L, Boogerd W, et al. Late effects of high-dose adjuvant chemotherapy on white and gray matter in breast cancer survivors: converging results from multimodal magnetic resonance imaging. Hum Brain Mapp. Dec 2012;33(12):2971-83. doi:10.1002/hbm.21422

15. Wardlaw JM, Valdes Hernandez MC, Munoz-Maniega S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. J Am Heart Assoc. Jun 23 2015;4(6):001140. doi:10.1161/JAHA.114.001140

16. Fernando MS, O'Brien JT, Perry RH, et al. Comparison of the pathology of cerebral white matter with post-mortem magnetic resonance imaging (MRI) in the elderly brain. Neuropathol Appl Neurobiol. Aug 2004;30(4):385-95. doi:10.1111/j.1365-2990.2004.00550.x

17. Kremer R, Williams A, Wardlaw J. Endothelial cells as key players in cerebral small vessel disease. Nat Rev Neurosci. Jan 2 2025;doi:10.1038/s41583-024-00892-0

18. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. Aug 2013;12(8):822-38. doi:10.1016/S1474-4422(13)70124-8

19. Banister HR, Hammond ST, Parr SK, et al. Lower endothelium-dependent microvascular function in adult breast cancer patients receiving radiation therapy. Cardiooncology. May 13 2021;7(1):18. doi:10.1186/s40959-021-00104-z

20. Hammond ST, Baumfalk DR, Parr SK, et al. Impaired microvascular reactivity in patients treated with 5-fluorouracil chemotherapy regimens: Potential role of endothelial dysfunction. Int J Cardiol Heart Vasc. Dec 2023;49:101300. doi:10.1016/j.ijcha.2023.101300

21. Terwoord JD, Beyer AM, Gutterman DD. Endothelial dysfunction as a complication of anti-cancer therapy. Pharmacol Ther. Sep 2022;237:108116. doi:10.1016/j.pharmthera.2022.108116

22. Koppelmans V, Vernooij MW, Boogerd W, et al. Prevalence of cerebral small-vessel disease in long-term breast cancer survivors exposed to both adjuvant radiotherapy and chemotherapy. J Clin Oncol. Feb 20 2015;33(6):588-93. doi:10.1200/JCO.2014.56.8345

23. Wang DY, Salem JE, Cohen JV, et al. Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis. JAMA Oncol. Dec 1 2018;4(12):1721-1728. doi:10.1001/jamaoncol.2018.3923

24. Fossa SD, Dahl AA, Myklebust TA, et al. Risk of positive selection bias in longitudinal surveys among cancer survivors: Lessons learnt from the national Norwegian Testicular Cancer Survivor Study. Cancer Epidemiol. Aug 2020;67:101744. doi:10.1016/j.canep.2020.101744

25. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. Nov 2013;37(7):658-65. doi:10.1002/gepi.21758

26. Larsson SC, Butterworth AS, Burgess S. Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J. Dec 14 2023;44(47):4913-4924. doi:10.1093/eurheartj/ehad736

27. Ference BA. How to use Mendelian randomization to anticipate the results of randomized trials. Eur Heart J. Feb 1 2018;39(5):360-362. doi:10.1093/eurheartj/ehx462

28. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. Apr 15 2008;27(8):1133-63. doi:10.1002/sim.3034

29. de Leeuw C, Savage J, Bucur IG, Heskes T, Posthuma D. Understanding the assumptions underlying Mendelian randomization. Eur J Hum Genet. Jun 2022;30(6):653-660. doi:10.1038/s41431-022-01038-5

30. Michailidou K, Lindstrom S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. Nature. Nov 2 2017;551(7678):92-94. doi:10.1038/nature24284

31. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: The STROBE-MR Statement. JAMA. Oct 26 2021;326(16):1614-1621. doi:10.1001/jama.2021.18236

32. Zhang H, Ahearn TU, Lecarpentier J, et al. Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. Nat Genet. Jun 2020;52(6):572-581. doi:10.1038/s41588-020-0609-2

33. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature. Oct 1 2015;526(7571):68-74. doi:10.1038/nature15393

34. Knox C, Wilson M, Klinger CM, et al. DrugBank 6.0: the DrugBank Knowledgebase for 2024. Nucleic Acids Res. Jan 5 2024;52(D1):D1265-D1275. doi:10.1093/nar/gkad976

35. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. Sep 11 2020;369(6509):1318-1330. doi:10.1126/science.aaz1776

36. Gill D, Georgakis MK, Walker VM, et al. Mendelian randomization for studying the effects of perturbing drug targets. Wellcome Open Res. 2021;6:16. doi:10.12688/wellcomeopenres.16544.2

37. Gill D, Dib MJ, Cronje HT, et al. Common pitfalls in drug target Mendelian randomization and how to avoid them. BMC Med. Oct 15 2024;22(1):473. doi:10.1186/s12916-024-03700-9

38. Schmidt AF, Finan C, Gordillo-Maranon M, et al. Genetic drug target validation using Mendelian randomisation. Nat Commun. Jun 26 2020;11(1):3255. doi:10.1038/s41467-020-16969-0

39. Sanderson E, Windmeijer F. A weak instrument [Formula: see text]-test in linear IV models with multiple endogenous variables. J Econom. Feb 2016;190(2):212-221. doi:10.1016/j.jeconom.2015.06.004

40. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol. Jun 2011;40(3):755-64. doi:10.1093/ije/dyr036

41. Rhodes B, Merriman ME, Harrison A, et al. A genetic association study of serum acute-phase C-reactive protein levels in rheumatoid arthritis: implications for clinical interpretation. PLoS Med. Sep 21 2010;7(9):e1000341. doi:10.1371/journal.pmed.1000341

42. Julia EP, Mando P, Rizzo MM, et al. Peripheral changes in immune cell populations and soluble mediators after anti-PD-1 therapy in non-small cell lung cancer and renal cell carcinoma patients. Cancer Immunol Immunother. Oct 2019;68(10):1585-1596. doi:10.1007/s00262-019-02391-z

43. Persyn E, Hanscombe KB, Howson JMM, Lewis CM, Traylor M, Markus HS. Genome-wide association study of MRI markers of cerebral small vessel disease in 42,310 participants. Nat Commun. May 1 2020;11(1):2175. doi:10.1038/s41467-020-15932-3

44. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. Oct 2018;562(7726):203-209. doi:10.1038/s41586-018-0579-z

45. Conroy MC, Lacey B, Besevic J, et al. UK Biobank: a globally important resource for cancer research. Br J Cancer. Feb 2023;128(4):519-527. doi:10.1038/s41416-022-02053-5

46. Duperron MG, Knol MJ, Le Grand Q, et al. Genomics of perivascular space burden unravels early mechanisms of cerebral small vessel disease. Nat Med. Apr 2023;29(4):950-962. doi:10.1038/s41591-023-02268-w

47. Knol MJ, Lu D, Traylor M, et al. Association of common genetic variants with brain microbleeds: A genome-wide association study. Neurology. Dec 15 2020;95(24):e3331-e3343. doi:10.1212/WNL.0000000000010852

48. Mishra A, Malik R, Hachiya T, et al. Stroke genetics informs drug discovery and risk prediction across ancestries. Nature. Nov 2022;611(7934):115-123. doi:10.1038/s41586-022-05165-3

49. Gagliano Taliun SA, VandeHaar P, Boughton AP, et al. Exploring and visualizing large-scale genetic associations by using PheWeb. Nat Genet. Jun 2020;52(6):550-552. doi:10.1038/s41588-020-0622-5

50. Walker VM, Zheng J, Gaunt TR, Smith GD. Phenotypic Causal Inference Using Genome-Wide Association Study Data: Mendelian Randomization and Beyond. Annu Rev Biomed Data Sci. Aug 10 2022;5:1-17. doi:10.1146/annurev-biodatasci-122120-024910

51. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. Jan 2023;613(7944):508-518. doi:10.1038/s41586-022-05473-8

52. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. May 2017;32(5):377-389. doi:10.1007/s10654-017-0255-x

53. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. May 2016;40(4):304-14. doi:10.1002/gepi.21965

54. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. May 2018;50(5):693-698. doi:10.1038/s41588-018-0099-7

55. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. Apr 2015;44(2):512-25. doi:10.1093/ije/dyv080

56. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol. Dec 1 2017;46(6):1985-1998. doi:10.1093/ije/dyx102

57. Babacic H, Lehtio J, Pico de Coana Y, Pernemalm M, Eriksson H. In-depth plasma proteomics reveals increase in circulating PD-1 during anti-PD-1 immunotherapy in patients with metastatic cutaneous melanoma. J Immunother Cancer. May 2020;8(1)doi:10.1136/jitc-2019-000204

58. Dijkshoorn ABC, van Stralen HE, Sloots M, Schagen SB, Visser-Meily JMA, Schepers VPM. Prevalence of cognitive impairment and change in patients with breast cancer: A systematic review of longitudinal studies. Psychooncology. May 2021;30(5):635-648. doi:10.1002/pon.5623

59. Cimprich B, So H, Ronis DL, Trask C. Pre-treatment factors related to cognitive functioning in women newly diagnosed with breast cancer. Psychooncology. Jan 2005;14(1):70-8. doi:10.1002/pon.821

60. Wei YC, Chen KF, Wu CL, et al. Stroke Rate Increases Around the Time of Cancer Diagnosis. Front Neurol. 2019;10:579. doi:10.3389/fneur.2019.00579

61. Deprez S, Amant F, Yigit R, et al. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp. Mar 2011;32(3):480-93. doi:10.1002/hbm.21033

62. Menning S, de Ruiter MB, Veltman DJ, et al. Changes in brain white matter integrity after systemic treatment for breast cancer: a prospective longitudinal study. Brain Imaging Behav. Apr 2018;12(2):324-334. doi:10.1007/s11682-017-9695-x

63. Whittaker AL, George RP, O'Malley L. Prevalence of cognitive impairment following chemotherapy treatment for breast cancer: a systematic review and meta-analysis. Sci Rep. Feb 8 2022;12(1):2135. doi:10.1038/s41598-022-05682-1

64. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. Neurosci Biobehav Rev. Jan 2011;35(3):729-41. doi:10.1016/j.neubiorev.2010.09.006

65. Drobni ZD, Alvi RM, Taron J, et al. Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque. Circulation. Dec 15 2020;142(24):2299-2311. doi:10.1161/CIRCULATIONAHA.120.049981

66. Farina A, Villagran-Garcia M, Fourier A, et al. Diagnostic and prognostic biomarkers in immune checkpoint inhibitor-related encephalitis: a retrospective cohort study. Lancet Reg Health Eur. Sep 2024;44:101011. doi:10.1016/j.lanepe.2024.101011

67. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol. Jul 2019;18(7):684-696. doi:10.1016/S1474-4422(19)30079-1

68. Mohn N, Wattjes MP, Adams O, et al. PD-1-inhibitor pembrolizumab for treatment of progressive multifocal leukoencephalopathy. Ther Adv Neurol Disord. 2021;14:1756286421993684. doi:10.1177/1756286421993684

69. Cortese I, Muranski P, Enose-Akahata Y, et al. Pembrolizumab Treatment for Progressive Multifocal Leukoencephalopathy. N Engl J Med. Apr 25 2019;380(17):1597-1605. doi:10.1056/NEJMoa1815039

70. Haroche J, Cohen Aubart F, Mokhtari K, et al. Immune Reconstitution Syndrome After Nivolumab for Progressive Multifocal Leukoencephalopathy. Neurol Clin Pract. Jun 2021;11(3):e376-e378. doi:10.1212/CPJ.0000000000000811

71. Parker J, Wolansky LJ, Khatry D, Geba GP, Molfino NA. Brain magnetic resonance imaging in adults with asthma. Contemp Clin Trials. Jan 2011;32(1):86-9. doi:10.1016/j.cct.2010.09.006

72. Fajgenbaum DC, June CH. Cytokine Storm. N Engl J Med. Dec 3 2020;383(23):2255-2273. doi:10.1056/NEJMra2026131

73. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol. Jun 2016;45(3):908-15. doi:10.1093/ije/dyw127

74. Bagnall-Moreau C, Chaudhry S, Salas-Ramirez K, Ahles T, Hubbard K. Chemotherapy-Induced Cognitive Impairment Is Associated with Increased Inflammation and Oxidative Damage in the Hippocampus. Mol Neurobiol. Oct 2019;56(10):7159-7172. doi:10.1007/s12035-019-1589-z

75. Cavalier AN, Clayton ZS, Hutton DA, et al. Accelerated aging of the brain transcriptome by the common chemotherapeutic doxorubicin. Exp Gerontol. Sep 2021;152:111451. doi:10.1016/j.exger.2021.111451

76. Gupta P, Makkar TK, Goel L, Pahuja M. Role of inflammation and oxidative stress in chemotherapy-induced neurotoxicity. Immunol Res. Dec 2022;70(6):725-741. doi:10.1007/s12026-022-09307-7

77. Parr SK, Liang J, Schadler KL, Gilchrist SC, Steele CC, Ade CJ. Anticancer Therapy-Related Increases in Arterial Stiffness: A Systematic Review and Meta-Analysis. J Am Heart Assoc. Jul 21 2020;9(14):e015598. doi:10.1161/JAHA.119.015598

78. Scheuermann BC, Parr SK, Schulze KM, et al. Associations of Cerebrovascular Regulation and Arterial Stiffness With Cerebral Small Vessel Disease: A Systematic Review and Meta-Analysis. J Am Heart Assoc. Dec 5 2023;12(23):e032616. doi:10.1161/JAHA.123.032616

Chapter 5 - Circulatory Links between Immune Checkpoint Inhibitors and Cerebral Small Vessel Disease: Integration of Single Cell RNA-Sequencing and Multiomic Mendelian Randomization

Abstract

Background: Immune checkpoint inhibitor (ICI) exposure is associated with neurotoxic adverse events that vary in incidence rates and severity. Many forms of these neurotoxicities share common traits with cerebral small vessel disease (CSVD), suggesting a possible link between ICI treatment and CSVD burden. However, investigations into this pathway have been limited. **Methods:** We leveraged single-cell RNA-sequencing data obtained from clear cell renal carcinoma patients who were (n=4) or were not (n=3) exposed to ICI treatment. Differentially expressed genes were identified specifically in endothelial cells to target the endothelium as a link between ICI exposure and CSVD. The set of differentially expressed genes were subsequently used to design exposures in multiomic Mendelian randomization analyses with CSVD outcomes. Both gene expression and methylation omics levels were entered as exposures using expression quantitative trait loci and methylation quantitative trait loci. Genes associated with CSVD markers in both levels were subsequently selected to identify potential therapeutics with opposing transcription signatures. **Results:** Single-cell RNA-seq analysis of renal vascular endothelial cells from clear cell carcinoma patients (untreated n=3, ICI-treated n=4) identified 1085 differentially expressed genes (DEGs) after ICI exposure, relating to pathways involving oxidative phosphorylation, protein folding chaperones, and cellular redox homeostasis. Five genes (*Cd55*, *Cltb*, *Htra1*, *Nme4*, *Xrcc6*) were associated with CSVD markers at both genomic

and epigenetic levels in Mendelian randomization analysis, highlighting biological mechanisms including DNA damage repair and complement/coagulation cascades. Finally, pharmaco-transcriptomic analyses suggested the strongest therapeutic potential was observed in statins.

Discussion: Our findings from the present study provide multiomic evidence supporting an association between ICI exposure and CSVD. These results were extended to explore potential pharmacological approaches, which identified statins as a strong possibility.

Introduction

Targeting the anti-tumor immune response through administration of immune checkpoint inhibitors (ICI) has provided treatment opportunities for many cancer tumor types, improving cancer control five-fold in some cases.[1] The ever-expanding list of successful trials, such as KEYNOTE-564 (NCT03142334) in renal-cell carcinoma[2] or the Impower150 trial (NCT02366143) in metastatic non-squamous non-small-cell lung cancer[3], underscore their growing contributions across a variety of oncology settings. As the rate of ICI prescription increases[4], so does the role of immune-vascular oncology research in determining the causes and consequences of ICI-related adverse effects on the cardiovascular system.[5] As pointed out by Cronin et al earlier this year[6], we are in "a new era of immunotherapy survivorship" that brings not only the hope of improving cancer outcomes, but also the reminder to consider the integrated physiological consequences of anti-cancer treatment.

One of the most severe adverse side-effects of ICI exposure is neurotoxicity.[7] While incidences tend to be rare[8], some reports suggest mortality rates ranging from 8% to 15% mortality rate after severe neurotoxic events.[9,10] The clinical presentations vary widely, indicating the possibility of multiple etiological mechanisms[11]; however, emerging case reports[12,13] and biomarker discovery studies[14-16] have implicated substantial overlaps with cerebral small vessel disease (CSVD). CSVD is a pathology primarily affecting the small penetrating arterioles of the cerebrovasculature detected on magnetic resonance imaging[17,18], associated with numerous physiological[19] and clinical sequelae.[20] An understanding of CSVD in the neurotoxicity associated with ICI treatment would likely advance monitoring and therapeutic strategies, yet findings remain limited for numerous reasons despite the vital importance.

The same publication that detailed the "new era of immunotherapy survivorship" also discussed several obstacles to studying adverse events in the short and long-term timeframes.[6] Predominant amongst these obstacles are the restricted adverse event reporting windows in clinical trials (typically ~90 days) and biases inherent to observational or retrospective studies.[21] Thus, in an effort to guide future prospective or clinical trials in this area, unique approaches are necessary such as using Mendelian randomization[22,23]. In the present study, we aimed to first identify vascular cell-specific effects of ICI treatment using single-cell RNA-sequencing data collected from patients who were or were not exposed to monoclonal antibodies targeting programmed death 1 (PD-1). Genes that were differentially expressed between ICI conditions were selected as a "gene set" that guided subsequent analyses. The gene set from single-cell RNA-sequencing was integrated with genetic and epigenetic Mendelian randomization analyses to estimate the effects of ICI-related vascular cell alterations on CSVD. Finally, vascular genes supported by robust evidence (i.e., found to be significant in both gene expression and methylation expression Mendelian randomization analyses) were selected for further exploration of potential therapeutic routes that may reverse the vascular effects of ICI treatment. The goals of the present work are two-fold: 1) to provide initial insight into possible mechanisms linking ICI exposure to CSVD through the effects ICIs have on the vasculature, and 2) to inform future investigations focusing on both understanding the etiology of neurotoxicity after ICI use and means to mitigate or treat the neurotoxicity.

Methods

Ethics Statement and Study Overview

The design, execution and reporting of this study were conducted in adherence with the Strengthening the Reporting of Observational studies in Epidemiology – Mendelian Randomization (STROBE-MR) guidelines.[24] All data used herein were sourced from published and publicly available databases that obtained ethical approval and participant informed consent; therefore, approval by local institutional research boards at Kansas State University was not required. All data and analyses can be found in this study or by reasonable request to the authors.

In this study, we aimed to identify biological pathways and processes contributing to the risk of CSVD in patients who have received ICI treatments, alongside the identification of possible therapeutic options. A visual schematic overview of the study design is presented in Figure 5-1.

scRNA-seq Analysis of Patient Tissue

Data source.

We used scRNA-seq data from the Zenodo database to evaluate differentially expressed genes (DEGs) between ICI treatment conditions in patients with clear cell carcinoma. Tumor and adjacent non-tumor bulk tissue samples were obtained in the study from Bi et al[25] and pre-processed as part of a publicly available dataset from Gondal et al.[26] The tissue samples were collected from untreated patients (n=3) and treated patients (n=4); the treated patients received some combination of anti-PD-1 blockade, anti-CTLA-4 blockade, and/or VEGF inhibitors. Patient characteristics are detailed in Table 5-1. Sample collection details can be found in the original publication.[25]

Table 5-1. Characteristics of patients who contributed tissue samples for the single-cell RNA sequencing data used in the present study. Data originally collected by Bi et al.[25]

Patient ID	Age (years)	Sex	Cancer Type	AJCC Stage	ICI Type	Response to ICI
No Immune Checkpoint Inhibitors						
P76	68	Female	Clear cell renal carcinoma	IV	N/A	N/A
P90	71	Female	Clear cell renal carcinoma	I	N/A	N/A
P916	61	Male	Clear cell renal carcinoma	IV	N/A	N/A
Treated with Immune Checkpoint Inhibitors						
P55	57	Male	Clear cell renal carcinoma	IV	Anti-PD-1	PR
P912	60	Male	Clear cell renal carcinoma	IV	Anti-PD-1	NE
P913	47	Male	Clear cell renal carcinoma	IV	Anti-PD-1	PR
P915	58	Male	Clear cell renal carcinoma	IV	Anti-PD-1 / Anti-CTLA-4	SD

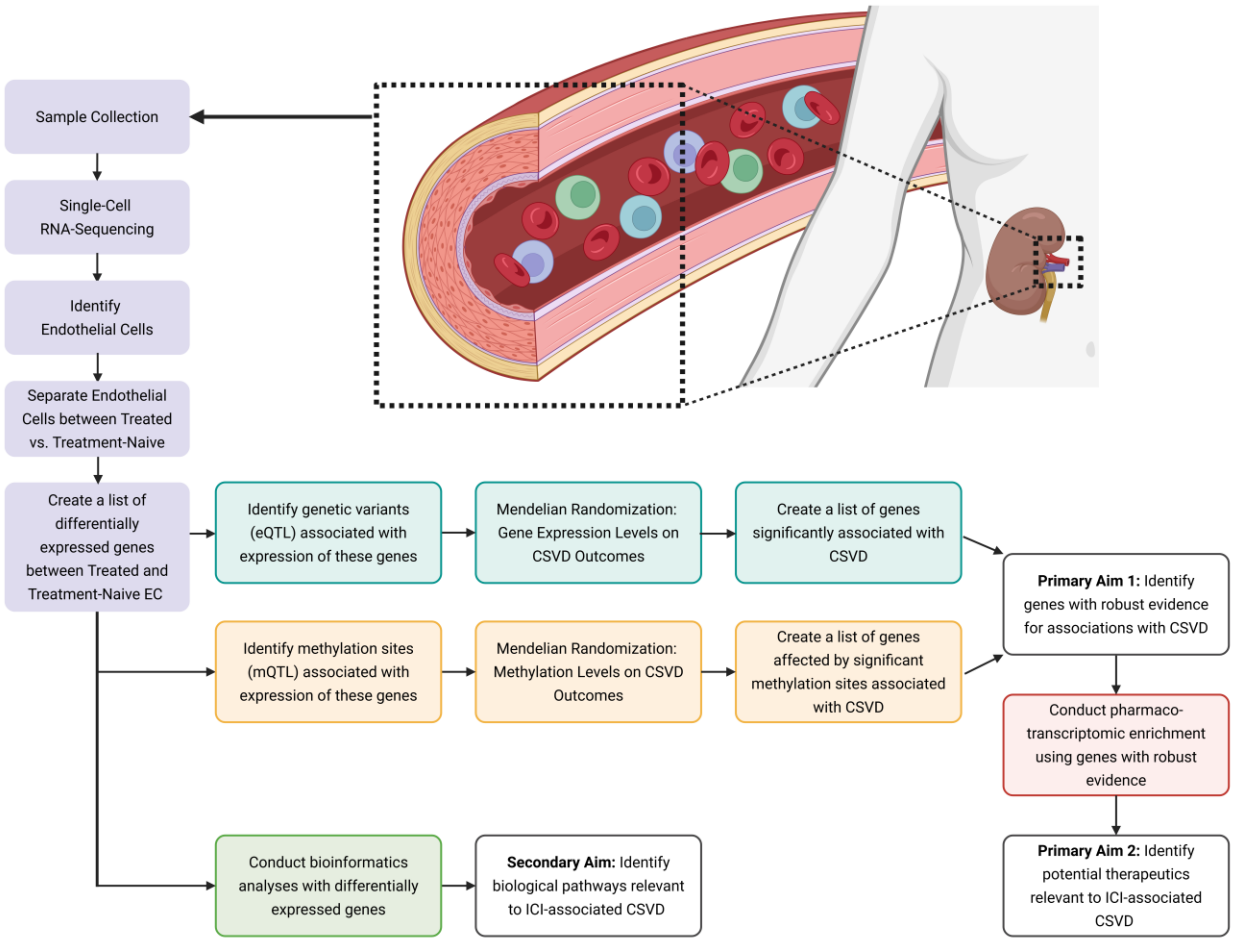


Figure 5-1. An overview schematic of the study design. ICI, immune checkpoint inhibitor; CSVD, cerebral small vessel disease. Figure created with BioRender.

Data processing.

Data processing and analysis were conducted using the *Seurat* package (version 5.2).[27,28] To ensure data quality, cells with >20% mitochondrial gene content or <5% ribosomal RNA expression were excluded. The *DoubletFinder* algorithm[29] was used to remove any doublets (more than one cell per bead). Further filtering removed any non-malignant cell types that had less than 20 cells.[26] Subsequently, data were normalized and scaled using the *NormalizeData* and *ScaleData* functions. Dimensionality reduction and Louvain unsupervised clustering were performed using the first 20 principal components. Cell types were identified using cell-specific molecular signatures through the *Vision* algorithm[30] alongside CellMarker 2.0.[31] Using patient clinical metadata, differential expressional analyses were performed between treatment-naïve and ICI-treated conditions within cell types. To specifically investigate ICI-induced vascular changes, endothelial cells were selected as the cell-type of primary interest since endothelial cells act as the interface between circulating biological or pharmacological factors and organ tissue.[32,33] Furthermore, endothelial dysfunction and impaired vascular reactivity are implicated as key factors in the early pathogenesis of CSVD.[34] Therefore, the endothelial cell cluster was deemed most relevant to investigating the effects of ICI treatment on the risk of CSVD in the face of limitations of not having cerebral tissue-specific analyses available. Markers used to identify the endothelial cell cluster are provided in Table 5-2.

Statistical analysis

The Wilcoxon rank sum test was used to compare genes between patient samples with or without ICI treatment. Differentially expressed genes within the endothelial cell type were identified at Bonferroni-adjusted P-values <0.05 to account for multiple testing.

Table 5-2. The top 15 markers used to identify the endothelial cell subcluster from the single-cell RNA-sequencing analysis on bulk tumor and non-tumor tissue derived from Bi et al. The "Proportion Expressed" column is the proportion of cells in the endothelial cell cluster that expressed the marker compared to the proportion of all cells in the other clusters that expressed the marker; p-values (adjusted for multiple comparisons) are provided. EC, endothelial cells; OC, other cells; PMID, PubMed identification number.

Marker ID	Marker Name	Proportion Expressed (EC:OC)	Adjusted P-value	Publication Reference PMIDs
EGFL7	EGF like domain multiple 7	0.827 : 0.075	$<1.00x10^{-100}$	30789893, 32483223
VWF	von Willebrand factor	0.755 : 0.007	$<1.00x10^{-100}$	29230012, 35168605, 33936064, 34687145
IGFBP7	insulin like growth factor binding protein 7	0.673 : 0.170	$<1.00x10^{-100}$	34775482
PLVAP	plasmalemma vesicle associated protein	0.664 : 0.005	$<1.00x10^{-100}$	32754283, 30093597
IFITM3	interferon induced transmembrane protein 3	0.659 : 0.360	$4.99x10^{-42}$	32236824
CRIP2	cysteine rich protein 2	0.600 : 0.091	$<1.00x10^{-100}$	32236824
FLT1	fms related receptor tyrosine kinase 1	0.595 : 0.008	$<1.00x10^{-100}$	34921160, 32754283
RAMP2	receptor activity modifying protein 2	0.568 : 0.003	$<1.00x10^{-100}$	34921160, 30789893
CLDN5	claudin 5	0.527 : 0.005	$<1.00x10^{-100}$	33936064
CDH5	cadherin 5	0.495 : 0.001	$<1.00x10^{-100}$	34843404
VWA1	von Willebrand factor A domain containing 1	0.414 : 0.036	$<1.00x10^{-100}$	33406409
TEK	TEK receptor tyrosine kinase	0.327 : 0.001	$<1.00x10^{-100}$	32107344
TJP1	tight junction protein 1	0.286 : 0.023	$<1.00x10^{-100}$	32236824
NOS3	nitric oxide synthase 3	0.127 : 0.003	$<1.00x10^{-100}$	35168605
ABCG2	ATP binding cassette subfamily G member 2 (JR blood group)	0.086 : 0.002	$<1.00x10^{-100}$	32236824

Pathway Enrichment Analysis of Differentially Expressed Genes

Differentially expressed genes identified as significant from analyses were selected as inputs for bioinformatics analyses. Only genes significant at a Bonferroni-adjusted $p<0.05$ were selected from scRNA-seq analyses. Protein-protein interaction maps were generated using Cytoscape (version 3.10.3) and the String database plugin (version 10.0). Enriched Gene Ontology (GO) Biological Processes[35], Reactome Pathways[36], and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified.[37] Pathways were visualized with GeneMania.[38] To further facilitate biological interpretation, the functionally related GO pathway terms were clustered using the ClueGO plugin (version 2.5.10) in Cytoscape.[39] Only genes with a $\log_2$(fold-change)$\geq$2.0 were imported into ClueGO. Network settings were GO Tree intervals between 3 and 10, the minimum number of genes involved per pathway set at 4, and at least 15% coverage of genes with associated terms. ClueGO cluster significance was adjusted using the Bonferroni step-down correction.

Endothelial Cell-Based Multiomic Associations with CSVD

Gene Expression Exposures

Gene expression quantitative trait loci (eQTL) connect genetic variants to the expression of specific genes across different tissue sites. To investigate gene expressions associated with CSVD, cis-variants (i.e., variants within ±100kb of target gene region) significantly associated with the expression a specific gene in whole blood were extracted from the Genotype Tissue Expression (GTEx v10) database[40] to form gene-specific eQTL. In sensitivity analysis, eQTL were also created for coronary artery tissue to determine the robustness of associations in another vascular tissue type. Variants were allowed to be in weak linkage disequilibrium ($r^2<0.2$, window

10000kb) to increase statistical power of analyses. The linkage disequilibrium reference used in the present study was the 1000 Genomes reference panel.[41] Instrument strength for each exposure was assessed using the F-statistic, calculated as [$F = (R^2 \times (N-2))/(1-R^2)$], where R^2 is the squared correlation of the exposure relationship and N is the sample size.[42-44] The overall F-statistic for each genetic instrument was taken as the average of all included individual genetic variant F-statistic values. An F-value>10 indicates sufficient instrument strength. Further details on variant selection in the GTEx database are provided in Supplementary Methods (Appendix C).

Methylation Exposures

Similar to eQTL, methylation quantitative trait loci (mQTL) quantitatively link genetic variants to methylation levels at specific DNA sites. The Accessible Resource for Integrated Epigenomics Studies (ARIES) project[45] generated epigenetic data on blood samples from 1,018 participants of the Avon Longitudinal Study of Parents and Children (ALSPAC) study.[46] Cis-variants associated with cytosine guanine dinucleotide (CpG) site methylation levels ($p<1x10^{-7}$, $r^2=0.001$, window 10000kb) were extracted to form methylation site-specific mQTL. Statistical criteria for variants were selected to be consistent with the ARIES study methods. Only cis-variants detected for both sexes were incorporated in current analyses. Instrument strength for each methylation exposure was assessed using the F-statistic, calculated using the same approach as described for gene expression exposures. Further details on variant selection in the ARIES database are provided in Supplementary Methods (Appendix C).

CSVD Outcomes

Imaging markers and clinical consequences of CSVD were selected as the outcome variables for the present study, including white matter hyperintensity (WMH) volume, perivascular spaces burden (PVS), cerebral microbleeds (CMB), and small vessel strokes (SVS). WMH data was obtained from a previously published GWAS[47] conducted using the UK Biobank cohort, which has been previously described elsewhere.[48,49] Genetic associations with PVS were obtained from a study combining 18 population-based cohorts.[50] Data for CMB was extracted from an Alzheimer's Disease Neuroimaging Initiative study incorporating 11 population-based studies and 3 case-control or control-only stroke cohorts.[51] SVS summary GWAS statistics were derived from a meta-analysis of >1 million individuals of European descent.[52] Cohort characteristics are presented in Table 5-3, and detailed information CSVD marker assessment methods can be found in the respective publications.[47,50-52]

Mendelian randomization

In brief, MR begins with identifying genetic variants associated with a selected exposure trait; these variants are used as a genetic instrumental variable. Using genetic summary statistics for an outcome trait, the association between the selected exposure and the target outcome can be assessed. If the core assumptions of MR are met, then this approach results in an estimate of the causal relationship under investigation.[53] There are three core assumptions: 1) relevance – the instrumental variable is associated with the exposure of interest; 2) independence – the instrumental variable is not related to any confounders, and 3) exclusion restriction – the instrumental variable is not related to the outcome measure.[54] Further description of MR theory is provided in the Supplementary Methods (Appendix C).

Table 5-3. Characteristics of the genome-wide association studies (GWAS) used in the present investigation.

Extracted Variable	Source GWAS	Predominant Ethnicity	Sample Size	Adjustments in Model [a]
Exposures				
Gene Expression – Whole Blood	PMID: 32913098	European	943	Sex, PCs, PEER[b] factors, sequencing platform, sequencing protocol.
Gene Expression – Coronary Artery	PMID: 32913098	European	943	Sex, PCs, PEER factors, sequencing platform, sequencing protocol.
Methylation – Whole Blood	PMID: 25869828	European	1018	Gestational age, sex, birth weight, parity, maternal age, maternal smoking, child delivery method.
Primary Outcomes				
White Matter Hyperintensity	PMID: 32358547	European	18,381	Age, sex, genotyping array, UK Biobank site, first 10 PCs, MRI head motion.
Perivascular Spaces	PMID: 37069360	European	40,095	Age, sex, intracranial volume, study site, PCs.
Cerebral Microbleeds	PMID: 32913026	European	25,862	Age, sex, PCs.
Small Vessel Stroke	PMID: 36180795	European	1,614,080	Age, sex, PCs, study-specific covariates.

[a] PCs, principal components used to adjust for population stratification.
[b] PEER, probabilistic estimation of expression residuals. A method used to identify a set of covariates based on sample size.

In the present study, MR was implemented both at the levels of gene expression and methylation. Differentially expressed genes in scRNA-seq analysis of endothelial cells were selected, and both gene expression data and methylation data for those genes were identified if available. The set of differentially expressed genes with gene expression and/or methylation data were entered into MR models with the CSVD markers as outcomes.

MR statistical analysis

Exposure-outcome associations were assessed using inverse-variance weighted methods, or with Wald ratio methods for exposure variables with only 1 genetic variant. To account for multiple testing within each CSVD marker, p-values were adjusted using the Benjamini-Hochberg method with a false discovery rate (FDR) <0.05. Analyses were run in RStudio (version 2024.12.0) using the *TwoSampleMR* package.[55] Results are presented as beta and 95% confidence intervals (95% CI) for continuous outcomes (WMH), or odds ratio (OR) and 95% CI for binary outcomes (PVS, CMB, SVS). These estimates are given per additional copy of the effect allele.

Identifying Potential Pharmacological Interventions

Differentially expressed genes from scRNA-seq analysis associated with at least one marker of CSVD in both gene expression and methylation MR analyses were selected as inputs for pharmaco-transcriptomics. These genes were queried against the Connectivity Map[56], a database of perturbation expression signatures based on human cell lines. A reduced statistical threshold (FDR<0.1) was implemented to ensure a sufficient number of differentially expressed genes. A total of 171 perturbation signature classes – grouped by pharmacological or biological

mechanisms of action – were queried. Embedded tests in the Connectivity Map assess each signature class against the input data, resulting in a normalized connectivity score ranging from -1 (reference signature is completely opposite of the input query) to +1 (reference signature completely mirrors the input query).[56] Connectivity Map analyses were conducted using the web tools (version 1.1.1.43) along with RStudio (version 2024.12.0). Drug classes identified through Connectivity Map query were subsequently described using the online webtool, DrugBank.[57]

Results

ICI-induced Differences in scRNA-seq of Patient Tissue

Overview of patient cohort

As described in detail in Table 5-1, samples were collected from patients either treated with immune checkpoint inhibitors (n=3) or not treated with ICI (n=4) for renal cell carcinoma. Treatment-naïve samples were collected from 1 man and 2 women (66.7 years, range: 61-71 years). Two patients were stage IV and one patient was stage I, based on American Joint Committee on Cancer staging criteria. ICI-treated patient samples were not collected from any individuals who had contributed treatment-naïve samples. ICI-treated patient samples were collected from 4 males (55.5 years, range: 47-60 years) who all had stage IV renal cell carcinoma. All ICI-treated patients contributing tissue had received anti-PD-1 blockade therapy, and samples were collected ~2 months (range: 1-3 months) after cessation of ICIs.

Differentially expressed genes and enriched pathways

Endothelial cells were identified based on cluster cell markers and stratified by treatment exposure, resulting in 74 treatment-naïve endothelial cells and 146 endothelial cells treated with anti-PD-1 (Figure 5-2; Appendix Figure C-1). A total of 1085 differentially expressed genes were identified in endothelial cells with or without ICI treatment, the majority of which were upregulated with exposure to ICIs (Figure 5-3A). Several of the top GO Biological Processes, ranked by FDR, indicated alterations in metabolism (Figure 5-3B). Major genes encoding for cytochrome proteins (*Mt-cyb*, *Mt-co2*) or other portions of the electron transport chain (*Mt-nd1, Atp5f1d, Ndufb7*) were differentially regulated, resulting in enriched pathways related to oxidative phosphorylation (GOBP:0006119) and cellular respiration (GOBP:00453333). This

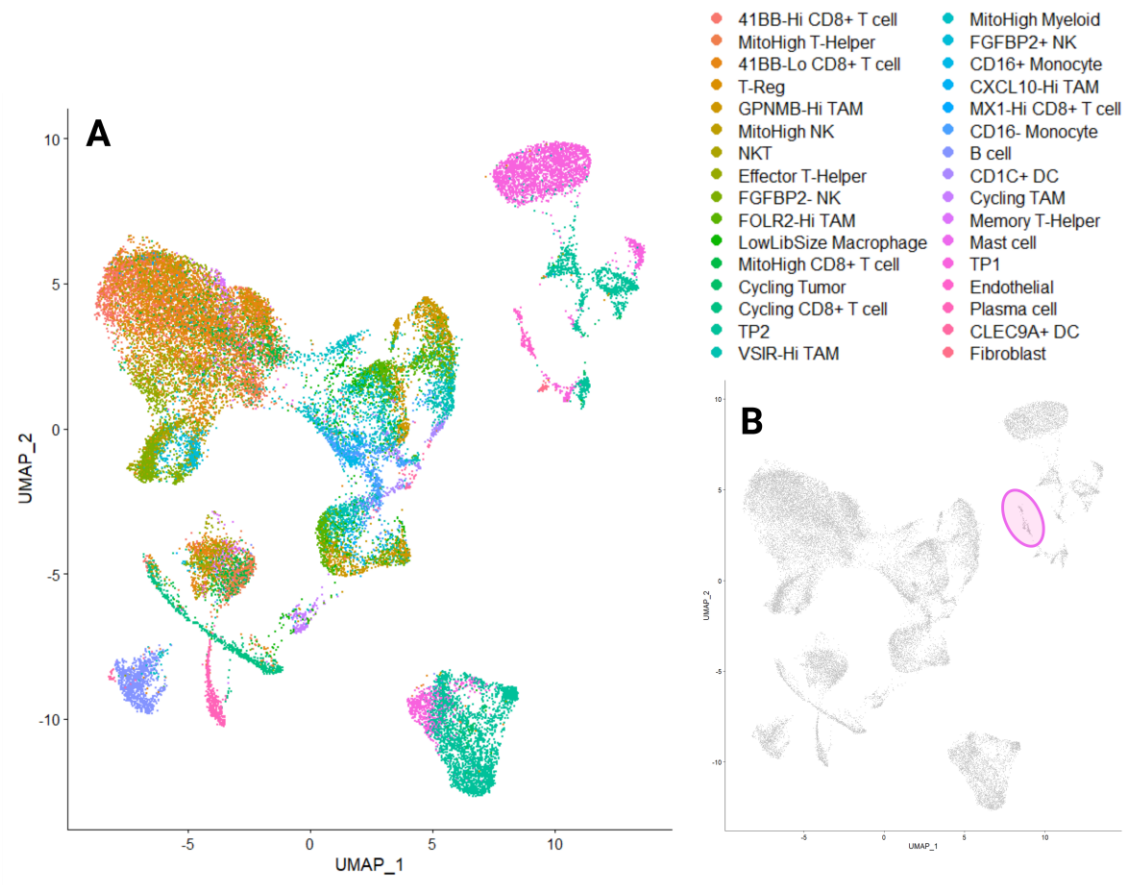


Figure 5-2. Cells from the single-cell RNA-sequencing data were clustered according to standard cell markers. The resulting clusters are presented using a uniform manifold projection and approximation (UMAP) plot (**A**). For clarity, the location of the endothelial cell cluster is highlighted (**B**).

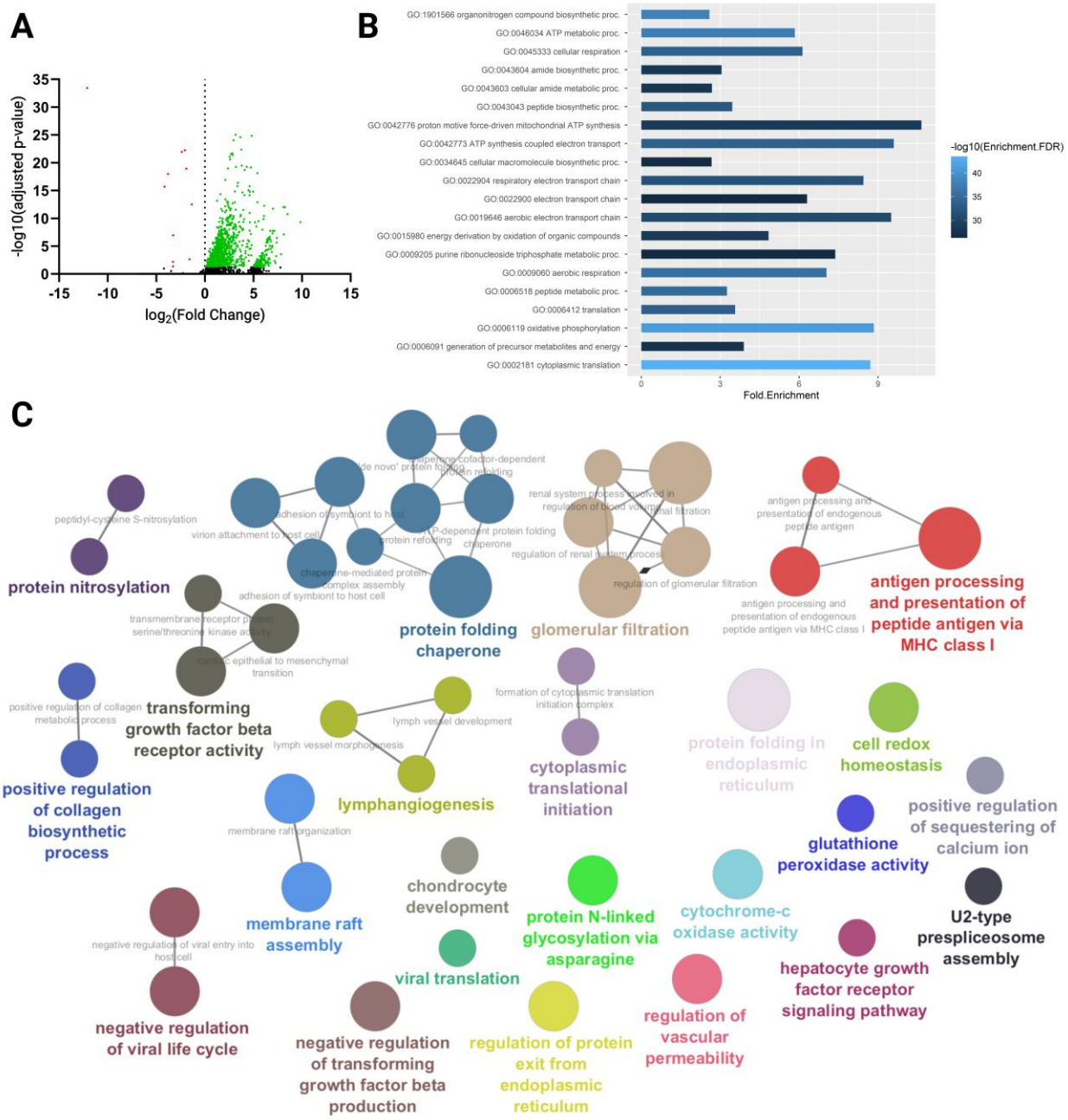


Figure 5-3. (**A**) Volcano plot depiction of endothelial cell-specific genes that were upregulated (green) or downregulated (red) after immune checkpoint inhibitor exposure. (**B**) Gene Ontology Biological Process (GOBP) pathways significantly enriched when comparing differentially expressed genes between control and immune checkpoint inhibitor-exposed endothelial cells. (**C**) Enriched pathways were clustered using the ClueGO software plugin as part of the Cytoscape suite of programs. Significant clusters are shown (**C**); statistics are reported in Appendix Table C-1.

was supported by enrichment analyses in KEGG and Reactome (Appendix Figures C-2, C-3). Interestingly, pathways related to cerebral diseases, such as Alzheimer's disease or Huntington's disease, were also enriched by the presence of genes implicated in oxidative stress (*Sod1*), cell structure and adhesion (*Tubb, Adrm1*) and cell transport processes (*Hspa5, Cav1, Slc25a5*).

Clustering analyses resulted in 23 distinct clusters that were largely consistent with pathway enrichment analyses. ClueGO analyses indicated that 19.6% of genes were involved in protein folding chaperones, 6.5% in transforming growth factor beta receptor activity, 4.4% in membrane raft assembly, 2.2% in cellular redox homeostasis and 2.2% in cytochrome c oxidase activity (Figure 5-3C; Appendix Table C-1).

Gene Expression Levels Associated with CSVD

Genetic eQTL instruments were analyzed in up to 619 differentially expressed genes identified from patient tissue analyses. All 619 instruments were available for the PVS outcome; in the remaining outcomes, <8% of instruments had to be excluded due to nonmatching genomic summary statistics in the outcome variable (Appendix Tables C-2, C-3). All genetic instruments demonstrated sufficient strength (average F-statistic: 53.5, range: 12.4 – 2282.4).

In MR analyses, a total of 38 unique genes were significantly associated with at least one marker of CSVD (Figure 5-4). Two genes (*Hla-c, Hla-drb1*) were associated with multiple CVSD outcomes – both WMH and PVS. Overall, the slight majority of gene-outcome pairs (n=22, 55%) of the significant individual models were positively related to a greater CSVD burden.

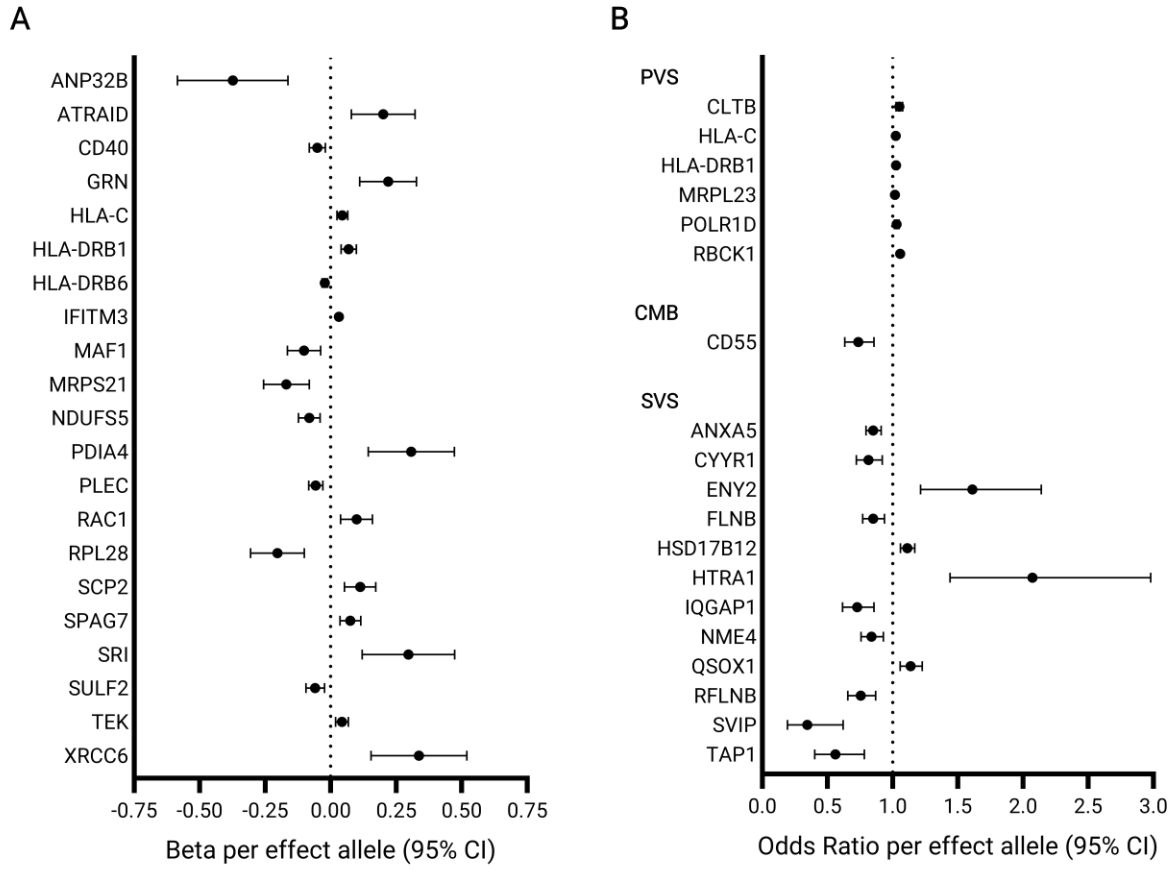


Figure 5-4. Gene expression-level results of Mendelian randomization analyses with imaging markers of cerebral small vessel disease outcomes. Panel **A** depicts results for white matter hyperintensity (WMH) volume. Panel **B** depicts results for perivascular space (PVS) burden, cerebral microbleeds (CMB), and small-vessel stroke (SVS). Results are presented for inverse variance weighted or Wald (if the instrument only had 1 variant) methods. 95% CI indicates that the plotted error bars show the 95% confidence interval.

Sensitivity analyses were conducted using eQTL in coronary artery tissue (instead of whole blood) at a nominal $p<0.05$. There were notably less significant gene-outcome associations in the coronary artery analyses after adjusting p-values for multiple testing (Supplementary Table C-4); however, the results were largely consistent with our primary findings. Several genes were supported with evidence from both whole blood and coronary artery eQTL, such as *Hla-c*, *Hla-drb1*, *Iqgap1* and *Ndufs5*. Importantly, the direction of effect was consistent across both eQTL analyses in the majority (83.5%) of cases.

Methylation Levels Associated with CSVD

In MR analyses, 531 methylation sites (mQTL) were eligible for testing in WMH; PVS (1.1%), CMB (1.3%) and SVS (4.1%) had some excluded instruments due to nonmatching genomic summary statistics in the outcome variable. This corresponded to 267 unique genes targeted by the methylation (mQTL) sites that were tested for WMH and PVS, 268 for CMB, and 262 for SVS (Appendix Tables C-5, C-6). All exposure instrument variables demonstrated sufficient strength assessed with F-statistics (mean: 146.9, range: 26.7 – 1407.5).

There were a total of 52 methylation sites associated with at least one marker of CSVD, with an even split (50.0%) of associations positively related to CSVD burden (Figure 5-5). Seven methylation sites were associated with ≥2 CSVD markers; two sites (*cg16958793*, *cg00472373*) were associated with three markers. A total of 38 unique genes were linked to the significant methylation sites. Six of those genes (*Cmip*, *Htra1*, *Bmpr2*, *Arpc4*, *St6gal1*, *Cdkn1a*) were significantly related to multiple CSVD outcomes.

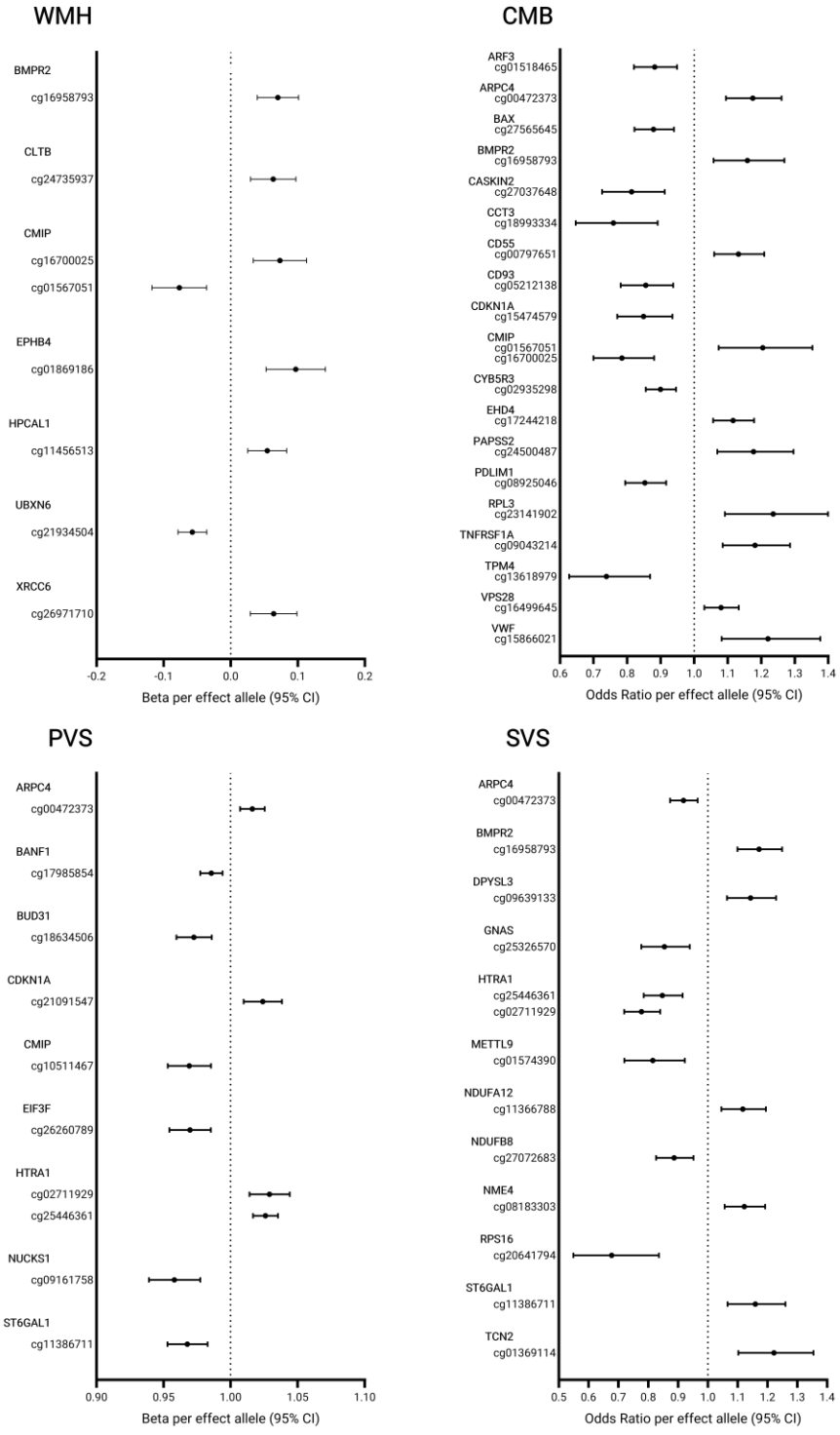


Figure 5-5. Methylation-level results of Mendelian randomization analyses with imaging markers of cerebral small vessel disease outcomes. Results are presented for inverse variance weighted or Wald (if the instrument only had 1 variant) methods. 95% CI indicates that the plotted error bars show the 95% confidence interval. WMH, white matter hyperintensity volume; PVS, perivascular space burden; CMB, cerebral microbleeds; SVS, small vessel stroke.

Integrated Evidence from Multiomic Analyses

Shown in Figure 5-6A, integrating genes from the eQTL and mQTL analyses resulted in five shared, high-priority targets: *Cd55* (cluster of differentiation 55 molecule), *Cltb* (clathrin light chain B), *Htra1* (high temperature requirement A serine peptidase 1), *Nme4* (NME/NM23 nucleoside diphosphate kinase 4), and *Xrcc6* (X-ray repair cross complementing 6). Gene loci and surrounding gene encoding regions are presented in Appendix Figures C-4 through C-8. Pathway enrichment of these genes (allowing for up to 10 additional interacting molecules) predominantly implicated DNA damage repair pathways (GOBP:0006302, FDR $p=9.9x10^{-5}$; GOBP:0006303, FDR $p=3.0x10^{-4}$) and activation of complement and coagulation cascades (GOBP:0006956, FDR p=0.036). The complement and coagulation pathways were supported by evidence from KEGG (hsa04610, FDR p=0.011) and Reactome (HSA-977606, FDR p=0.018) databases. The protein-protein interaction map is presented in Figure 5-6B.

Pharmaco-transcriptomic Enrichment

We used a reduced threshold (FDR $p<0.10$) to integrate results from MR analysis of eQTL and mQTL for pharmaco-transcriptomic analysis. The 5 genes above (*Cd55*, *Cltb*, *Htra1*, *Nme4*, and *Xrcc6*) were included, as were 8 additional genes (*Arf3*, *Ephb4*, *Ifitm3*, *Mvp*, *Ndufb8*, *Qsox1*, *Scp2*, and *Ssbp4*). This list of 13 genes (all upregulated by ICI treatment in endothelial cells based on scRNA-seq results) were queried in the Connectivity Map database against 2385 combinations of pharmacological classes tested in different human cell lines. We ranked significant results (FDR $p<0.05$) separately by positive (similar directions of results from this study and reference perturbation) and negative (opposing directions of results from this study and reference perturbation) connectivity score.

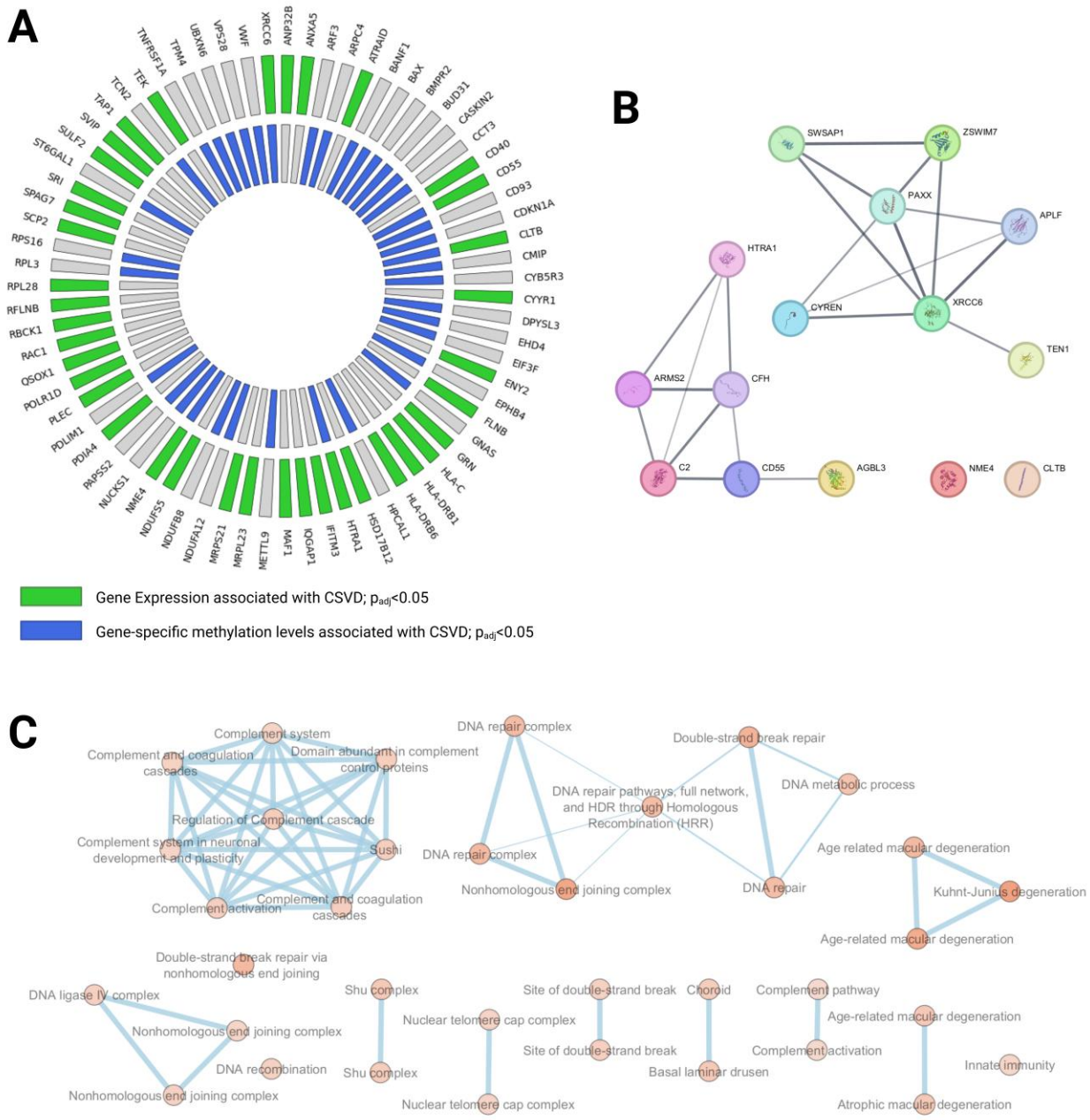


Figure 5-6. Integration of evidence across multiple omics levels. (**A**) Circle plot depicting genes that were significantly associated with cerebral small vessel disease at the genomic (green) and/or methylomic (blue) levels. (**B**) Protein-protein interaction mapping of the genes that were significantly associated with cerebral small vessel disease in both omics and were therefore considered "robust". (**C**) Pathway enrichment results for the "robust" genes associated with cerebral small vessel disease.

The top 10 significant, opposing results are shown in Table 5-4. Notably, the top hit for negative connectivity scores was a standard pharmaceutical in cardiovascular risk prevention – 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) inhibitors, or statins (Connectivity Score: -0.72, FDR $p=2.2x10^{-16}$). Several other classes with negative scores were investigational drugs without regulatory approval (Table 5-3). Pharmacological classes with positive connectivity scores consisted largely of anti-cancer therapeutics (Appendix Table C-7), as well as selective estrogen receptor modulators (FDR p=0.0042) and vitamin D receptor agonists (FDR p=0.046).

Table 5-4. The top 10 (ranked by connectivity score) pharmacological perturbation signatures opposing the effects of immune checkpoint inhibitor treatment on endothelial cell gene expression in the gene set robustly associated with cerebral small vessel disease.

Pharmacological Class	**Cell Types Tested**	**Raw Connectivity Score**	**Adjusted P-value**	**Regulatory Status**
HMGCR Inhibitors	HEPG2	-0.72	$2.2x10^{-16}$	FDA-approved
DNA Inhibitors	HEK293	-0.69	0.004	FDA-approved
ATPase Inhibitors	ASC	-0.66	$2.2x10^{-16}$	Investigational
XIAP Inhibitors	A375	-0.65	0.0011	Investigational
Aurora Kinase Inhibitors	HCC515	-0.63	$2.2x10^{-16}$	Investigational
GABA Receptor Antagonists	HCC515	-0.57	0.0098	FDA-approved
HSP Inhibitors	HCC515	-0.47	0.021	Investigational
JAK Inhibitors	HA1E	-0.43	0.048	FDA-approved
Protein Synthesis Inhibitors	PC3	-0.36	0.0018	FDA-approved
Ubiquitin-specific Protease Inhibitors	N/A	-0.33	0.037	FDA-approved

Discussion

In the present study, we integrated findings from scRNA-seq, gene expression (eQTL) and methylation (mQTL) analyses to 1) explore links between the effects of ICIs on endothelial cells and the risk of CSVD, and 2) identify potential therapeutic approaches to mitigate ICI-related CSVD. Our results indicated 5 genes that were consistently associated with one or more CSVD markers: *Cd55*, *Cltb*, *Htra1*, *Nme4*, and *Xrcc6.* Furthermore, including these 5 genes and 8 more genes at a less strict statistical threshold in a query targeting pharmacological routes identified possible therapeutic options, such as statins. Taken in sum, our study provides multiomic evidence supporting vascular and circulation-specific pathological links between ICI treatment and CSVD as well as possible means of mitigating this phenomenon.

The gene implicated most frequently across CSVD markers was *Htra1*, which has been demonstrated in several studies to contain mutations that drive a rare familial form of CSVD called cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL).[58,59] However, emerging evidence suggests that polymorphisms in the *Htra1* gene region may also lead to sporadic, non-familial forms of CSVD.[60] The HTRA family of proteins act as both chaperones and serine proteases[61] – HTRA1 degrades components of the extracellular matrix such as fibronectin and type II collagen, playing a key role in vascular integrity[62], inflammation, and immune cell infiltration.[63] HTRA1 also mediates transforming growth factor beta (TGF-β) signaling, which is believed to underly HTRA1-specific CSVD.[64] Interestingly, pre-clinical models of CSVD have suggested that HTRA1 protein expression was increased only in animal models of cerebral amyloid angiopathy, not in models of hypertensive CSVD.[65] This finding aligns with reports that HTRA1 variants are associated with CSVD independent of hypertension, even in sporadic CSVD.[60] While not

apparent in the present approach, literature supports an association between *Htra1* mutations and local cerebral arteriosclerosis, regardless of whether the mutations are homozygous or heterozygous.[66] Further research is needed to better understand the role of *Htra1* in sporadic CSVD.

Three of the five key genes identified (*Htra1*, *Nme4*, *Xrcc6*) have been implicated as contributors to cellular senescence. Levels of HTRA1 protein appear to regulate cell cycle processes; for example, upregulation of HTRA1 is associated with accelerated cellular senescence.[67] While the exact mechanisms are not fully known, evidence in an HTRA1 loss-of-function model may compromise the function of cell cycle checkpoints as they relate to DNA damage.[68] This overlaps with senescence-related properties of both the *Nme4* and *Xrcc6* - both have been linked to DNA damage and DNA repair pathways[69,70], albeit via different routes. The *Xrcc6* gene encodes Ku70, part of the Ku heterodimer that aids in repairing DNA through non-homologous end joining.[71] Impairment in the *Xrcc6* gene results in cell death characterized by severe telomere shortening[69], a stereotypical presentation of accelerated cellular aging and cellular senescence.[72,73] *Nme4* is also implicated in telomere length and cellular senescence. However, as it encodes a mitochondrial protein, reports indicate pathways involving *Nme4* likely include dysregulated metabolism and generation of reactive oxygenation species.[70] In connection to CSVD, cellular senescence has been linked to injury in the cerebrovasculature and several of the subsequent manifestations in multiple preclinical studies.[74-76] However, the potential impacts of these pathophysiological mechanisms remain largely unknown in patients.

In our analysis of endothelial cell gene expression, ICI treatment induced an upregulation in the *Cd55* and *Cltb* genes. This is not necessarily a surprising finding in the context of ICI-induced immune activation. It has previously been established that complement activation results

in an increased CD55 protein expression.[77] Similarly, CLTB expression is enhanced as part of the lysosome signaling cascades that regulate cell death and autophagy in immune responses.[78] CLTB is also associated with atherosclerosis - a key factor in the pathogenesis of sporadic CSVD[79] - through lysosome-mediated lipid metabolism. Low-density lipoprotein receptor (LDLR) endocytosis is mediated through clathrin-associated pathways[80] in the endothelium, and subsequent release of LDL into the subendothelial space can lead to atherogenesis.[81] This process may be exacerbated in the context of overt immune activation, such as the systemic response to ICI treatment. A previous report provided evidence to support this concept of an immune role by demonstrating that *apoE*$^{-/-}$/*Cd55*$^{-/-}$ mice had reduced plaque development compared to *apoE*$^{-/-}$ controls.[82] Their findings in this atherosclerosis-prone preclinical model lacking CD55 were unexpected - the loss of CD55, as an inhibitor of the complement system, was hypothesized to increase atherosclerosis. However, this paradoxical finding has been reproduced in other studies.[83] A better understanding of the complement and immune systems in CSVD and atherosclerosis contributions to CSVD is warranted, especially as investigations continue to report accelerated atherosclerotic development after ICI treatment.[5,84]

Considering the variety of biological pathways implicated in our present findings, a pharmacological therapeutic with established benefits in a variety of conditions - and limited side effects - is ideal. The results of our pharmaco-transcriptomics analysis identified several possibilities; however, the potential repurposing of statins may present a route that satisfies the above criteria and is more feasible from a regulatory viewpoint.[85] Beyond their lipid-lowering effects, statins have demonstrated beneficial effects on reducing cellular senescence[86] and atherosclerosis.[87,88] Indeed, statins are often prescribed on the basis of atherosclerotic cardiovascular disease prevention.[89] While statins have been described as beneficial to improve

cerebrovascular regulation[90], which is associated with CSVD[19], few studies have investigated statin use directly for the treatment of CSVD.[91] Nevertheless, their pleiotropic effects and limited adverse effect profile[92] posits drugs of the statin class as encouraging candidates for reducing cerebrovascular complications, such as CSVD, in ICI-treated patients. American Heart Association calculators that predict 10-year risk of atherosclerotic cardiovascular disease have not yet accounted for the heightened risk that anti-cancer treatments induce, but reports of their validity in general[93] and cancer-specific contexts[94] suggest that this may be adapted for statin prescription in cardio-oncology settings. Research into the links between statin use, immune regulation, and CSVD may provide further insight.[95]

Strengths and Limitations

The primary strength of this study lies in its triangulation of evidence using scRNA-seq data, multiomics data and genetic analyses to provide robust results.[96] Additionally, our use of combined publicly available data and genetic analyses allows us to address a gap in the literature where current investigations into long-term ICI-associated toxicities are hampered by the sparse nature of available data from prospective or retrospective studies.[6] However, there are limitations to the present study that are worth noting. First, the GWAS summary statistics used in this study were primarily conducted in individuals of European descent. Therefore, generalizability to other ethnicities may be limited. Second, the summary statistics used in the present work were collected in mixed sex samples, precluding the investigation of sex-specific effects. As suggested recently[97], this limitation may bias results towards the null due to unknown sex differences in genetic variants associated with CSVD outcomes. Third, the results of scRNA-seq analyses were derived from renal vascular endothelial cells, and not cerebrovascular endothelial cells which

limits generalizability.[98] While the two vascular beds have several distinct characteristics, both are high-flow, low-impedance regions of the cardiovascular system with unique barrier properties and vascular regulatory mechanisms.[99,100] The use of renal samples also avoids the substantial challenges making collecting cerebral tissue samples difficult in human patients. Finally, despite our sensitivity analyses, it remains possible that there is heterogeneity or pleiotropy not accounted for in our results.

Conclusions

Findings from our present study suggest that genetically predicted gene expression and methylation levels of 5 genes (*Cd55*, *Ctlb*, *Htra1*, *Nme4*, *Xrcc6*) derived from scRNA-seq analysis of patients with or without ICI treatment are associated with CSVD. Furthermore, integration of multiomic data revealed potential therapeutic options, including statins. Expanded research is needed to elucidate underlying biological mechanisms linking ICI treatment with CSVD and to evaluate mitigation strategies.

References

1. Carlino MS, Larkin J, Long GV. Immune checkpoint inhibitors in melanoma. Lancet. 2021;398:1002-1014. doi: 10.1016/S0140-6736(21)01206-X
2. Powles T, Tomczak P, Park SH, Venugopal B, Ferguson T, Symeonides SN, Hajek J, Gurney H, Chang YH, Lee JL, et al. Pembrolizumab versus placebo as post-nephrectomy adjuvant therapy for clear cell renal cell carcinoma (KEYNOTE-564): 30-month follow-up analysis of a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2022;23:1133-1144. doi: 10.1016/S1470-2045(22)00487-9
3. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Rodriguez-Abreu D, Moro-Sibilot D, Thomas CA, Barlesi F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med. 2018;378:2288-2301. doi: 10.1056/NEJMoa1716948
4. Haslam A, Gill J, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for Immune Checkpoint Inhibitor Drugs. JAMA Netw Open. 2020;3:e200423. doi: 10.1001/jamanetworkopen.2020.0423
5. Drobni ZD, Alvi RM, Taron J, Zafar A, Murphy SP, Rambarat PK, Mosarla RC, Lee C, Zlotoff DA, Raghu VK, et al. Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque. Circulation. 2020;142:2299-2311. doi: 10.1161/CIRCULATIONAHA.120.049981
6. Cronin C, O'Sullivan A, Naidoo J. Long-Term Toxicity of Immune Checkpoint Inhibitors-Time to Widen the Lens. JAMA Oncol. 2025. doi: 10.1001/jamaoncol.2024.6809

7. Pan PC, Haggiagi A. Neurologic Immune-Related Adverse Events Associated with Immune Checkpoint Inhibition. Curr Oncol Rep. 2019;21:108. doi: 10.1007/s11912-019-0859-2

8. Bruna J, Argyriou AA, Anastopoulou GG, Alemany M, Nadal E, Kalofonou F, Piulats JM, Simo M, Velasco R, Kalofonos HP. Incidence and characteristics of neurotoxicity in immune checkpoint inhibitors with focus on neuromuscular events: Experience beyond the clinical trials. J Peripher Nerv Syst. 2020;25:171-177. doi: 10.1111/jns.12371

9. Placais L, Michot JM, Champiat S, Romano-Martin P, Baldini C, Joao MS, Marabelle A, Voisin AL, Not A, Labeyrie C, et al. Neurological complications induced by immune checkpoint inhibitors: a comprehensive descriptive case-series unravelling high risk of long-term sequelae. Brain Commun. 2021;3:fcab220. doi: 10.1093/braincomms/fcab220

10. Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, Zhao S, Das S, Beckermann KE, Ha L, et al. Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis. JAMA Oncol. 2018;4:1721-1728. doi: 10.1001/jamaoncol.2018.3923

11. Garcia CR, Robertson IJ, Gregory TA, Zahid A, Amini B, Kamiya-Matsuoka C, Tummala S. Differential diagnosis in immune checkpoint inhibitors neurotoxicity. J Neurol. 2025;272:116. doi: 10.1007/s00415-024-12872-6

12. Lasocki A, Kee D. Clinical and radiological evolution of cerebral amyloid angiopathy-related inflammation in the context of anti-PD-1 immunotherapy. Melanoma Res. 2020;30:608-612. doi: 10.1097/CMR.0000000000000683

13. Severijns C, Drion E, Bianchi E, Maquet P. Cerebral Amyloid Angiopathy-Related Inflammation following Multiple Cancers and Chemotherapies. Case Rep Neurol. 2022;14:149-156. doi: 10.1159/000522308

14. Gattringer T, Pinter D, Enzinger C, Seifert-Held T, Kneihsl M, Fandler S, Pichler A, Barro C, Grobke S, Voortman M, et al. Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology. 2017;89:2108-2114. doi: 10.1212/WNL.0000000000004645

15. Bjursten S, Zhao Z, Al Remawi H, Studahl M, Pandita A, Simren J, Zetterberg H, Lundell AC, Rudin A, Ny L, et al. Concentrations of S100B and neurofilament light chain in blood as biomarkers for checkpoint inhibitor-induced CNS inflammation. EBioMedicine. 2024;100:104955. doi: 10.1016/j.ebiom.2023.104955

16. Qu Y, Tan CC, Shen XN, Li HQ, Cui M, Tan L, Dong Q, Yu JT, Alzheimer's Disease Neuroimaging I. Association of Plasma Neurofilament Light With Small Vessel Disease Burden in Nondemented Elderly: A Longitudinal Study. Stroke. 2021;52:896-904. doi: 10.1161/STROKEAHA.120.030302

17. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-838. doi: 10.1016/S1474-4422(13)70124-8

18. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol. 2019;18:684-696. doi: 10.1016/S1474-4422(19)30079-1

19. Scheuermann BC, Parr SK, Schulze KM, Kunkel ON, Turpin VG, Liang J, Ade CJ. Associations of Cerebrovascular Regulation and Arterial Stiffness With Cerebral Small

Vessel Disease: A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2023;12:e032616. doi: 10.1161/JAHA.123.032616

20. Pasi M, Cordonnier C. Clinical Relevance of Cerebral Small Vessel Diseases. Stroke. 2020;51:47-53. doi: 10.1161/STROKEAHA.119.024148

21. Fossa SD, Dahl AA, Myklebust TA, Kiserud CE, Nome R, Klepp OH, Brydoy M, Haugnes HS. Risk of positive selection bias in longitudinal surveys among cancer survivors: Lessons learnt from the national Norwegian Testicular Cancer Survivor Study. Cancer Epidemiol. 2020;67:101744. doi: 10.1016/j.canep.2020.101744

22. Ference BA. How to use Mendelian randomization to anticipate the results of randomized trials. Eur Heart J. 2018;39:360-362. doi: 10.1093/eurheartj/ehx462

23. Larsson SC, Butterworth AS, Burgess S. Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J. 2023;44:4913-4924. doi: 10.1093/eurheartj/ehad736

24. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, VanderWeele TJ, Higgins JPT, Timpson NJ, Dimou N, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: The STROBE-MR Statement. JAMA. 2021;326:1614-1621. doi: 10.1001/jama.2021.18236

25. Bi K, He MX, Bakouny Z, Kanodia A, Napolitano S, Wu J, Grimaldi G, Braun DA, Cuoco MS, Mayorga A, et al. Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma. Cancer Cell. 2021;39:649-661 e645. doi: 10.1016/j.ccell.2021.02.015

26. Gondal MN, Cieslik M, Chinnaiyan AM. Integrated cancer cell-specific single-cell RNA-seq datasets of immune checkpoint blockade-treated patients. Sci Data. 2025;12:139. doi: 10.1038/s41597-025-04381-6

27. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, 3rd, Zheng S, Butler A, Lee MJ, Wilk AJ, Darby C, Zager M, et al. Integrated analysis of multimodal single-cell data. Cell. 2021;184:3573-3587 e3529. doi: 10.1016/j.cell.2021.04.048

28. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, Srivastava A, Molla G, Madad S, Fernandez-Granda C, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nat Biotechnol. 2024;42:293-304. doi: 10.1038/s41587-023-01767-y

29. McGinnis CS, Murrow LM, Gartner ZJ. DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. Cell Syst. 2019;8:329-337 e324. doi: 10.1016/j.cels.2019.03.003

30. DeTomaso D, Jones MG, Subramaniam M, Ashuach T, Ye CJ, Yosef N. Functional interpretation of single cell similarity maps. Nat Commun. 2019;10:4376. doi: 10.1038/s41467-019-12235-0

31. Hu C, Li T, Xu Y, Zhang X, Li F, Bai J, Chen J, Jiang W, Yang K, Ou Q, et al. CellMarker 2.0: an updated database of manually curated cell markers in human/mouse and web tools based on scRNA-seq data. Nucleic Acids Res. 2023;51:D870-D876. doi: 10.1093/nar/gkac947

32. Fricker G, Miller DS. Modulation of drug transporters at the blood-brain barrier. Pharmacology. 2004;70:169-176. doi: 10.1159/000075545

33. Yazdani S, Jaldin-Fincati JR, Pereira RVS, Klip A. Endothelial cell barriers: Transport of molecules between blood and tissues. Traffic. 2019;20:390-403. doi: 10.1111/tra.12645

34. Kremer R, Williams A, Wardlaw J. Endothelial cells as key players in cerebral small vessel disease. Nat Rev Neurosci. 2025;26:179-188. doi: 10.1038/s41583-024-00892-0

35. The Gene Ontology C. Expansion of the Gene Ontology knowledgebase and resources. Nucleic Acids Res. 2017;45:D331-D338. doi: 10.1093/nar/gkw1108

36. Milacic M, Beavers D, Conley P, Gong C, Gillespie M, Griss J, Haw R, Jassal B, Matthews L, May B, et al. The Reactome Pathway Knowledgebase 2024. Nucleic Acids Res. 2024;52:D672-D678. doi: 10.1093/nar/gkad1025

37. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28:27-30. doi: 10.1093/nar/28.1.27

38. Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader GD, Morris Q. GeneMANIA update 2018. Nucleic Acids Res. 2018;46:W60-W64. doi: 10.1093/nar/gky311

39. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009;25:1091-1093. doi: 10.1093/bioinformatics/btp101

40. The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. 2020;369:1318-1330. doi: 10.1126/science.aaz1776

41. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, et al. A global reference for human genetic variation. Nature. 2015;526:68-74. doi: 10.1038/nature15393

42. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol. 2011;40:755-764. doi: 10.1093/ije/dyr036

43. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37:658-665. doi: 10.1002/gepi.21758

44. Sanderson E, Glymour MM, Holmes MV, Kang H, Morrison J, Munafo MR, Palmer T, Schooling CM, Wallace C, Zhao Q, et al. Mendelian randomization. Nat Rev Methods Primers. 2022;2. doi: 10.1038/s43586-021-00092-5

45. Relton CL, Gaunt T, McArdle W, Ho K, Duggirala A, Shihab H, Woodward G, Lyttleton O, Evans DM, Reik W, et al. Data Resource Profile: Accessible Resource for Integrated Epigenomic Studies (ARIES). Int J Epidemiol. 2015;44:1181-1190. doi: 10.1093/ije/dyv072

46. Golding J, Pembrey M, Jones R, Team AS. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol. 2001;15:74-87. doi: 10.1046/j.1365-3016.2001.00325.x

47. Persyn E, Hanscombe KB, Howson JMM, Lewis CM, Traylor M, Markus HS. Genome-wide association study of MRI markers of cerebral small vessel disease in 42,310 participants. Nat Commun. 2020;11:2175. doi: 10.1038/s41467-020-15932-3

48. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562:203-209. doi: 10.1038/s41586-018-0579-z

49. Conroy MC, Lacey B, Besevic J, Omiyale W, Feng Q, Effingham M, Sellers J, Sheard S, Pancholi M, Gregory G, et al. UK Biobank: a globally important resource for cancer research. Br J Cancer. 2023;128:519-527. doi: 10.1038/s41416-022-02053-5

50. Duperron MG, Knol MJ, Le Grand Q, Evans TE, Mishra A, Tsuchida A, Roshchupkin G, Konuma T, Tregouet DA, Romero JR, et al. Genomics of perivascular space burden unravels early mechanisms of cerebral small vessel disease. Nat Med. 2023;29:950-962. doi: 10.1038/s41591-023-02268-w

51. Knol MJ, Lu D, Traylor M, Adams HHH, Romero JRJ, Smith AV, Fornage M, Hofer E, Liu J, Hostettler IC, et al. Association of common genetic variants with brain microbleeds: A genome-wide association study. Neurology. 2020;95:e3331-e3343. doi: 10.1212/WNL.0000000000010852

52. Mishra A, Malik R, Hachiya T, Jurgenson T, Namba S, Posner DC, Kamanu FK, Koido M, Le Grand Q, Shi M, et al. Stroke genetics informs drug discovery and risk prediction across ancestries. Nature. 2022;611:115-123. doi: 10.1038/s41586-022-05165-3

53. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27:1133-1163. doi: 10.1002/sim.3034

54. de Leeuw C, Savage J, Bucur IG, Heskes T, Posthuma D. Understanding the assumptions underlying Mendelian randomization. Eur J Hum Genet. 2022;30:653-660. doi: 10.1038/s41431-022-01038-5

55. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7. doi: 10.7554/eLife.34408

56. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313:1929-1935. doi: 10.1126/science.1132939

57. Knox C, Wilson M, Klinger CM, Franklin M, Oler E, Wilson A, Pon A, Cox J, Chin NEL, Strawbridge SA, et al. DrugBank 6.0: the DrugBank Knowledgebase for 2024. Nucleic Acids Res. 2024;52:D1265-D1275. doi: 10.1093/nar/gkad976

58. Hara K, Shiga A, Fukutake T, Nozaki H, Miyashita A, Yokoseki A, Kawata H, Koyama A, Arima K, Takahashi T, et al. Association of HTRA1 mutations and familial ischemic cerebral small-vessel disease. N Engl J Med. 2009;360:1729-1739. doi: 10.1056/NEJMoa0801560

59. Uemura M, Nozaki H, Kato T, Koyama A, Sakai N, Ando S, Kanazawa M, Hishikawa N, Nishimoto Y, Polavarapu K, et al. HTRA1-Related Cerebral Small Vessel Disease: A Review of the Literature. Front Neurol. 2020;11:545. doi: 10.3389/fneur.2020.00545

60. Mishra A, Chauhan G, Violleau MH, Vojinovic D, Jian X, Bis JC, Li S, Saba Y, Grenier-Boley B, Yang Q, et al. Association of variants in HTRA1 and NOTCH3 with MRI-defined extremes of cerebral small vessel disease in older subjects. Brain. 2019;142:1009-1023. doi: 10.1093/brain/awz024

61. Eigenbrot C, Ultsch M, Lipari MT, Moran P, Lin SJ, Ganesan R, Quan C, Tom J, Sandoval W, van Lookeren Campagne M, et al. Structural and functional analysis of HtrA1 and its subdomains. Structure. 2012;20:1040-1050. doi: 10.1016/j.str.2012.03.021

62. Muratoglu SC, Belgrave S, Hampton B, Migliorini M, Coksaygan T, Chen L, Mikhailenko I, Strickland DK. LRP1 protects the vasculature by regulating levels of

connective tissue growth factor and HtrA1. Arterioscler Thromb Vasc Biol. 2013;33:2137-2146. doi: 10.1161/ATVBAHA.113.301893

63. Lu Z, Lin V, May A, Che B, Xiao X, Shaw DH, Su F, Wang Z, Du H, Shaw PX. HTRA1 synergizes with oxidized phospholipids in promoting inflammation and macrophage infiltration essential for ocular VEGF expression. PLoS One. 2019;14:e0216808. doi: 10.1371/journal.pone.0216808

64. Shiga A, Nozaki H, Yokoseki A, Nihonmatsu M, Kawata H, Kato T, Koyama A, Arima K, Ikeda M, Katada S, et al. Cerebral small-vessel disease protein HTRA1 controls the amount of TGF-beta1 via cleavage of proTGF-beta1. Hum Mol Genet. 2011;20:1800-1810. doi: 10.1093/hmg/ddr063

65. Schrader JM, Stanisavljevic A, Xu F, Van Nostrand WE. Distinct Brain Proteomic Signatures in Cerebral Small Vessel Disease Rat Models of Hypertension and Cerebral Amyloid Angiopathy. J Neuropathol Exp Neurol. 2022;81:731-745. doi: 10.1093/jnen/nlac057

66. Lee YC, Chung CP, Chao NC, Fuh JL, Chang FC, Soong BW, Liao YC. Characterization of Heterozygous HTRA1 Mutations in Taiwanese Patients With Cerebral Small Vessel Disease. Stroke. 2018;49:1593-1601. doi: 10.1161/STROKEAHA.118.021283

67. Supanji, Shimomachi M, Hasan MZ, Kawaichi M, Oka C. HtrA1 is induced by oxidative stress and enhances cell senescence through p38 MAPK pathway. Exp Eye Res. 2013;112:79-92. doi: 10.1016/j.exer.2013.04.013

68. Schillinger J, Severin K, Kaschani F, Kaiser M, Ehrmann M. HTRA1-Dependent Cell Cycle Proteomics. J Proteome Res. 2018;17:2679-2694. doi: 10.1021/acs.jproteome.8b00129

69. Kim S, Bi X, Czarny-Ratajczak M, Dai J, Welsh DA, Myers L, Welsch MA, Cherry KE, Arnold J, Poon LW, et al. Telomere maintenance genes SIRT1 and XRCC6 impact age-related decline in telomere length but only SIRT1 is associated with human longevity. Biogerontology. 2012;13:119-131. doi: 10.1007/s10522-011-9360-5

70. Sharma S, Sengupta A, Chowdhury S. Emerging Molecular Connections between NM23 Proteins, Telomeres and Telomere-Associated Factors: Implications in Cancer Metastasis and Ageing. Int J Mol Sci. 2021;22. doi: 10.3390/ijms22073457

71. Wang Z, Lin H, Hua F, Hu ZW. Repairing DNA damage by XRCC6/KU70 reverses TLR4-deficiency-worsened HCC development via restoring senescence and autophagic flux. Autophagy. 2013;9:925-927. doi: 10.4161/auto.24229

72. Zhu Y, Liu X, Ding X, Wang F, Geng X. Telomere and its role in the aging pathways: telomere shortening, cell senescence and mitochondria dysfunction. Biogerontology. 2019;20:1-16. doi: 10.1007/s10522-018-9769-1

73. Liu J, Wang L, Wang Z, Liu JP. Roles of Telomere Biology in Cell Senescence, Replicative and Chronological Ageing. Cells. 2019;8. doi: 10.3390/cells8010054

74. Kiss T, Nyul-Toth A, Balasubramanian P, Tarantini S, Ahire C, DelFavero J, Yabluchanskiy A, Csipo T, Farkas E, Wiley G, et al. Single-cell RNA sequencing identifies senescent cerebromicrovascular endothelial cells in the aged mouse brain. Geroscience. 2020;42:429-444. doi: 10.1007/s11357-020-00177-1

75. Csik B, Nyul-Toth A, Gulej R, Patai R, Kiss T, Delfavero J, Nagaraja RY, Balasubramanian P, Shanmugarama S, Ungvari A, et al. Senescent Endothelial Cells in Cerebral Microcirculation Are Key Drivers of Age-Related Blood-Brain Barrier

Disruption, Microvascular Rarefaction, and Neurovascular Coupling Impairment in Mice. Aging Cell. 2025:e70048. doi: 10.1111/acel.70048

76. Ungvari A, Nyul-Toth A, Patai R, Csik B, Gulej R, Nagy D, Shanmugarama S, Benyo Z, Kiss T, Ungvari Z, et al. Cerebromicrovascular senescence in vascular cognitive impairment: does accelerated microvascular aging accompany atherosclerosis? Geroscience. 2025. doi: 10.1007/s11357-025-01621-w

77. Ruiz-Arguelles A, Llorente L. The role of complement regulatory proteins (CD55 and CD59) in the pathogenesis of autoimmune hemocytopenias. Autoimmun Rev. 2007;6:155-161. doi: 10.1016/j.autrev.2006.09.008

78. Thelen AM, Zoncu R. Emerging Roles for the Lysosome in Lipid Metabolism. Trends Cell Biol. 2017;27:833-850. doi: 10.1016/j.tcb.2017.07.006

79. Csiszar A, Ungvari A, Patai R, Gulej R, Yabluchanskiy A, Benyo Z, Kovacs I, Sotonyi P, Kirkpartrick AC, Prodan CI, et al. Atherosclerotic burden and cerebral small vessel disease: exploring the link through microvascular aging and cerebral microhemorrhages. Geroscience. 2024;46:5103-5132. doi: 10.1007/s11357-024-01139-7

80. Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu Rev Cell Biol. 1985;1:1-39. doi: 10.1146/annurev.cb.01.110185.000245

81. Mundi S, Massaro M, Scoditti E, Carluccio MA, van Hinsbergh VWM, Iruela-Arispe ML, De Caterina R. Endothelial permeability, LDL deposition, and cardiovascular risk factors-a review. Cardiovasc Res. 2018;114:35-52. doi: 10.1093/cvr/cvx226

82. Lewis RD, Perry MJ, Guschina IA, Jackson CL, Morgan BP, Hughes TR. CD55 deficiency protects against atherosclerosis in ApoE-deficient mice via C3a modulation of lipid metabolism. Am J Pathol. 2011;179:1601-1607. doi: 10.1016/j.ajpath.2011.06.015

83. An G, Miwa T, Song WL, Lawson JA, Rader DJ, Zhang Y, Song WC. CD59 but not DAF deficiency accelerates atherosclerosis in female ApoE knockout mice. Mol Immunol. 2009;46:1702-1709. doi: 10.1016/j.molimm.2009.02.009

84. Suero-Abreu GA, Zanni MV, Neilan TG. Atherosclerosis With Immune Checkpoint Inhibitor Therapy: Evidence, Diagnosis, and Management: JACC: CardioOncology State-of-the-Art Review. JACC CardioOncol. 2022;4:598-615. doi: 10.1016/j.jaccao.2022.11.011

85. Parvathaneni V, Kulkarni NS, Muth A, Gupta V. Drug repurposing: a promising tool to accelerate the drug discovery process. Drug Discov Today. 2019;24:2076-2085. doi: 10.1016/j.drudis.2019.06.014

86. Ota H, Eto M, Kano MR, Kahyo T, Setou M, Ogawa S, Iijima K, Akishita M, Ouchi Y. Induction of endothelial nitric oxide synthase, SIRT1, and catalase by statins inhibits endothelial senescence through the Akt pathway. Arterioscler Thromb Vasc Biol. 2010;30:2205-2211. doi: 10.1161/ATVBAHA.110.210500

87. van Rosendael AR, van den Hoogen IJ, Gianni U, Ma X, Tantawy SW, Bax AM, Lu Y, Andreini D, Al-Mallah MH, Budoff MJ, et al. Association of Statin Treatment With Progression of Coronary Atherosclerotic Plaque Composition. JAMA Cardiol. 2021;6:1257-1266. doi: 10.1001/jamacardio.2021.3055

88. Mhaimeed O, Burney ZA, Schott SL, Kohli P, Marvel FA, Martin SS. The importance of LDL-C lowering in atherosclerotic cardiovascular disease prevention: Lower for longer is better. Am J Prev Cardiol. 2024;18:100649. doi: 10.1016/j.ajpc.2024.100649

89. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140:e596-e646. doi: 10.1161/CIR.0000000000000678

90. Murakami M, Fujioka S, Hirata Y, Kuratsu J. Low-dose of statin treatment improves cerebrovascular reactivity in patients with ischemic stroke: single photon emission computed tomography analysis. J Stroke Cerebrovasc Dis. 2008;17:16-22. doi: 10.1016/j.jstrokecerebrovasdis.2007.09.005

91. Guo Y, Li Y, Liu X, Cui Y, Zhao Y, Sun S, Jia Q, Chai Q, Gong G, Zhang H, et al. Assessing the effectiveness of statin therapy for alleviating cerebral small vessel disease progression in people >/=75 years of age. BMC Geriatr. 2020;20:292. doi: 10.1186/s12877-020-01682-w

92. Satoh M, Takahashi Y, Tabuchi T, Minami Y, Tamada M, Takahashi K, Itoh T, Morino Y, Nakamura M. Cellular and molecular mechanisms of statins: an update on pleiotropic effects. Clin Sci (Lond). 2015;129:93-105. doi: 10.1042/CS20150027

93. Scheuermann B, Brown A, Colburn T, Hakeem H, Chow CH, Ade C. External Validation of the American Heart Association PREVENT Cardiovascular Disease Risk Equations. JAMA Netw Open. 2024;7:e2438311. doi: 10.1001/jamanetworkopen.2024.38311

94. Pereira MA, Warner BM, Knobloch TJ, Weghorst CM, Lubet RA, Steele VE, Casto BC. Chemoprevention of mouse lung and colon tumors by suberoylanilide hydroxamic acid and atorvastatin. Int J Cancer. 2012;131:1277-1286. doi: 10.1002/ijc.27395

95. Tousoulis D, Psarros C, Demosthenous M, Patel R, Antoniades C, Stefanadis C. Innate and adaptive inflammation as a therapeutic target in vascular disease: the emerging role of statins. J Am Coll Cardiol. 2014;63:2491-2502. doi: 10.1016/j.jacc.2014.01.054

96. Munafo MR, Higgins JPT, Smith GD. Triangulating Evidence through the Inclusion of Genetically Informed Designs. Cold Spring Harb Perspect Med. 2021;11. doi: 10.1101/cshperspect.a040659

97. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol. 2016;45:908-915. doi: 10.1093/ije/dyw127

98. Miao Z, Humphreys BD, McMahon AP, Kim J. Multi-omics integration in the age of million single-cell data. Nat Rev Nephrol. 2021;17:710-724. doi: 10.1038/s41581-021-00463-x

99. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ Res. 2007;100:174-190. doi: 10.1161/01.RES.0000255690.03436.ae

100. Aird WC. Endothelial cell heterogeneity. Cold Spring Harb Perspect Med. 2012;2:a006429. doi: 10.1101/cshperspect.a006429

Appendix A - Supplementary Materials for Chapter 2

Supplementary Tables

Appendix Table A-1. Electronic search string for cerebrovascular reactivity and cerebral small vessel disease.

MEDLINE	**WEB OF SCIENCE**
("cerebrovascular reactivity" OR "cerebral vascular reactivity" OR "cerebral vasoreactivity" OR "CVR" OR "cerebrovascular reserve" OR "cerebral vasomotor reactivity")	ALL = ("cerebrovascular reactivity" OR "cerebral vascular reactivity" OR "cerebral vasoreactivity" OR "CVR" OR "cerebrovascular reserve" OR "cerebral vasomotor reactivity")
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	AND ALL = ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)
EMBASE	
("cerebrovascular reactivity" OR "cerebral vascular reactivity" OR "cerebral vasoreactivity" OR "CVR" OR "cerebrovascular reserve" OR "cerebral vasomotor reactivity")	
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	

Appendix Table A-2. Electronic search string for cerebral autoregulation and cerebral small vessel disease.

MEDLINE	**WEB OF SCIENCE**
(“cerebral autoregulation” OR “cerebrovascular autoregulation” OR “cerebral vascular autoregulation”)	ALL = (“cerebral autoregulation” OR “cerebrovascular autoregulation” OR “cerebral vascular autoregulation”)
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	AND ALL = ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)
EMBASE	
(“cerebral autoregulation” OR “cerebrovascular autoregulation” OR “cerebral vascular autoregulation”)	
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	

Appendix Table A-3. Electronic search string for arterial stiffness and cerebral small vessel disease.

MEDLINE	**WEB OF SCIENCE**
(“arterial stiffness” OR “pulse-wave velocity” OR “PWV” OR “arterial elasticity” OR “aortic stiffness” OR “carotid stiffness”)	ALL = (“arterial stiffness” OR “pulse-wave velocity” OR “PWV” OR “arterial elasticity” OR “aortic stiffness” OR “carotid stiffness”)
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	AND ALL = ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)
EMBASE	
(“arterial stiffness” OR “pulse-wave velocity” OR “PWV” OR “arterial elasticity” OR “aortic stiffness” OR “carotid stiffness”)	
AND ("cerebral small vessel disease*" OR “lacun* infarct*" OR "subcortical stroke" OR microinfarct* OR "subcortical lesion*" OR “cerebrovascular disorder” OR "perivascular space*" OR "Virchow-Robin space*" OR “leukoaraiosis” OR "white matter lesion*" OR "white matter hyperintens*" OR "cerebral microbleed*" OR “white matter disease*” OR “cerebral atrophy”)	

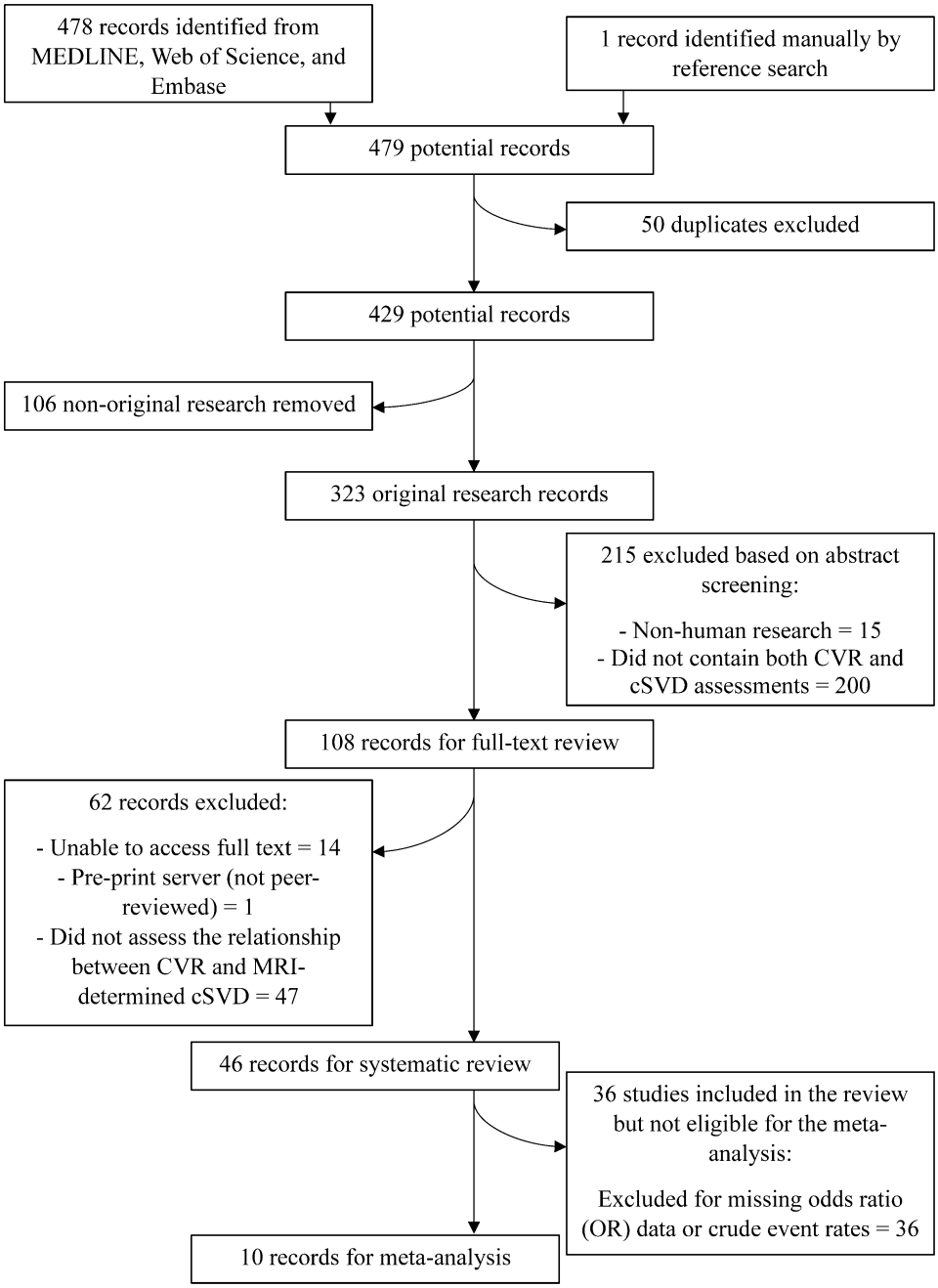
Appendix Table A-4. Results from multivariate meta-regression secondary analysis adjusted for three risk factors (hypertension, diabetes mellitus, and hyperlipidemia).

Predictor	**Variable**	**Point Estimate (95% CI)**	**P-Value**
Continuous Arterial Stiffness			
	Hypertension	0.0026 (-0.0014, 0.0065)	0.2
	Diabetes Mellitus	0.0082 (0.0022, 0.0142)	**0.01**
	Hyperlipidemia	-0.0026 (-0.0061, 0.0010)	0.16

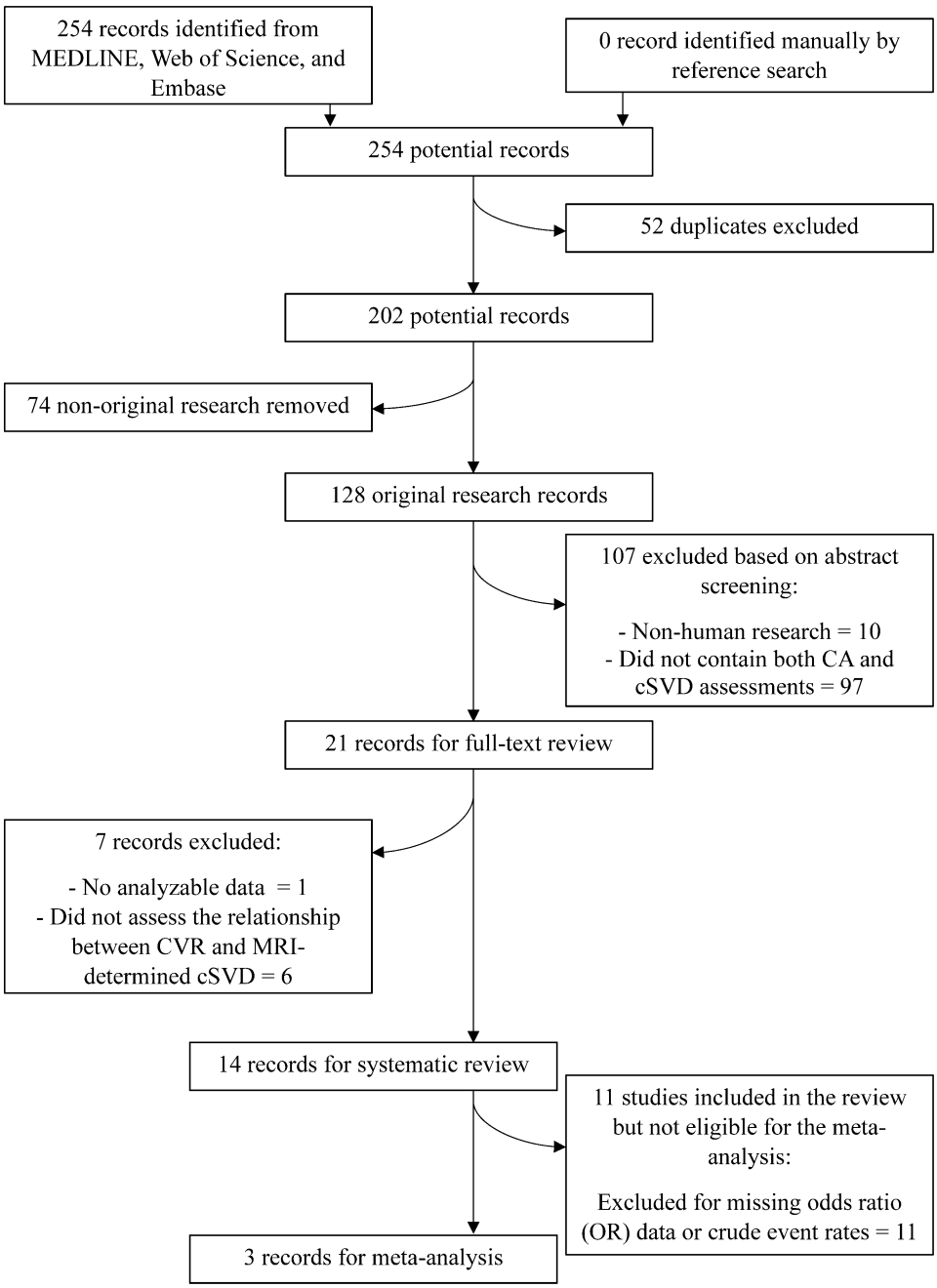
Appendix Table A-5. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) scores for certainty of evidence.

Certainty of Evidence Analyses							**Overall Certainty Assessment**
No. of Studies	**Study Designs**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other Considerations**	
Cerebrovascular Reactivity Association with cSVD – Dichotomous Exposure Variable							
7	Observational	Not serious	Serious	Not serious	Not serious	Potential Confounding, Large Effect Size, Small Sample Size	Low
Arterial Stiffness Association with cSVD – Continuous Exposure Variable							
37	Observational	Serious	Serious	Not serious	Not serious	Potential Confounding, Evidence of a Gradient	Low
Arterial Stiffness Association with cSVD – Dichotomous Exposure Variable							
13	Observational	Not serious	Serious	Not serious	Not serious	Potential Confounding	Low

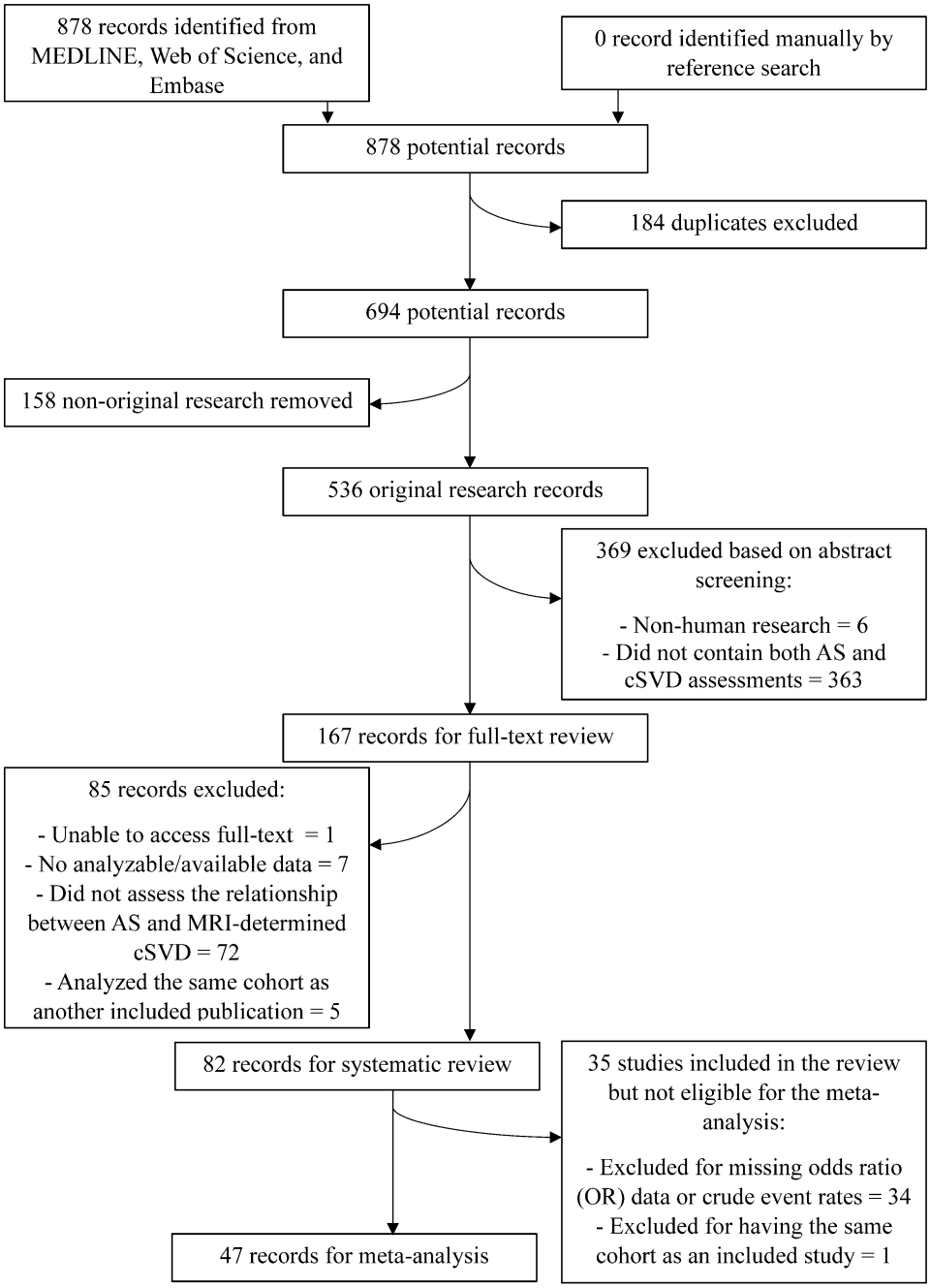
Supplementary Figures



Appendix Figure A-1. Flow chart of the selection process for eligible cerebrovascular reactivity studies. CVR indicates cerebrovascular reactivity.

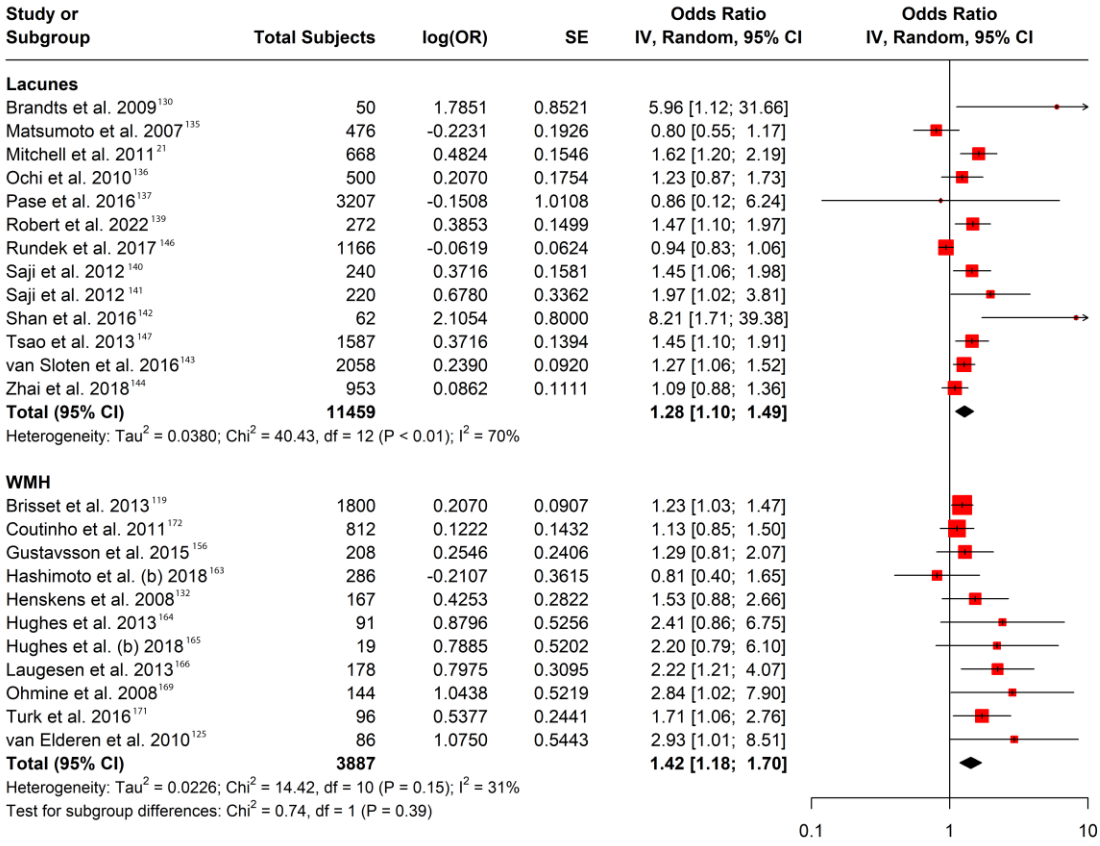


Appendix Figure A-2. Flow chart of the selection process for eligible cerebral autoregulation studies. CA indicates cerebral autoregulation.

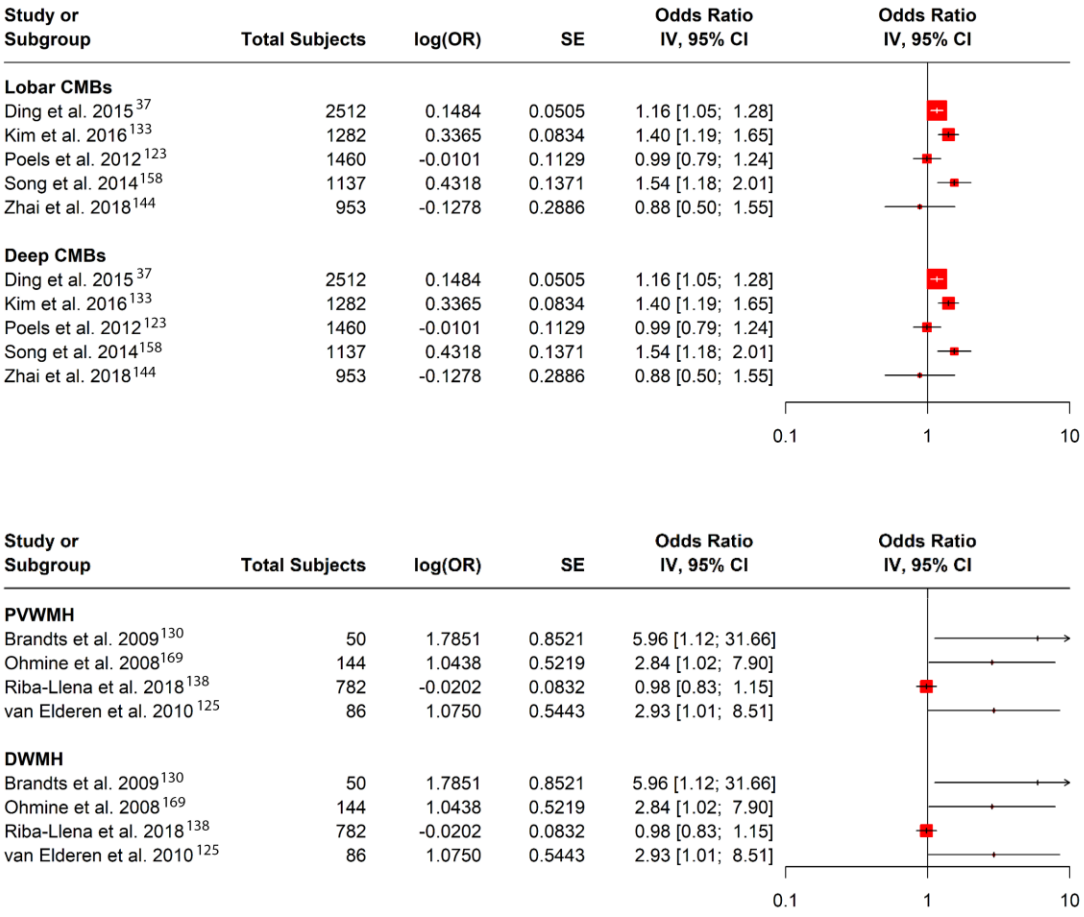


Appendix Figure A-3. Flow chart of the selection process for eligible arterial stiffness studies. AS indicates arterial stiffness.

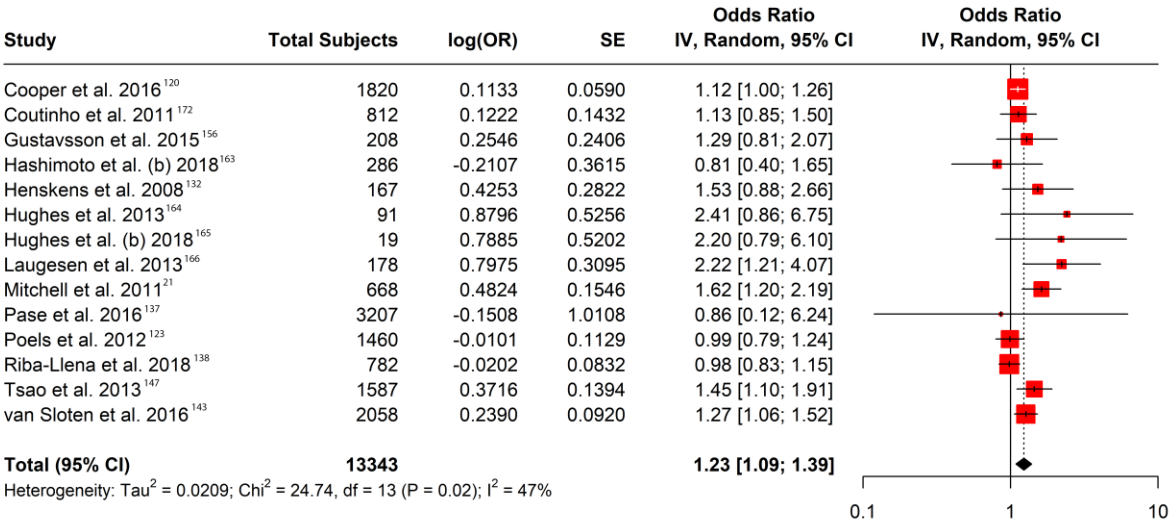
Appendix Figure A-4. Individual study results from longitudinal studies. Forest plot illustrating the individual effect sizes reported for each longitudinal study. Three studies were included for continuous arterial stiffness, while dichotomous cerebrovascular reactivity and dichotomous arterial stiffness only had one longitudinal study each. AS indicates arterial stiffness, while CVR indicates cerebrovascular reactivity.



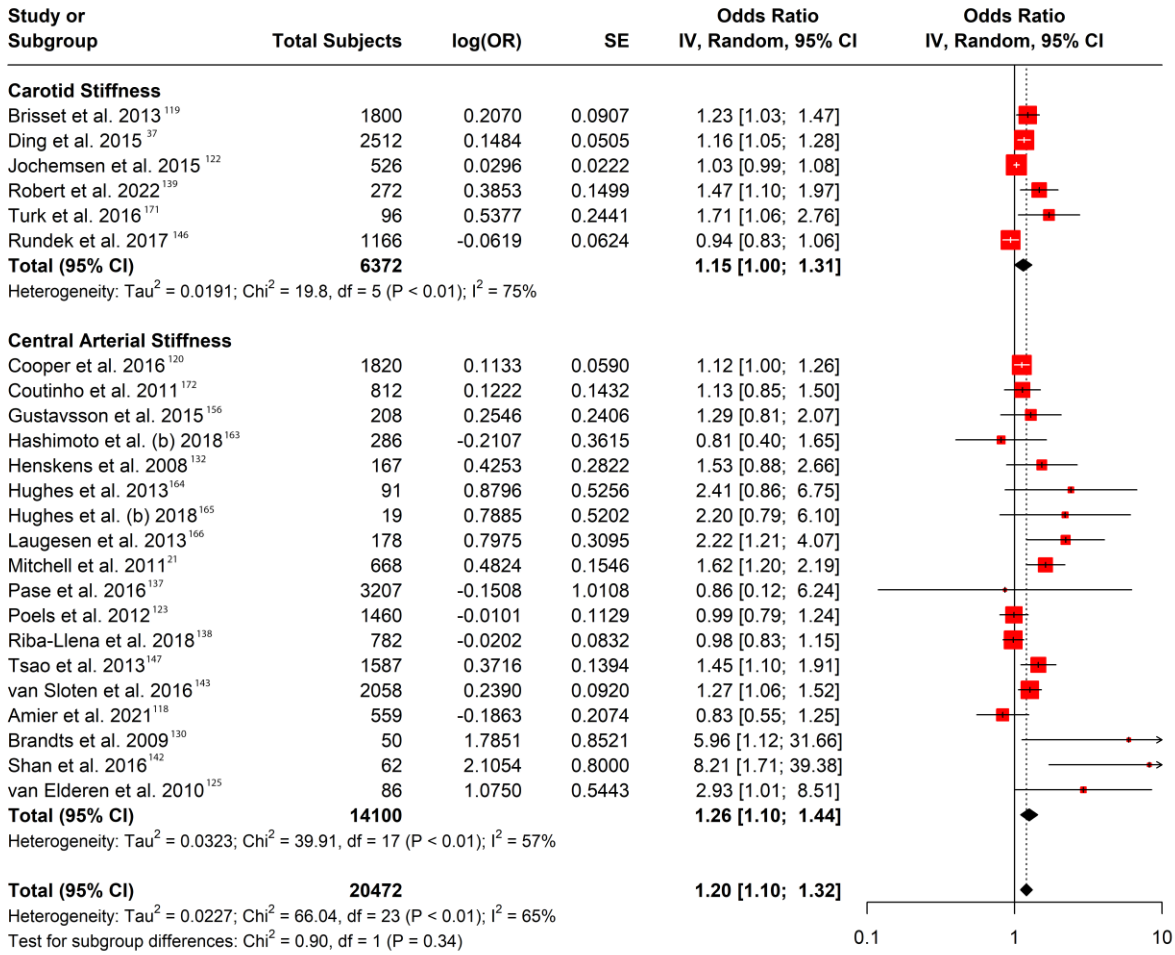
Appendix Figure A-5. Odds ratio results from secondary analyses of studies reporting arterial stiffness as a continuous exposure variable in relation to white matter hyperintensities or lacunes. Forest plot illustrating the effect sizes for studies reporting continuous arterial stiffness in relation to the presence of white matter hyperintensity or lacunes. Effect sizes are reported per 1-SD increase. The separate overall effects suggested an increasing arterial stiffness was associated with a greater presence of lacunes (OR: 1.28, 95% CI: 1.10-1.49) and a greater burden of white matter hyperintensities (OR: 1.42, 95% CI: 1.18-170). WMH, white matter hyperintensities; OR, odds ratio; SE, standard error; IV, inverse-variance.



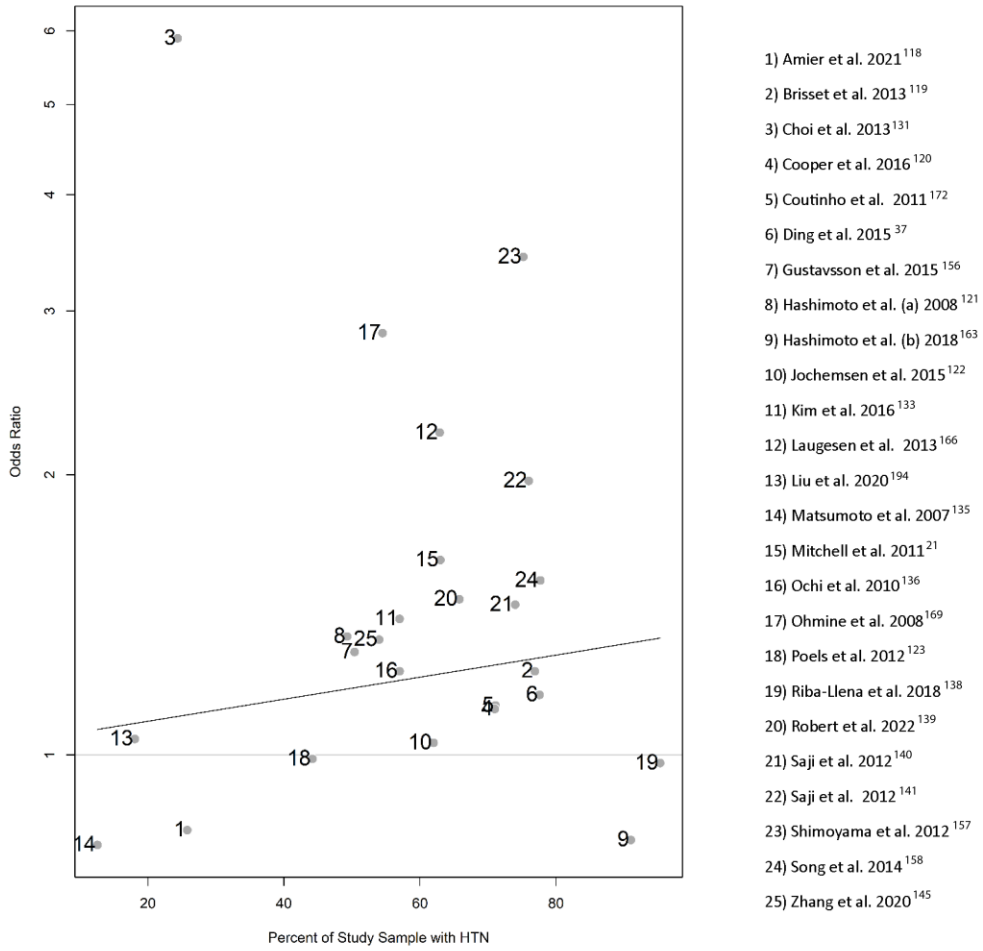
Appendix Figure A-6. Individual study results from continuous arterial stiffness studies that differentiated by region within a cSVD subtype. Forest plot illustrating the individual effect sizes reported for studies that divided the presence of lacunes into strictly lobar or deep lacunes (*top panel*) and studies that divided white matter hyperintensity burden into periventricular regions or deep regions (*bottom panel*). CMBs, cerebral microbleeds; PVWMH, periventricular white matter hyperintensities; DWMH, deep white matter hyperintensities; OR, odds ratio; SE, standard error; IV, inverse-variance.



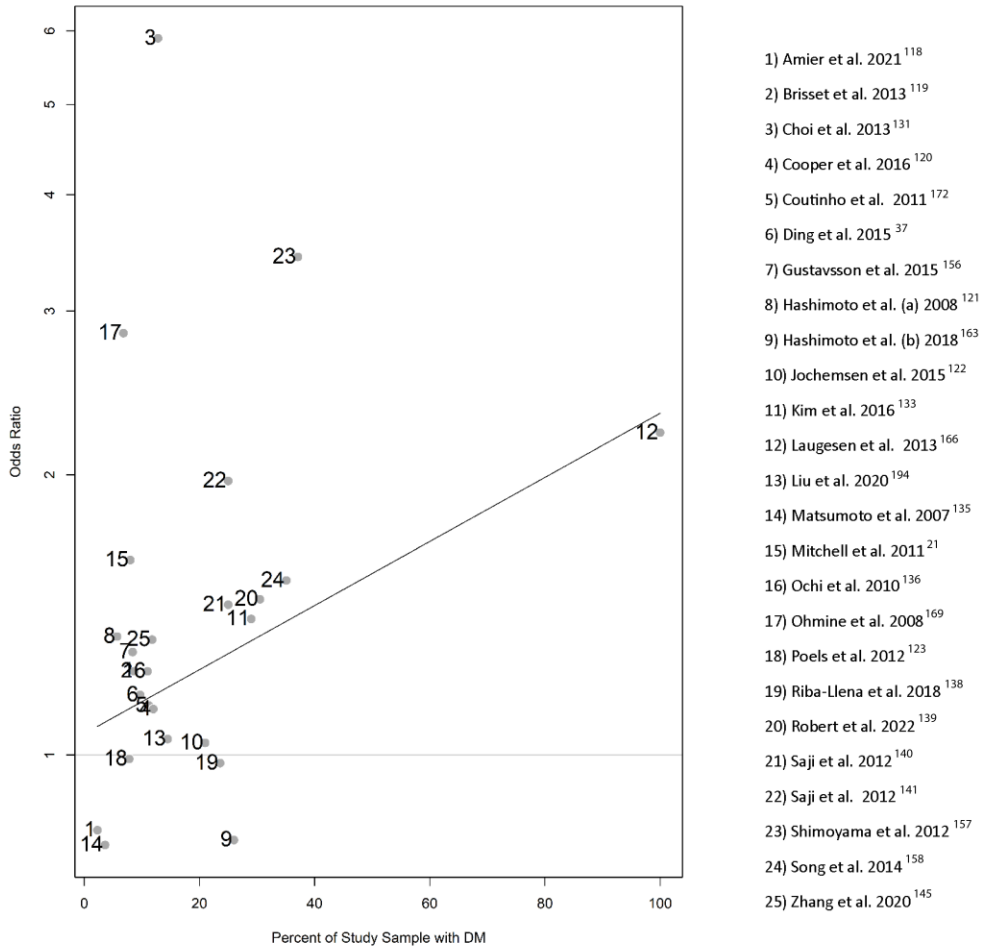
Appendix Figure A-7. Odds ratio results from secondary analyses of only the studies that assessed arterial stiffness as carotid-femoral pulse wave velocity. Forest plot illustrating the effect sizes for studies reporting carotid-femoral pulse wave velocity (cfPWV) in relation to cSVD burden. Effect sizes are reported per 1-SD increase. The separate overall effects suggested an increasing arterial stiffness was associated with a greater presence of cSVD (OR: 1.23, 95% CI: 1.09-1.39). OR, odds ratio; SE, standard error; IV, inverse-variance.



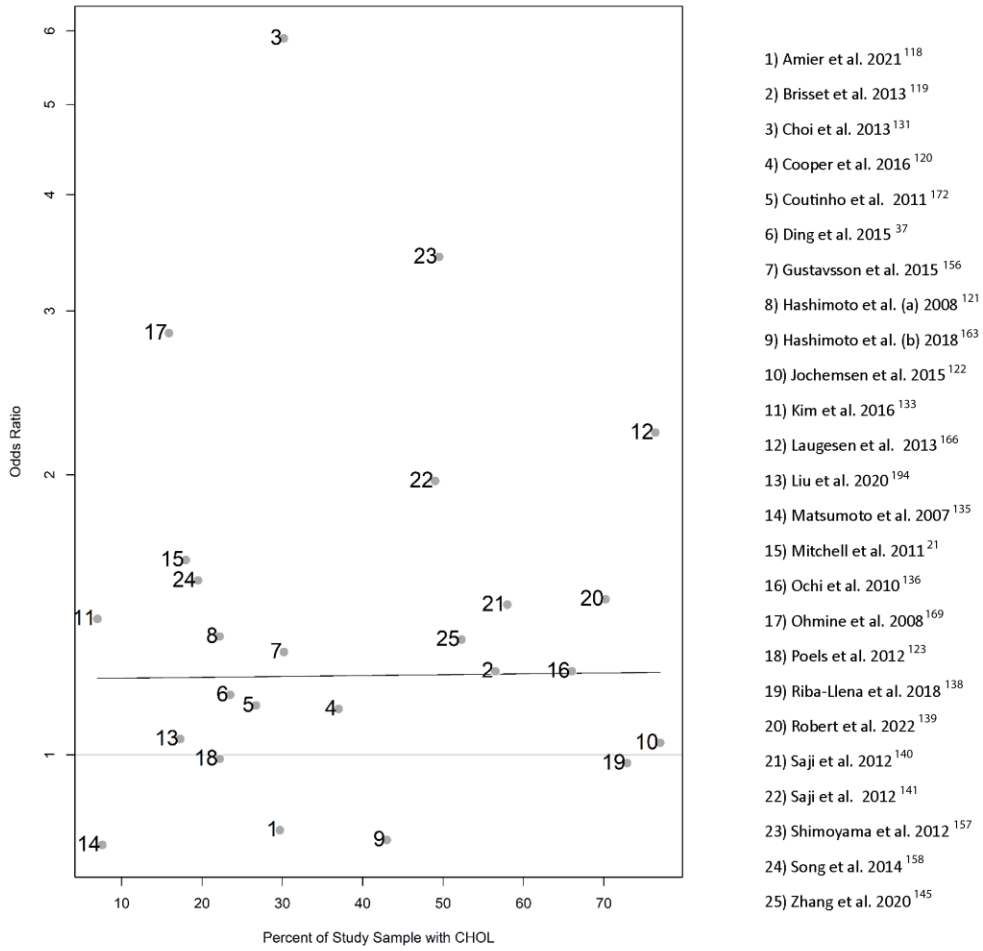
Appendix Figure A-8. Odds ratio results from secondary analyses comparing the studies that assessed central artery stiffness with the studies that assessed carotid artery stiffness. Forest plot illustrating the effect sizes for studies reporting central artery stiffness and for studies reporting carotid artery stiffness. Effect sizes are reported per 1-SD increase. The separate overall effects suggested that both an increasing central arterial stiffness (OR: 1.26, 95% CI: 1.10-1.44) and an increasing carotid artery stiffness (OR: 1.15, 95% CI: 1.00-1.31) were associated with a greater presence of cSVD. No significant differences were observed between central artery stiffness and carotid artery stiffness (P=0.34). OR, odds ratio; SE, standard error; IV, inverse-variance.



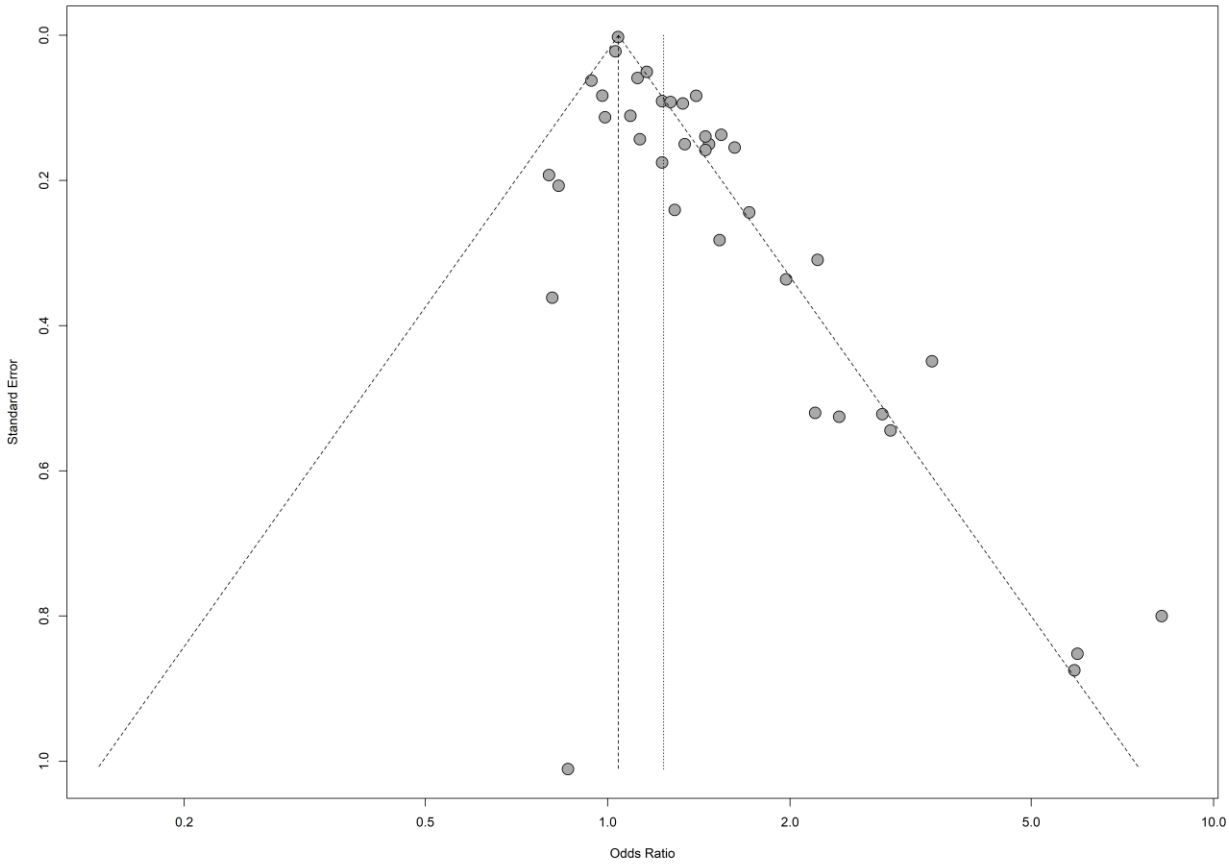
Appendix Figure A-9. Bubble plot from meta-regression showing the study proportion of hypertensive (HTN) participants as a study-level covariate.



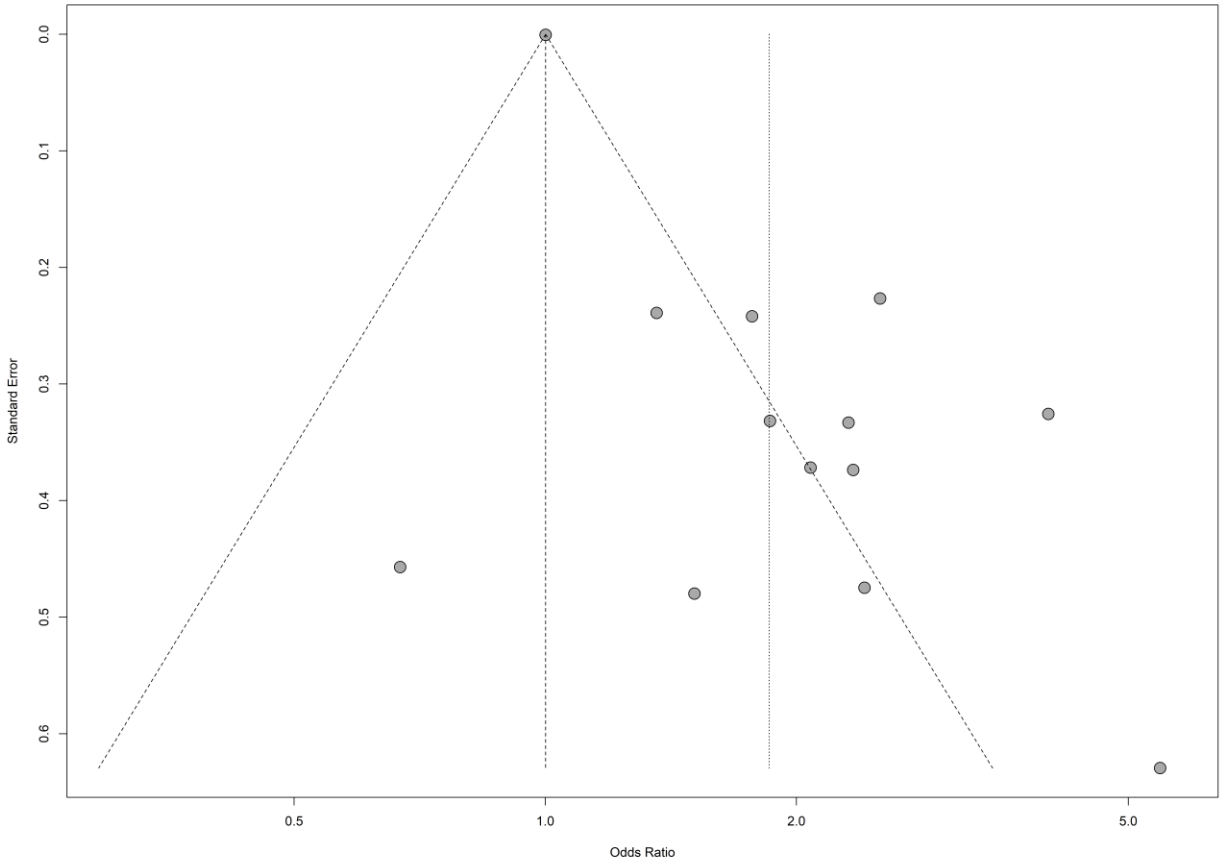
Appendix Figure A-10. Bubble plot from meta-regression showing the study proportion of diabetic (DM) participants as a study-level covariate.



Appendix Figure A-11. Bubble plot from meta-regression showing the study proportion of participants with hypercholesterolemia (HTN) as a study-level covariate.



Appendix Figure A-12. Funnel plot for studies reporting continuous arterial stiffness measures.



Appendix Figure A-13. Funnel plot for studies reporting dichotomous arterial stiffness measures.

Appendix B - Supplementary Materials for Chapter 4

Strengthening Reporting of Observational Studies in Epidemiology – Mendelian Randomization (STROBE-MR) Guidelines

Item No.	Section	Checklist item	Page No.
1	TITLE and ABSTRACT	Indicate Mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study	123
	INTRODUCTION		
2	Background	Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question	125, 126
3	Objectives	State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects	127
	METHODS		
4	Study design and data sources	Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:	128
	a)	Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.	129-132
	b)	Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis	129-132
	c)	Describe measurement, quality control and selection of genetic variants	129-132
	d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	129-132
	e)	Provide details of ethics committee approval and participant informed consent, if relevant	N/A
5	Assumptions	Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis	Appendix B
6	Statistical methods: main analysis	Describe statistical methods and statistics used	132-133
	a)	Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)	132-133
	b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	132-133
	c)	Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples	132-133
	d)	Explain how missing data were addressed	N/A
	e)	If applicable, indicate how multiple testing was addressed	N/A
7	Assessment of assumptions	Describe any methods or prior knowledge used to assess the assumptions or justify their validity	132-133
8	Sensitivity analyses and additional analyses	Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations)	132-133
9	Software and pre-registration		

	a)	Name statistical software and package(s), including version and settings used	133
	b)	State whether the study protocol and details were pre-registered (as well as when and where)	N/A
	RESULTS		
10	Descriptive data		
	a)	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram	N/A
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	134-135
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	N/A
	d)	For two-sample MR:	N/A
		i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples	
		ii. Provide information on the number of individuals who overlap between the exposure and outcome studies	
11	Main results		
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	136
	b)	Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference	136
	c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
	d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	137-138
12	Assessment of assumptions		
	a)	Report the assessment of the validity of the assumptions	Appendix B
	b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	Appendix B
13	Sensitivity analyses and additional analyses		
	a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	136
	b)	Report results from other sensitivity analyses or additional analyses	136
	c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	N/A
	d)	When relevant, report and compare with estimates from non-MR analyses	N/A
	e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	Appendix B
	DISCUSSION		
14	Key results	Summarize key results with reference to study objectives	139-140
15	Limitations	Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them	141
16	Interpretation		
	a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	139-141
	b)	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions	139-141

	c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	139-141
17	Generalizability	Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure	141
	OTHER INFORMATION		
18	Funding	Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based	
19	Data and data sharing	Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where	
20	Conflicts of Interest	All authors should declare all potential conflicts of interest	

Supplementary Methods

Core Assumptions of Mendelian Randomization

The core assumptions of two-sample Mendelian randomization studies are as follows: 1) relevance, 2) exchangeability, and 3) exclusion-restriction.[1] The relevance assumption is satisfied by demonstrating that the instrumental variable (the single genetic variant or multiple genetic variants used to proxy the exposure variable) is associated with the actual exposure of interest (Appendix Figure B-6, Panel A). In the scientific methods, this is done by selecting genetic variants that have previously been shown to associate with the exposure of interest at genome-wide standard threshold criteria (usually $p<5x10^{-8}$).[2] The exchangeability assumption says that there are no confounding pathways, mechanisms, or phenotypes between the instrumental variable and the outcome of interest that act independently of the exposure of interest (Appendix Figure B-6, Panel B). Genetic variation (aside from somatic mutations) are determined at conception, so many traditional confounding factors do not have an effect in this context. However, genome-specific confounders like population stratification and dynastic effects may play some role. To a degree, these are adjusted for in genome-wide association studies; some degree of confounding is always still possible.[1,3] Finally, the exclusion-restriction assumption states that the instrumental variable only affects the outcome through the exposure of interest, and there are no other traits that have downstream effects on the outcome variable (Appendix Figure B-6, Panel C). In our methods, this assumption is tested by checking both horizontal pleiotropy in the statistical Mendelian randomization models and by using phenome-wide analyses to identify potential confounding phenotypes.[4] However, it is important to note

that while sensitivity analyses may help address this third assumption, it cannot be truly and fully confirmed as there is always the possibility of some degree of unmeasured confounding.[5]

Details on Genetic Variant Identification Methods

For identifying and selecting genetic variants to proxy the effects of immune checkpoint inhibition, we followed the analytical and statistical methods of the GTEx consortium.[6] In brief, the GTEx protocol primarily used FastQTL for processing and analysis.[7] Variants were considered cis-variants if they were ±1Mb from a gene transcription start site (which we reduced to ±100kb), and all variants with a minor allele frequency ≥ 0.01 were incorporated. Genes with at least one eQTL (expression quantitative trait loci) variant were identified at a false discovery rate (FDR) ≤ 0.05 and clustered together as "eGenes". To then identify cis-variants significantly associated with expression of their target gene, first an empirical genome-wide p-value threshold (p_t) was set based on the empirical p-value of the eGene closest to FDR=0.05. This p_t value was used to establish gene-specific significance thresholds for each eGene. Cis-variants with a nominal p-value less than their target eGene significance threshold were considered statistically significant eQTL and reported as "significant gene-variant pairs".[6] These pairs were extracted from GTEx v10 data for present analyses.

Supplementary Tables

Appendix Table B-1. Mendelian randomization primary and sensitivity results for the effects of any breast cancer, estrogen receptor positive (ER+), estrogen receptor negative (ER-), and triple-negative (TN) breast cancer subtypes on cerebral small vessel disease outcomes. Results as reported as beta and 95% confidence interval for white matter hyperintensity (WMH) volume, and as odds ratio and 95% confidence interval for perivascular space (PVS) burden, cerebral microbleeds (CMB), and small vessel stroke (SVS).

		Cerebral Small Vessel Disease Marker			
Exposure	*MR Method*	*WMH*	*PVS*	*CMB*	*SVS*
Any Breast Cancer	IVW	0.015 (-0.023-0.053)	1.01 (0.99-1.02)	0.95 (0.86-1.06)	0.97 (0.90-1.05)
	MR-PRESSO	0.015 (-0.023-0.053)	1.01 (0.99-1.02)	0.95 (0.86-1.06)	0.97 (0.90-1.05)
	Weighted Median	0.014 (-0.034-0.061)	1.00 (0.98-1.02)	1.07 (0.92-1.23)	1.00 (0.89-1.11)
	MR-Egger	0.012 (-0.077-0.102)	1.00 (0.97-1.03)	0.94 (0.73-1.22)	1.08 (0.88-1.31)
ER+ Breast Cancer	IVW	0.026 (-0.01-0.062)	1.00 (0.99-1.02)	0.96 (0.88-1.06)	0.96 (0.89-1.03)
	MR-PRESSO	0.026 (-0.01-0.062)	1.00 (0.99-1.02)	0.96 (0.88-1.06)	0.96 (0.89-1.03)
	Weighted Median	0.014 (-0.029-0.058)	1.00 (0.98-1.02)	1.05 (0.93-1.19)	1.00 (0.90-1.10)
	MR-Egger	-0.004 (-0.082-0.074)	1.00 (0.98-1.03)	1.02 (0.83-1.25)	1.04 (0.89-1.21)
ER- Breast Cancer	IVW	0.001 (-0.048-0.051)	1.01 (0.99-1.03)	1.12 (0.92-1.36)	0.90 (0.76-1.07)
	MR-PRESSO	0.019 (-0.127-0.165)	1.01 (0.15-6.71)	1.12 (0.92-1.36)	0.90 (0.76-1.07)
	Weighted Median	-0.008 (-0.071-0.055)	1.01 (0.98-1.03)	1.14 (0.90-1.44)	1.03 (0.85-1.23)

	MR-Egger	-0.065 (-0.227-0.097)	1.03 (0.97-1.1)	1.56 (0.88-2.76)	1.29 (0.84-1.97)
TN Breast Cancer	IVW	-0.01 (-0.050-0.031)	1.00 (0.98-1.01)	0.96 (0.84-1.11)	1.02 (0.92-1.13)
	MR-PRESSO	-0.01 (-0.050-0.031)	1.00 (0.98-1.01)	0.96 (0.84-1.11)	1.02 (0.92-1.13)
	Weighted Median	-0.009 (-0.063-0.045)	1.00 (0.98-1.03)	1.01 (0.84-1.21)	1.00 (0.87-1.13)
	MR-Egger	0.002 (-0.110-0.114)	1.03 (0.99-1.07)	1.26 (0.87-1.83)	1.04 (0.77-1.42)

Appendix Table B-2. Variants included in the genetic instrument designed to proxy the effects of pharmacological immune checkpoint inhibition.

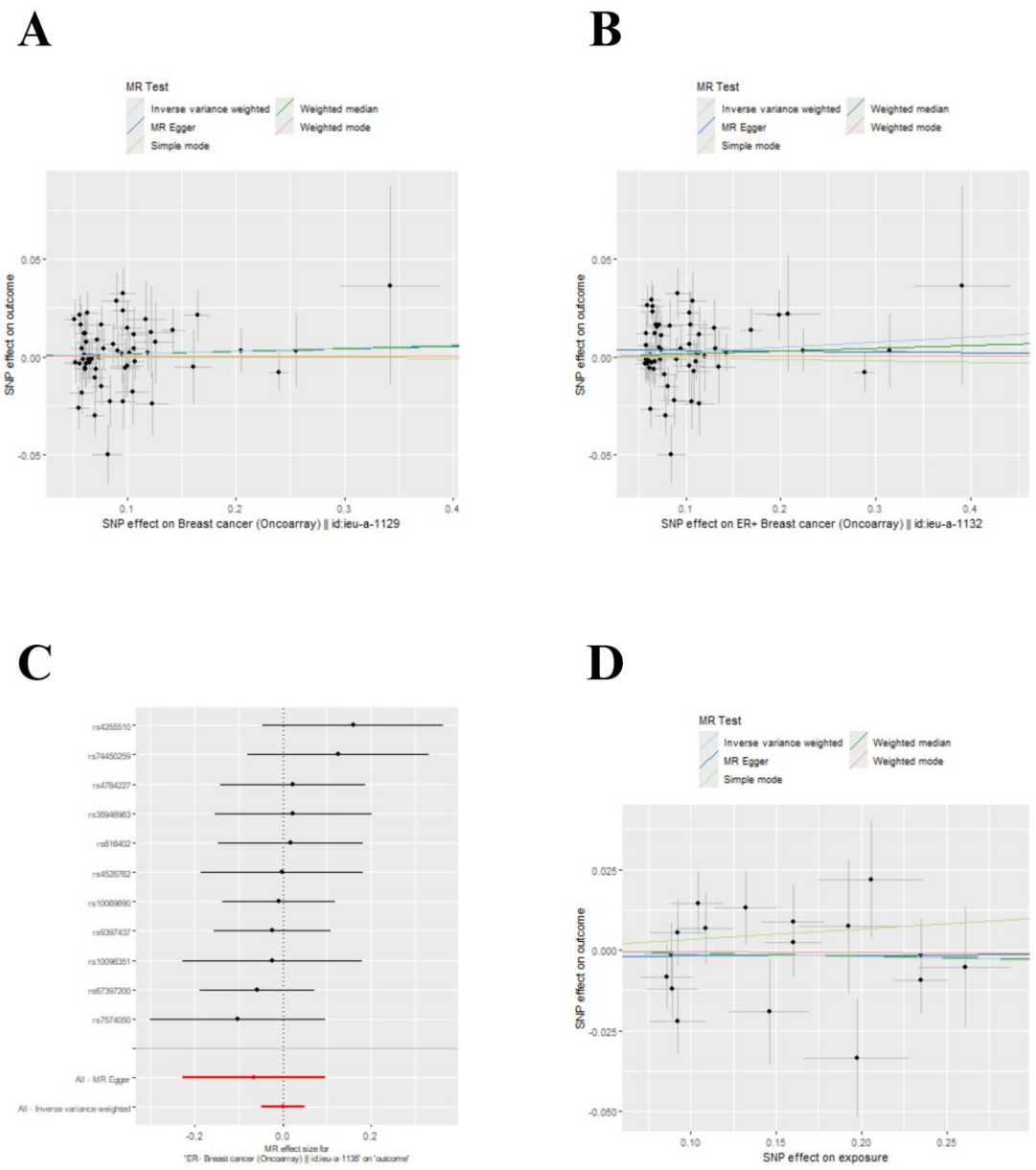
rsID	Gene Name	Variant ID	Distance (kb) [a]	MAF [b]	F-Statistic
rs115775475	*PDCD1*	chr2_241919740_A_C_b38	60846	0.095	13.6
rs13415837	*PDCD1*	chr2_241810383_G_C_b38	-48511	0.239	16.4
rs145228326	*PDCD1*	chr2_241901623_G_A_b38	42729	0.043	26.2
rs34667857	*PDCD1*	chr2_241808501_C_T_b38	-50393	0.419	42.9
rs3749155	*PDCD1*	chr2_241814768_C_T_b38	-44126	0.167	14.3
rs4441512	*PDCD1*	chr2_241822219_G_A_b38	-36675	0.417	32
rs62191180	*PDCD1*	chr2_241908700_G_A_b38	49806	0.157	13.5
rs62193087	*PDCD1*	chr2_241808893_A_C_b38	-50001	0.27	16.8
rs73011941	*PDCD1*	chr2_241777177_C_A_b38	-81717	0.034	14.4
rs74003953	*PDCD1*	chr2_241928080_C_G_b38	69186	0.549	24.2
rs76356158	*PDCD1*	chr2_241810882_C_G_b38	-48012	0.504	49.1
rs147055142	*CD274*	chr9_5476045_C_T_b38	25542	0.126	20.7
rs1970000	*CD274*	chr9_5465036_C_A_b38	14533	0.506	55.9
rs2890658	*CD274*	chr9_5465130_C_A_b38	14627	0.101	65.6
rs59906468	*CD274*	chr9_5466085_A_G_b38	15582	0.236	115.4
rs62560214	*CD274*	chr9_5471193_T_C_b38	20690	0.046	33.5
rs73399174	*CD274*	chr9_5487288_G_A_b38	36785	0.05	14.8

[a] Distance from gene-encoding region. A negative value indicates that the variant is upstream of the transcription start site.
[b] MAF, minor allele frequency (evaluated in the GTEx study cohort).

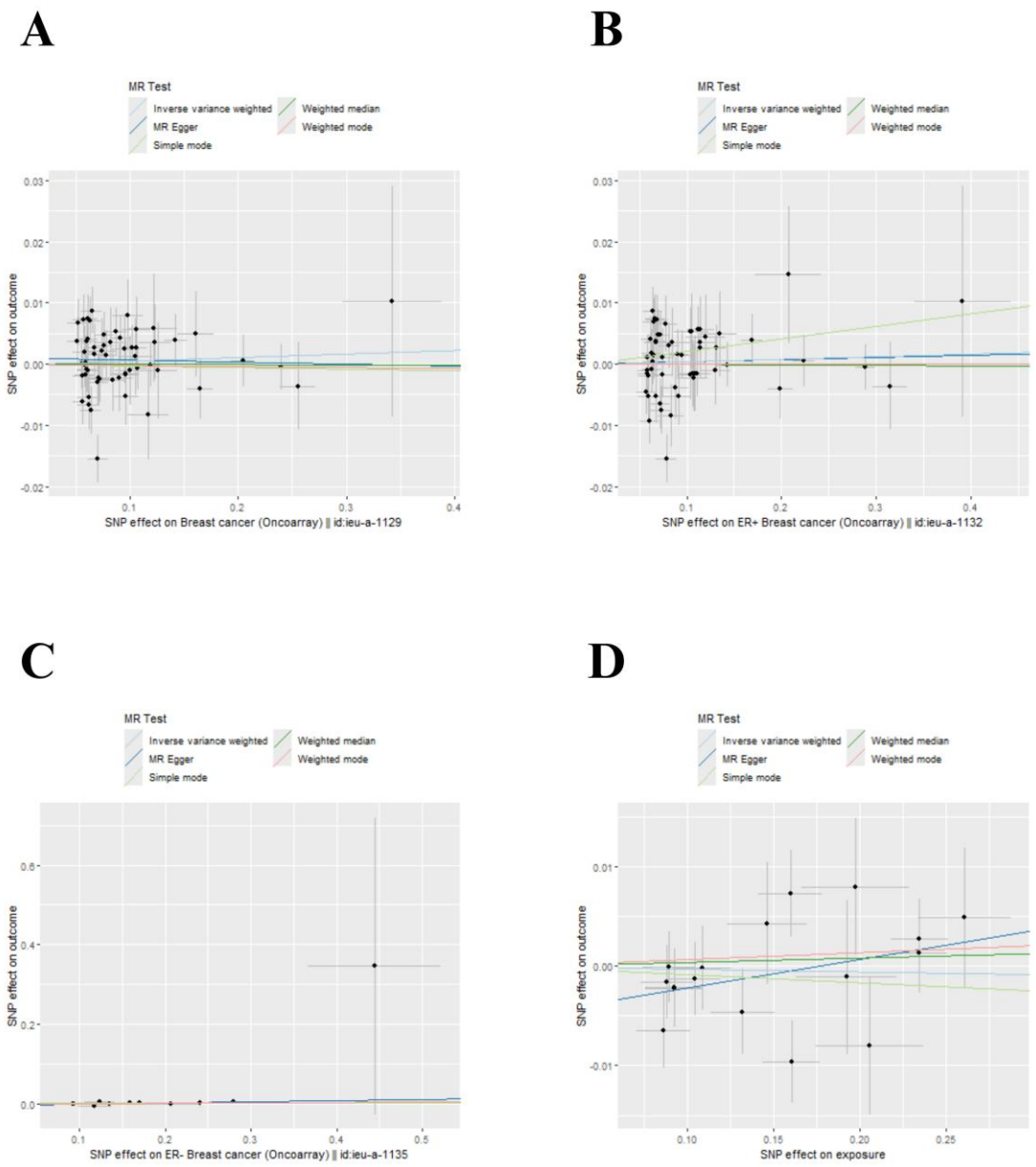
Appendix Table B-3. Mendelian randomization primary and sensitivity results for the effects of immune checkpoint inhibition on cerebral small vessel disease markers. Results as reported as beta and 95% confidence interval for white matter hyperintensity (WMH) volume, and as odds ratio and 95% confidence interval for perivascular space (PVS) burden, cerebral microbleeds (CMB), and small vessel stroke (SVS). Values are bolded for those that achieved significance ($p<0.05$).

		Cerebral Small Vessel Disease Marker			
Exposure	***MR Method***	***WMH***	***PVS***	***CMB***	***SVS***
Immune Checkpoint Inhibition	IVW	0.045 (0.003-0.087)	1.02 (1.00-1.03)	1.12 (0.93-1.34)	1.02 (0.86-1.22)
	MR-PRESSO	0.045 (0.017-0.074)	1.02 (1.00-1.03)	1.12 (1.01-1.23)	1.02 (0.86-1.22)
	Weighted Median	0.021 (-0.037-0.078)	1.02 (1.00-1.05)	1.12 (0.90-1.41)	1.07 (0.90-1.26)
	MR-Egger	-0.034 (-0.164-0.096)	1.01 (0.97-1.06)	1.43 (0.69-2.98)	1.62 (1.01-2.59)

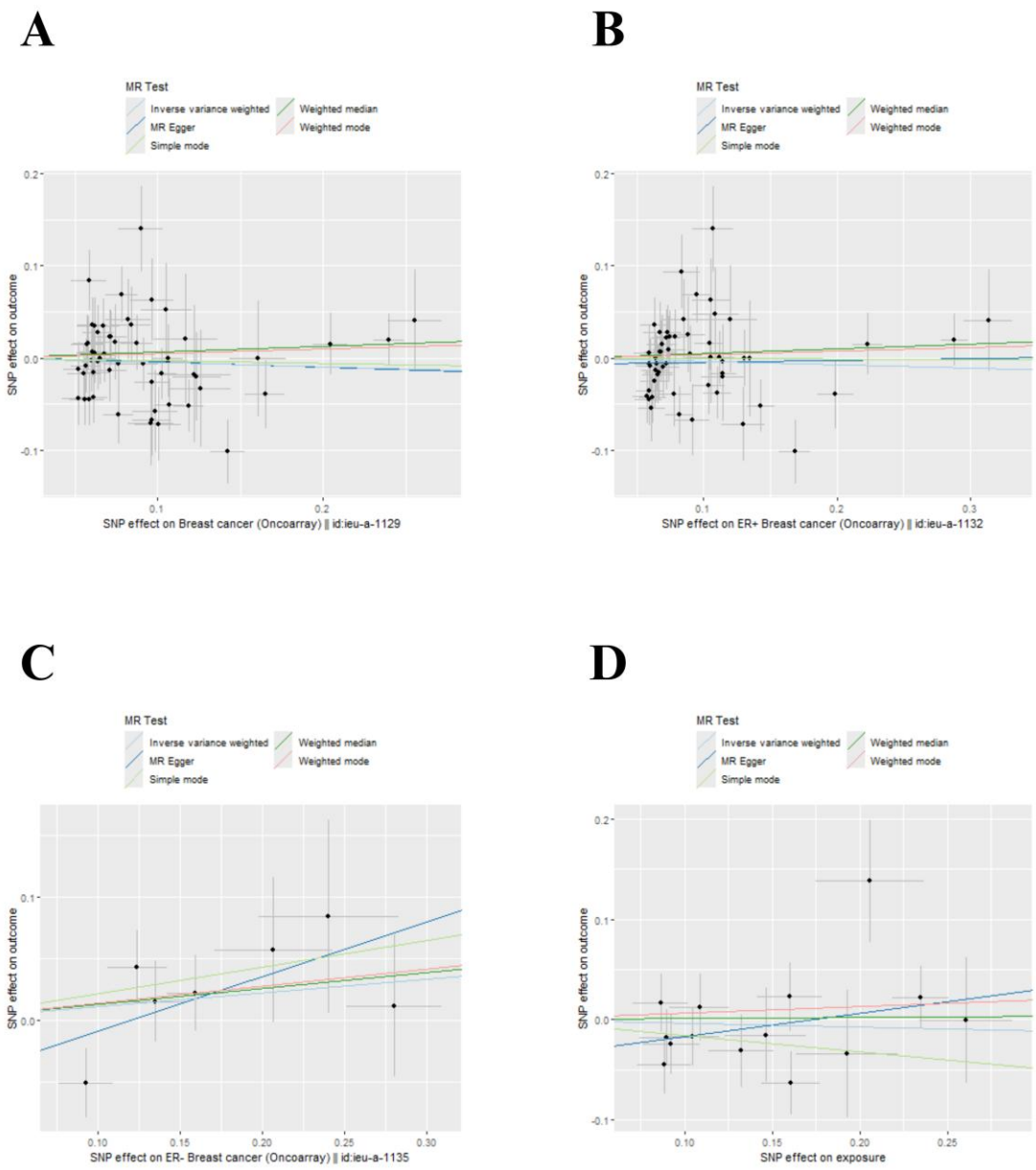
Supplementary Figures



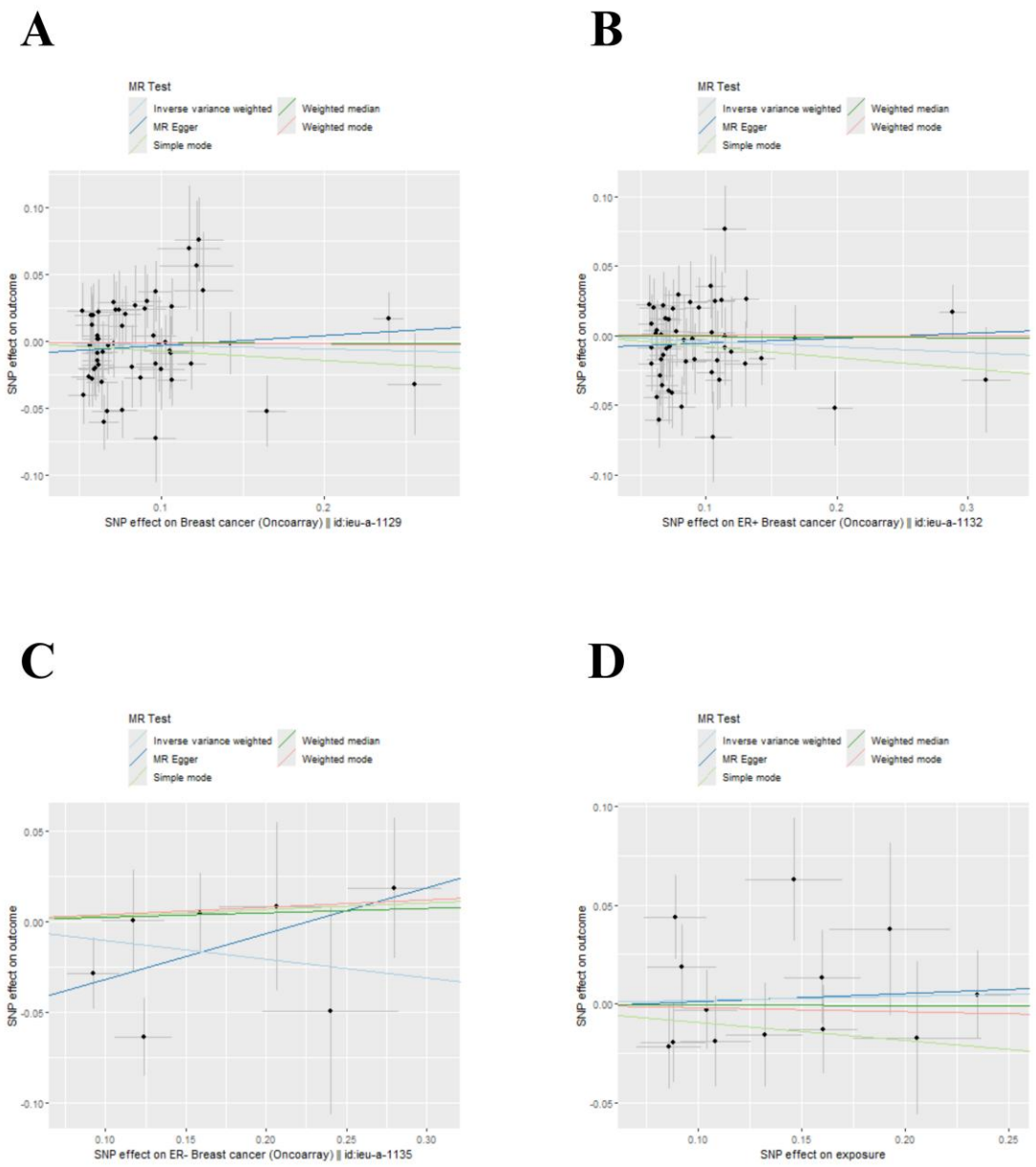
Appendix Figure B-1. Scatter plots depicting the different Mendelian randomization methods applied to assess the relationship between overall breast cancer (A), estrogen-receptor positive breast cancer (B), estrogen-receptor negative breast cancer (C) and triple-negative (TN) breast cancer (D) and white matter hyperintensity volume.



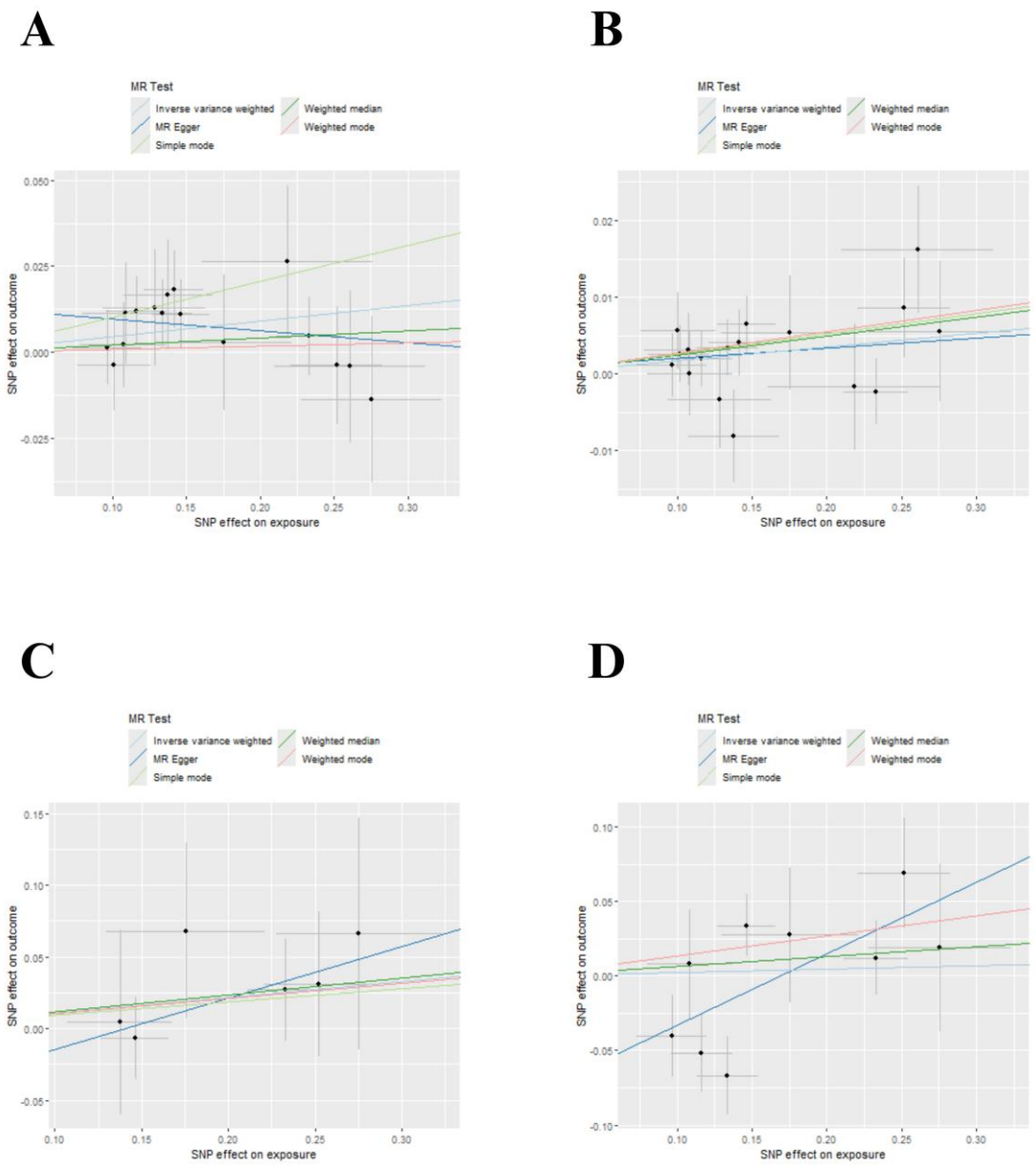
Appendix Figure B-2. Scatter plots depicting the different Mendelian randomization methods applied to assess the relationship between overall breast cancer (A), estrogen-receptor positive breast cancer (B), estrogen-receptor negative breast cancer (C) and triple-negative (TN) breast cancer (D) and perivascular space burden.



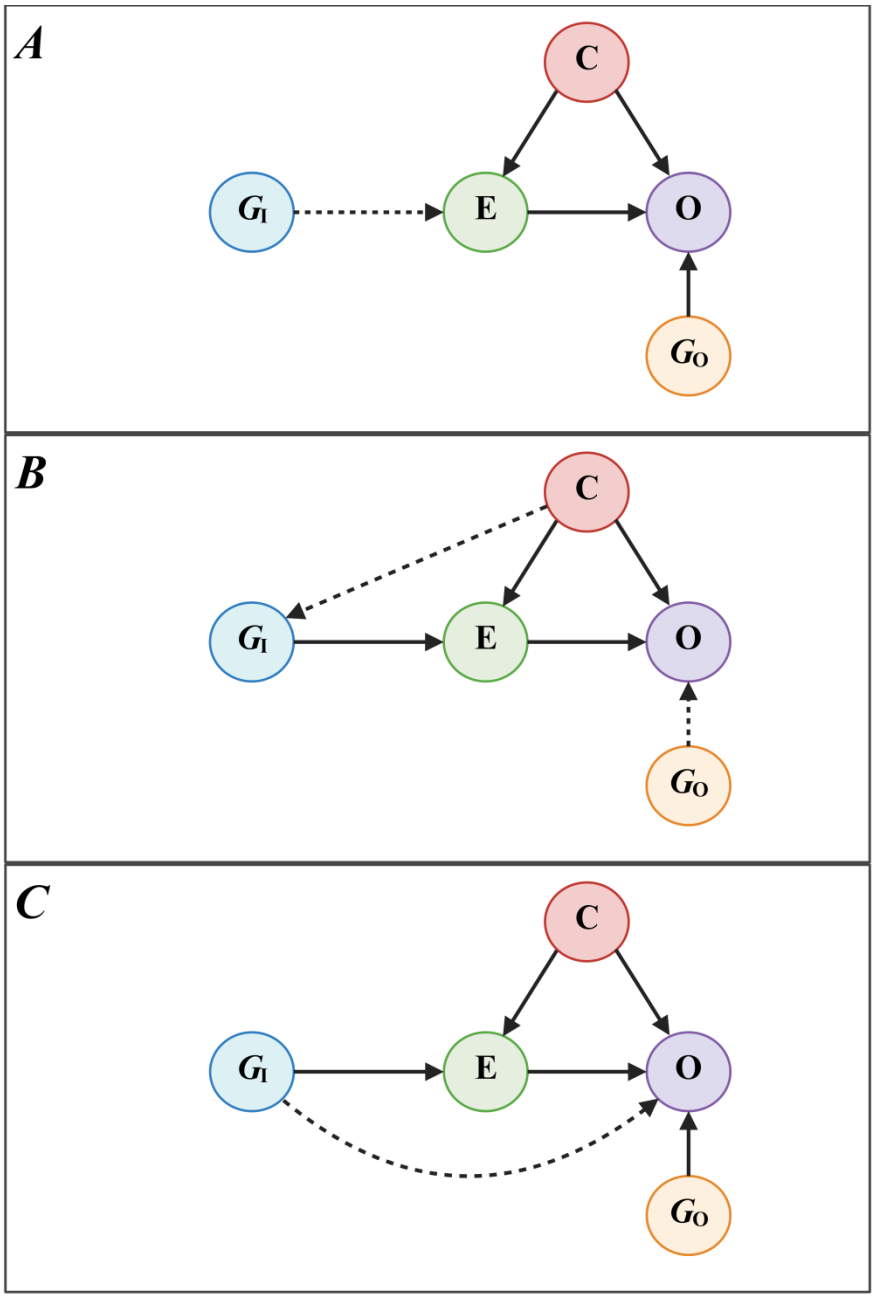
Appendix Figure B-3. Scatter plots depicting the different Mendelian randomization methods applied to assess the relationship between overall breast cancer (A), estrogen-receptor positive breast cancer (B), estrogen-receptor negative breast cancer (C) and triple-negative (TN) breast cancer (D) and cerebral microbleeds.



Appendix Figure B-4. Scatter plots depicting the different Mendelian randomization methods applied to assess the relationship between overall breast cancer (A), estrogen-receptor positive breast cancer (B), estrogen-receptor negative breast cancer (C) and triple-negative (TN) breast cancer (D) and the risk of small vessel stroke.



Appendix Figure B-5. Scatter plots depicting the different Mendelian randomization methods applied to assess the relationship between immune checkpoint inhibition and (A) white matter hyperintensity volume, (B) perivascular space burden, (C) cerebral microbleeds, and (D) the risk of small vessel stroke.



Appendix Figure B-6. Schematic demonstration of Mendelian randomization principles, as well as the core assumptions allowing for causal estimates to be derived. Panel A demonstrates the relevance assumption, Panel B depicts the exchangeability assumption, and Panel C shows the exclusion-restriction assumption. G_I, genetic variants used as instrumental variables for the exposure; G_O, genetic variants used as instrumental variables for the outcome; E, exposure variable; O, outcome variable; C, potential unmeasured confounding variables.

Supplementary References

1. Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. Nat Rev Methods Primers. Feb 10 2022;2doi:10.1038/s43586-021-00092-5
2. Zeng P, Zhao Y, Qian C, et al. Statistical analysis for genome-wide association study. J Biomed Res. Jul 2015;29(4):285-97. doi:10.7555/JBR.29.20140007
3. Brumpton B, Sanderson E, Heilbron K, et al. Avoiding dynastic, assortative mating, and population stratification biases in Mendelian randomization through within-family analyses. Nat Commun. Jul 14 2020;11(1):3519. doi:10.1038/s41467-020-17117-4
4. Walker VM, Zheng J, Gaunt TR, Smith GD. Phenotypic Causal Inference Using Genome-Wide Association Study Data: Mendelian Randomization and Beyond. Annu Rev Biomed Data Sci. Aug 10 2022;5:1-17. doi:10.1146/annurev-biodatasci-122120-024910
5. Glymour MM, Tchetgen Tchetgen EJ, Robins JM. Credible Mendelian randomization studies: approaches for evaluating the instrumental variable assumptions. Am J Epidemiol. Feb 15 2012;175(4):332-9. doi:10.1093/aje/kwr323
6. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. Sep 11 2020;369(6509):1318-1330. doi:10.1126/science.aaz1776
7. Ongen H, Buil A, Brown AA, Dermitzakis ET, Delaneau O. Fast and efficient QTL mapper for thousands of molecular phenotypes. Bioinformatics. May 15 2016;32(10):1479-85. doi:10.1093/bioinformatics/btv722

Appendix C - Supplementary Materials for Chapter 5

Strengthening Reporting of Observational Studies in Epidemiology – Mendelian Randomization (STROBE-MR) Guidelines

Item No.	Section	Checklist item	Page No.
1	TITLE and ABSTRACT	Indicate Mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study	154
	INTRODUCTION		
2	Background	Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question	156-157
3	Objectives	State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects	156-157
	METHODS		157, 160
4	Study design and data sources	Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:	157, 160
	a)	Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.	159, 166
	b)	Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis	159, 166
	c)	Describe measurement, quality control and selection of genetic variants	163-164
	d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	163-165
	e)	Provide details of ethics committee approval and participant informed consent, if relevant	N/A
5	Assumptions	Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis	165
6	Statistical methods: main analysis	Describe statistical methods and statistics used	167
	a)	Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)	167
	b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	167
	c)	Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples	167
	d)	Explain how missing data were addressed	N/A
	e)	If applicable, indicate how multiple testing was addressed	167
7	Assessment of assumptions	Describe any methods or prior knowledge used to assess the assumptions or justify their validity	167
8	Sensitivity analyses and additional analyses	Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations)	167

9	Software and pre-registration		
	a)	Name statistical software and package(s), including version and settings used	167
	b)	State whether the study protocol and details were pre-registered (as well as when and where)	N/A
	RESULTS		
10	Descriptive data		
	a)	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram	159, 166
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	166
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	N/A
	d)	For two-sample MR:	N/A
		i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples	
		ii. Provide information on the number of individuals who overlap between the exposure and outcome studies	
11	Main results		
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	172, 174
	b)	Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference	172-175
	c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
	d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	173, 175
12	Assessment of assumptions		
	a)	Report the assessment of the validity of the assumptions	N/A
	b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	N/A
13	Sensitivity analyses and additional analyses		
	a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	N/A
	b)	Report results from other sensitivity analyses or additional analyses	N/A
	c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	N/A
	d)	When relevant, report and compare with estimates from non-MR analyses	N/A
	e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	N/A
	DISCUSSION		
14	Key results	Summarize key results with reference to study objectives	180-183
15	Limitations	Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them	183
16	Interpretation		
	a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	180-183
	b)	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions	180-183

	c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	180-183
17	Generalizability	Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure	183
	OTHER INFORMATION		
18	Funding	Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based	
19	Data and data sharing	Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where	
20	Conflicts of Interest	All authors should declare all potential conflicts of interest	

Supplementary Methods

Details on Genetic Variant Identification Methods

Gene Expression Quantitative Trait Loci. For identifying and selecting genetic variants to proxy the effects of immune checkpoint inhibition, we followed the analytical and statistical methods of the GTEx consortium.[1] In brief, the GTEx protocol primarily used FastQTL for processing and analysis.[2] Variants were considered cis-variants if they were ±1Mb from a gene transcription start site (which we reduced to ±100kb), and all variants with a minor allele frequency ≥ 0.01 were incorporated. Genes with at least one eQTL (expression quantitative trait loci) variant were identified at a false discovery rate (FDR) ≤ 0.05 and clustered together as "eGenes". To then identify cis-variants significantly associated with expression of their target gene, first an empirical genome-wide p-value threshold (p_t) was set based on the empirical p-value of the eGene closest to FDR=0.05. This p_t value was used to establish gene-specific significance thresholds for each eGene. Cis-variants with a nominal p-value less than their target eGene significance threshold were considered statistically significant eQTL and reported as "significant gene-variant pairs".[1] These pairs were extracted from GTEx v10 data for present analyses.

Methylation Quantitative Trait Loci. Methylation quantitative trait loci (mQTL) data were retrieved from the ARIES (Accessible Resource for Integrated Epigenomic Studies) data resource.[3] ARIES included data from a subset of participants enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC).[4] Specifically, 1018 mother-offspring pairs were included based on the availability of samples for DNA analysis. Mothers were analyzed at two separate time points: pregnancy (average age of mother: 29.2 yrs) and at a follow-up point on

average 15.5 years later. Offspring were assessed at three timepoints: birth (average gestational age: 40 weeks), childhood (average age of offspring: 7.5 yrs), and adolescence (average age of offspring: 17.1 yrs). Blood samples were processed, and genome-wide methylation status was analyzed at over 485000 methylation sites using the Illumina 450K system (Illumina Inc., CA, USA). Samples were distributed across arrays semi-randomly to reduce batch effect biases[5], and quality control metrics were implemented. Genome-wide significance was set at $p<5x10^{-7}$ to account for the number of methylation sites assessed. Data for mQTLs were extracted using the *MRInstruments* package in R.[6]

Core Assumptions of Mendelian Randomization

The core assumptions of two-sample Mendelian randomization studies are as follows: 1) relevance, 2) exchangeability, and 3) exclusion-restriction.[7] The relevance assumption is satisfied by demonstrating that the instrumental variable (the single genetic variant or multiple genetic variants used to proxy the exposure variable) is associated with the actual exposure of interest (Supplementary Figure 9, Panel A). In the scientific methods, this is done by selecting genetic variants that have previously been shown to associate with the exposure of interest at genome-wide standard threshold criteria (usually $p<5x10^{-8}$).[8] The exchangeability assumption says that there are no confounding pathways, mechanisms, or phenotypes between the instrumental variable and the outcome of interest that act independently of the exposure of interest (Supplementary Figure 9, Panel B). Genetic variation (aside from somatic mutations) are determined at conception, so many traditional confounding factors do not have an effect in this context. However, genome-specific confounders like population stratification and dynastic effects may play some role. To a degree, these are adjusted for in genome-wide association

studies; some degree of confounding is always still possible.[7,9] Finally, the exclusion-restriction assumption states that the instrumental variable only affects the outcome through the exposure of interest, and there are no other traits that have downstream effects on the outcome variable (Supplementary Figure 9, Panel C). In our methods, this assumption is tested by checking both horizontal pleiotropy in the statistical Mendelian randomization models and by using phenome-wide analyses to identify potential confounding phenotypes.[10] However, it is important to note that while sensitivity analyses may help address this third assumption, it cannot be truly and fully confirmed as there is always the possibility of some degree of unmeasured confounding.[11]

Supplementary Tables

Appendix Table C-1. Results of clustering analysis performed with endothelial cell-specific differentially expressed genes obtained from single cell RNA-sequencing in samples obtained from patients with or without exposure to immune checkpoint inhibitors.

Cluster Name	**Cluster P-Value**
Cytochrome-c oxidase activity	3.43E-03
Glutathione peroxidase activity	5.93E-03
Regulation of vascular permeability	5.25E-04
Cell redox homeostasis	1.63E-03
Hepatocyte growth factor receptor signaling pathway	7.13E-03
Positive regulation of sequestering of calcium ion	5.85E-03
Regulation of protein exit from endoplasmic reticulum	3.63E-03
Negative regulation of transforming growth factor beta production	2.75E-03
U2-type prespliceosome assembly	7.61E-03
Chondrocyte development	6.90E-03
Protein N-linked glycosylation via asparagine	3.43E-03
Viral translation	7.13E-03
Protein folding in endoplasmic reticulum	1.11E-04
Protein nitrosylation	6.25E-03
Negative regulation of viral life cycle	5.01E-04
Cytoplasmic translational initiation	4.30E-03
Membrane raft assembly	3.43E-03
Positive regulation of collagen metabolic process	4.41E-03
Transforming growth factor beta receptor activity	5.27E-03
Antigen processing and presentation of endogenous peptide antigen	4.04E-04
Lymphangiogenesis	6.86E-03
Regulation of glomerular filtration	4.63E-04
Protein folding chaperone	1.10E-07

Appendix Table C-2. List of unique genes included as instruments in the Mendelian randomization analyses using gene expression exposures and cerebral small vessel disease outcomes.

ACKR1	ACTR1A	ACTR2	ACTR3	ADAM15	ADH5	AGTRAP	AHNAK	AHR	ALDH2	ANP32B
ANXA2	ANXA5	AP2M1	AP2S1	APLP2	APOL1	APP	APRT	ARF3	ARL6IP1	ARL6IP5
ARPC1B	ARPC2	ARPC3	ARPC4	ASRGL1	ATOX1	ATP1A1	ATP1B3	ATP2B4	ATP5F1A	ATP5F1C
ATP5F1D	ATP5IF1	ATP5MC1	ATP5PD	ATP5PO	ATP6V0B	ATP6V1G1	ATRAID	B2M	BANF1	BAX
BCL7B	BLOC1S1	BMPR2	BNIP2	BRD4	BRI3	BRK1	BSG	BTG1	BTG2	BUD31
BZW1	C12orf57	C19orf53	C1QBP	C4orf3	CACYBP	CALCRL	CALM1	CALM3	CALR	CALU
CAMLG	CAPNS1	CARD16	CARHSP1	CASKIN2	CAST	CAV2	CAVIN3	CCDC12	CCDC69	CCDC85B
CCT3	CCT4	CCT5	CCT6A	CCT7	CD151	CD320	CD40	CD55	CD59	CD63
CD81	CD93	CDC26	CDC42	CDC42EP3	CDIPT	CDK2AP1	CDKN1A	CFL1	CHCHD2	CHD4
CHMP3	CHMP4B	CHSY1	CLDN5	CLEC2B	CLIC1	CLIC4	CLPP	CLTB	CMIP	CNPY2
COL15A1	COL6A2	COMMD6	COPE	COPS6	COX4I1	COX7A2	COX7A2L	COX8A	CRIM1	CRIP2
CRTAP	CTNNA1	CTNNAL1	CTSL	CUTA	CYB5R3	CYYR1	DAD1	DAZAP2	DCTN3	DDRGK1
DDX18	DDX24	DECR1	DEGS1	DGUOK	DNAJC1	DNAJC15	DNAJC3	DPP7	DPYSL2	DPYSL3
DSTN	DUSP23	DUT	DYNC1I2	EBPL	ECE1	EEF2	EHD4	EIF3D	EIF3F	EIF3G
EIF4B	EIF4G2	EIF4H	EIF5	EIF5A	EIF6	EMC4	EMC7	EMP1	ENG	ENY2
EPAS1	EPHB4	ERAP2	ERH	ESM1	ETFB	ETS2	F2R	FAM107A	FAM167B	FAM177A1
FAM43A	FAU	FCGRT	FERMT2	FIBP	FIS1	FKBP1A	FLNB	FLOT1	FLYWCH2	FRG1
FSTL1	FURIN	FXYD5	GANAB	GAS6	GBP4	GDI2	GHITM	GIMAP1	GIMAP4	GIMAP6
GIMAP7	GINM1	GLUD1	GNA11	GNAI2	GNAS	GNG11	GNG5	GNS	GOLGA7	GRAMD1A
GRN	GSTK1	GSTO1	GSTP1	GTF3A	GYPC	HEBP2	HEG1	HIGD2A	HINT1	HLA-A
HLA-B	HLA-C	HLA-DRB1	HLA-DRB6	HLA-E	HMGN1	HMGN3	HNRNPM	HPCAL1	HSBP1	HSD17B12
HSP90B1	HSPA1B	HSPB1	HTRA1	ICAM2	IER3	IER3IP1	IFITM2	IFITM3	IGFBP2	IGFBP4
IGFBP7	IGHG1	IGLC2	IGLC3	IK	IKBIP	INSR	IQGAP1	ISCU	ISG15	ISG20
ITGA2	JAK1	KANK3	KCNE3	KDELR2	KLF10	KRT10	LAMTOR1	LAMTOR4	LAMTOR5	LAP3
LDHA	LEPROT	LMAN1	LMAN2	LMNA	LMO2	LRP10	LRPAP1	LRRC32	LSM1	LSM2
LSM4	LSM6	LSM7	LSM8	LTC4S	LXN	LY6E	MAF1	MAPK3	MCFD2	MESD
METTL26	METTL9	MFNG	MGST3	MICOS10	MIDN	MLEC	MOB2	MORF4L1	MPHOSPH8	MRFAP1
MRPL20	MRPL23	MRPL27	MRPL33	MRPL51	MRPL54	MRPS21	MTLN	MTPN	MVP	MYADM
MYL12A	MYL12B	NAA38	NCL	NDUFA11	NDUFA12	NDUFA2	NDUFA3	NDUFA6	NDUFAB1	NDUFB1
NDUFB10	NDUFB2	NDUFB5	NDUFB8	NDUFB9	NDUFC1	NDUFC2	NDUFS5	NDUFS8	NEAT1	NECTIN2
NENF	NFIC	NME1	NME4	NNMT	NOP10	NOP53	NOP56	NPDC1	NQO1	NRN1
NSA2	NUB1	NUCB1	NUCB2	NUCKS1	OCIAD1	OLA1	OS9	OSTC	PABPC4	PAK2
PAPSS2	PCMTD1	PDAP1	PDCD5	PDGFB	PDIA3	PDIA4	PDIA6	PDLIM1	PET100	PFKL
PGAM1	PHB2	PHPT1	PIM3	PKIG	PKN1	PLEC	PLPP3	PLSCR1	PLXND1	PNKD
PNRC1	POLE4	POLR1D	POLR2E	POLR2J	POLR2L	POMP	PON2	PPA1	PPDPF	PPIA
PPIB	PPIG	PPP1R18	PPP2CA	PPP2CB	PRCP	PRDX1	PRDX2	PRDX5	PRDX6	PRELID1
PRKCSH	PRMT1	PROS1	PRPF6	PSENEN	PSMB1	PSMB3	PSMB5	PSMB8	PSMB9	PSMC1
PSMC3	PSMC5	PSMD8	PSME1	PTPN12	PTPRM	PTTG1IP	PXDN	QSOX1	RAB2A	RAB31

RABAC1	RAC1	RACK1	RALA	RALBP1	RAMP3	RAP1B	RAPGEF1	RASIP1	RBCK1	RBM8A
RBPMS	RCN1	RDX	REEP5	RFLNB	RHOC	RIPOR1	RNASE1	RNF181	RPL11	RPL13
RPL14	RPL18A	RPL27	RPL28	RPL29	RPL3	RPL30	RPL32	RPL36	RPL36AL	RPL8
RPLP0	RPLP2	RPN1	RPS10	RPS13	RPS14	RPS16	RPS18	RPS19	RPS2	RPS20
RPS24	RPS26	RPS28	RPS3	RPS5	RPS6	RPS8	RPS9	RTF1	RTN3	RWDD1
S100A10	S100A11	S100A13	S100A6	SBDS	SCAND1	SCP2	SDF4	SDHC	SEC11A	SELENOK
SELENOM	SELENON	SELENOT	SELP	SEPHS2	SERF2	SERINC1	SERINC3	SERPINB1	SERPING1	SERPINH1
SF3B5	SH3BGRL3	SH3BP5	SHISA5	SIVA1	SLC25A37	SLC44A2	SLIRP	SLU7	SMAD1	SMARCA4
SNHG5	SNRK	SNRPB	SNRPB2	SNRPC	SNRPD1	SNRPD2	SNRPE	SNRPF	SNTB2	SNX17
SOCS3	SOD1	SPAG7	SPCS1	SPCS2	SPG21	SPTBN1	SPTLC2	SQSTM1	SRI	SRP14
SRP72	SSBP4	SSU72	ST13	ST6GAL1	STAB1	SUGT1	SULF2	SUMO2	SVIP	SYNGR2
TAGLN2	TAP1	TAP2	TAPBP	TAX1BP1	TAX1BP3	TBCA	TBCB	TCIM	TCN2	TEK
TGOLN2	THBD	THRAP3	TIMM13	TKT	TMA7	TMBIM1	TMED2	TMED9	TMEM109	TMEM123
TMEM147	TMEM14C	TMEM179B	TMEM204	TMEM230	TMEM255B	TMEM258	TMEM30A	TMEM50A	TMOD3	TMTC1
TNFRSF1A	TOMM7	TPGS1	TPM3	TPM4	TPST2	TRAM1	TRAPPC1	TRIM28	TRIM8	TRIOBP
TRMT112	TSPAN4	TSPAN9	TSPO	TUBA1A	TUBB	TUFM	TXN	TXNDC17	TYROBP	UBE2B
UBE2L3	UBE2L6	UBE2R2	UBXN6	UFC1	UQCR10	URM1	VAMP3	VAMP5	VAT1	VCP
VDAC2	VKORC1	VPS28	VWF	WDR1	XRCC5	XRCC6	YIPF3	YWHAB	YWHAG	YY1
ZC3H13	ZFP36L1	ZNHIT1								

Appendix Table C-3. Genes excluded for each CSVD outcome in the Mendelian randomization analyses using gene expression exposures and cerebral small vessel disease outcomes.

Excluded for WMH	Excluded for PVS	Excluded for CMB	Excluded for SVS
DPP7		ATP2B4	APLP2
NPDC1		BSG	ATP1A1
		CLIC1	ATP2B4
		COL15A1	BUD31
		FAM107A	C12orf57
		FLOT1	CLIC1
		FRG1	COL15A1
		GAS6	CRIM1
		HLA-A	FAM107A
		HLA-C	GSTO1
		HLA-DRB1	HLA-A
		HLA-DRB6	HLA-E
		HLA-E	HNRNPM
		HSPA1B	HSPA1B
		IER3	HSPB1
		IGHG1	IER3
		ISG15	IGHG1
		LSM2	LSM1
		LTC4S	LSM2
		MRPL20	LSM6
		PPP1R18	LTC4S
		PRMT1	MIDN
		PSMC5	MRPL20
		RAP1B	MRPL33
		RBM8A	MTLN
		RPL18A	OSTC
		RPS13	PDAP1
		S100A6	PPP1R18
		SBDS	PRCP
		SELENON	PRMT1
		TPGS1	PSMC5
		TUBB	PTPRM
		UBE2B	RAP1B
			RBM8A
			RPL18A
			RPL29
			RPL36
			RPS13
			SELENON
			SERINC3
			TMEM179B
			TRAPPC1
			TUBB
			UBE2B
			YY1

Appendix Table C-4. Sensitivity analyses performed using coronary artery-specific gene expression exposures and cerebral small vessel disease outcomes. Genes listed were associated with the respective cerebral small vessel disease marker at a nominal $p<0.05$; bolded genes were also significant after correction for multiple testing.

Associated with WMH	**Associated with PVS**	**Associated with CMB**	**Associated with SVS**
HLA-DRB6	HLA-C	CCT3	HSD17B12
HLA-C	RBCK1	SPG21	IQGAP1
NDUFS5	HLA-DRB6	TRIOBP	ERAP2
CALCRL	NUCKS1	DDX18	ANXA5
MRPS21	MRPL32	NDUFA3	NME4
PDCD5	NRP2	CAVIN3	SPG21
ANXA5	MRPL23	MRPS21	ACTR3
HLA-B	RCN1	MRPL32	LRPAP1
DYNC1I2	NEAT1	CARHSP1	IKBIP
LRPAP1	LAMTOR4	MRPL23	TCN2
PPA1	RPS9	ATRAID	PRDX1
RPS18	METTL26	PSMB9	PLEC
SPAG7	BANF1	CAMLG	SIVA1
PLEC	MRPS21	SPARC	CDK2AP1
HLA-DRB1	MCFD2	CD59	NSA2
CARHSP1	URM1		EBPL
ARL6IP5	LTC4S		ATRAID
RPL28	QSOX1		MRPL32
ATP5PO	GAS6		ACTR2
LSM2	POLR1D		CD59
PPP1R18	VPS28		PSMB5
FAM107A	RPS24		
ATRAID	C7orf50		
ARHGDIB	IKBIP		
GSTK1	FIS1		
SPG21	RPS18		
	HLA-A		
	CAVIN3		
	TUFM		
	MOB2		

Appendix Table C-5. List of unique methylation sites included as instruments in the Mendelian randomization analyses using gene expression exposures and cerebral small vessel disease outcomes.

cg00061031	cg00166216	cg00323915	cg00347643	cg00348858	cg00356499	cg00375581	cg00395296	cg00423487	cg00431565	cg00472373
cg00524220	cg00551647	cg00581603	cg00684283	cg00685014	cg00726046	cg00785193	cg00797651	cg00836007	cg00836091	cg00854314
cg00883689	cg00900524	cg00923807	cg00935119	cg00962304	cg01054402	cg01091099	cg01114900	cg01284415	cg01284869	cg01285435
cg01286133	cg01299997	cg01328473	cg01359822	cg01369114	cg01422274	cg01439670	cg01459453	cg01514075	cg01517384	cg01518465
cg01529207	cg01567051	cg01574390	cg01591881	cg01629329	cg01667702	cg01741836	cg01758539	cg01770362	cg01827781	cg01869186
cg02065750	cg02072170	cg02075820	cg02101833	cg02152968	cg02183531	cg02232751	cg02276665	cg02338345	cg02427764	cg02573566
cg02652597	cg02711929	cg02792877	cg02799643	cg02814118	cg02871659	cg02935298	cg03038418	cg03043406	cg03063057	cg03079761
cg03110633	cg03141158	cg03183936	cg03309967	cg03355213	cg03394764	cg03437912	cg03480605	cg03589001	cg03633268	cg03714916
cg03853587	cg03861347	cg03863486	cg03864128	cg03870261	cg03888447	cg03957109	cg04053108	cg04059188	cg04070771	cg04188351
cg04226276	cg04351776	cg04506190	cg04552852	cg04614290	cg04633683	cg04663916	cg04696780	cg04728402	cg04731861	cg04833034
cg04865442	cg05046996	cg05083128	cg05147525	cg05163057	cg05212138	cg05231098	cg05232215	cg05236720	cg05241015	cg05380558
cg05498726	cg05532239	cg05587400	cg05638426	cg05638821	cg05834899	cg05868183	cg05965490	cg05977523	cg06018095	cg06046490
cg06070002	cg06151968	cg06285241	cg06355652	cg06409856	cg06419659	cg06422947	cg06463313	cg06483076	cg06521852	cg06524039
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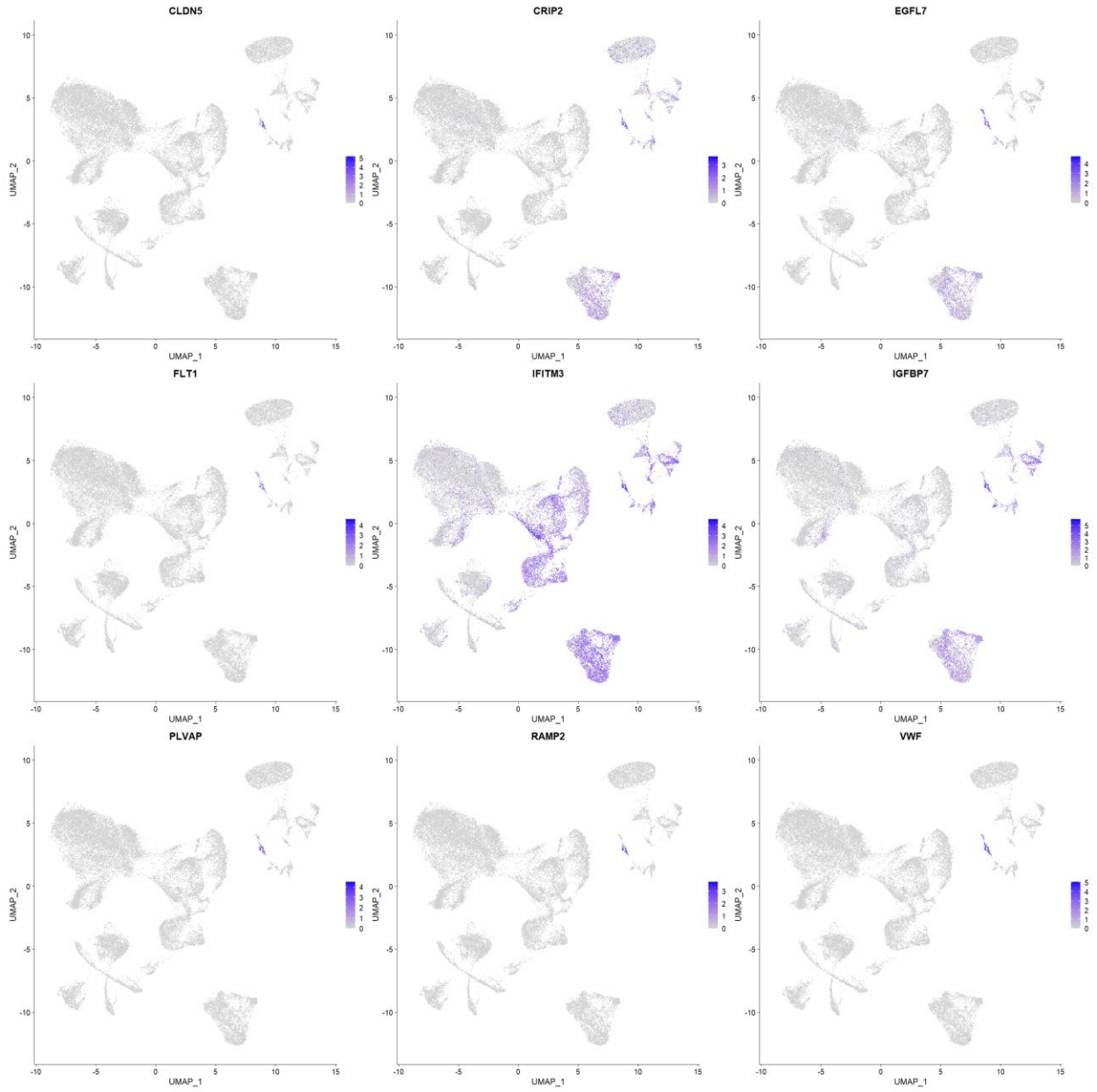
Appendix Table C-6. Genes excluded for each CSVD outcome in the Mendelian randomization analyses using methylation site exposures and cerebral small vessel disease outcomes.

Excluded for WMH	Excluded for PVS	Excluded for CMB	Excluded for SVS
ANXA5	ANXA5	CDKN1A	ANP32B
DNAJC15	CDKN1A	CRIP2	ANXA5
FIS1	CRIP2	FIS1	ARPC2
HLA-C	DNAJC15	GNA11	BUD31
NPDC1	GNA11	HLA-B	CDKN1A
TRIOBP	HLA-C	HLA-DRB6	CRIP2
	KANK3	IGFBP4	DNAJC15
	PHB2	PHB2	FIS1
	PNKD	PNKD	GNA11
	TRIOBP	SQSTM1	HLA-C
	TSPAN4	TSPAN4	HLA-DRB6
	VPS28	VPS28	IGFBP4
			KANK3
			LRPAP1
			PFKL
			PHB2
			PIM3
			PNKD
			PXDN
			RNASE1
			S100A13
			SQSTM1
			SSU72
			ST13
			TRIOBP
			TSPAN4
			VPS28

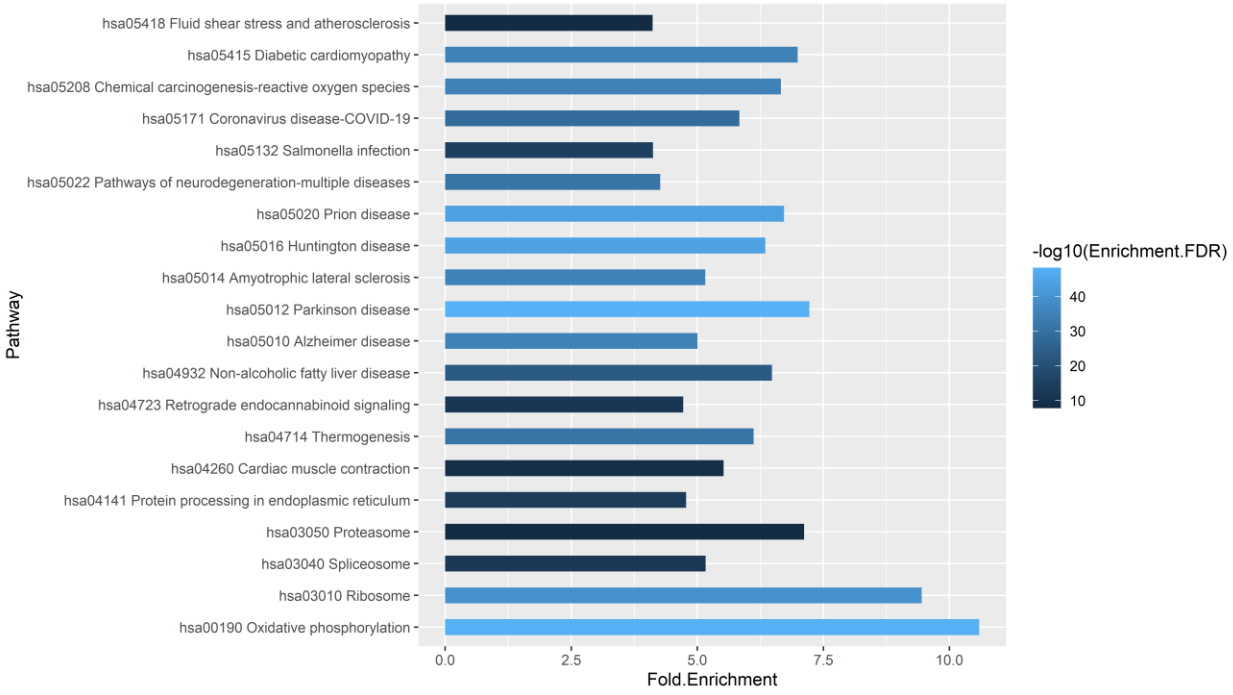
Appendix Table C-7. The top 10 (ranked by connectivity scores) pharmacological perturbation signatures in the same direction as the effects of immune checkpoint inhibitor treatment on endothelial cell gene expression in the gene set robustly associated with cerebral small vessel disease.

Pharmacological Class	**Cell Types Tested**	**Raw Connectivity Score**	**Adjusted P-value**	**Regulatory Status**
Selective Estrogen Receptor Modulators	MCF7	0.81	0.0042	FDA-approved
ALK Tyrosine Kinase Receptor Inhibitors	MCF10A	0.81	0.046	FDA-approved
Vitamin D Receptor Agonists	MCF10A	0.76	0.046	FDA-approved
Dihydrofolate Reductase Inhibitors	HA1E	0.74	0.0052	FDA-approved
MEK Inhibitors	MCF10A	0.7	0.0036	FDA-approved
BCR-ABL Kinase Inhibitors	HT29	0.69	0.0039	FDA-approved
DNA Methyltransferase Inhibitors	HA1E	0.66	0.046	FDA-approved
RET Tyrosine Kinase Inhibitors	YAPC	0.66	0.035	FDA-approved
RAF Inhibitors	HT29	0.65	0.0024	FDA-approved
FLT3 Inhibitors	HT29	0.59	0.0029	FDA-approved

Supplementary Figures

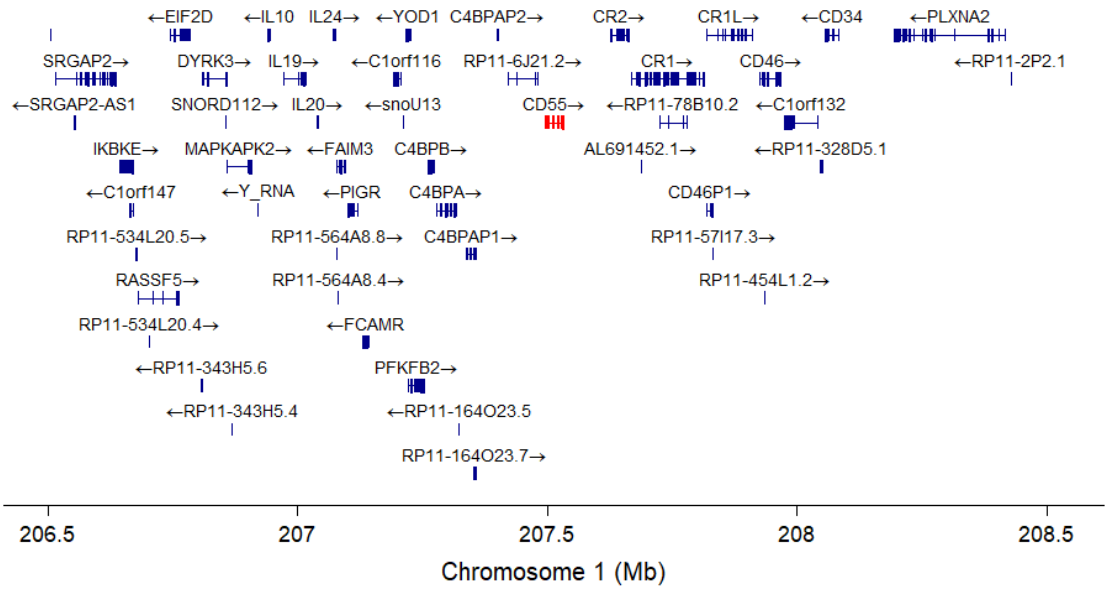


Appendix Figure C-1. Uniform manifold approximation and projection (UMAP) plots for 9 of the top markers used to identify the cluster of endothelial cells from single-cell RNA-sequencing data collected in clear cell renal carcinoma patients.

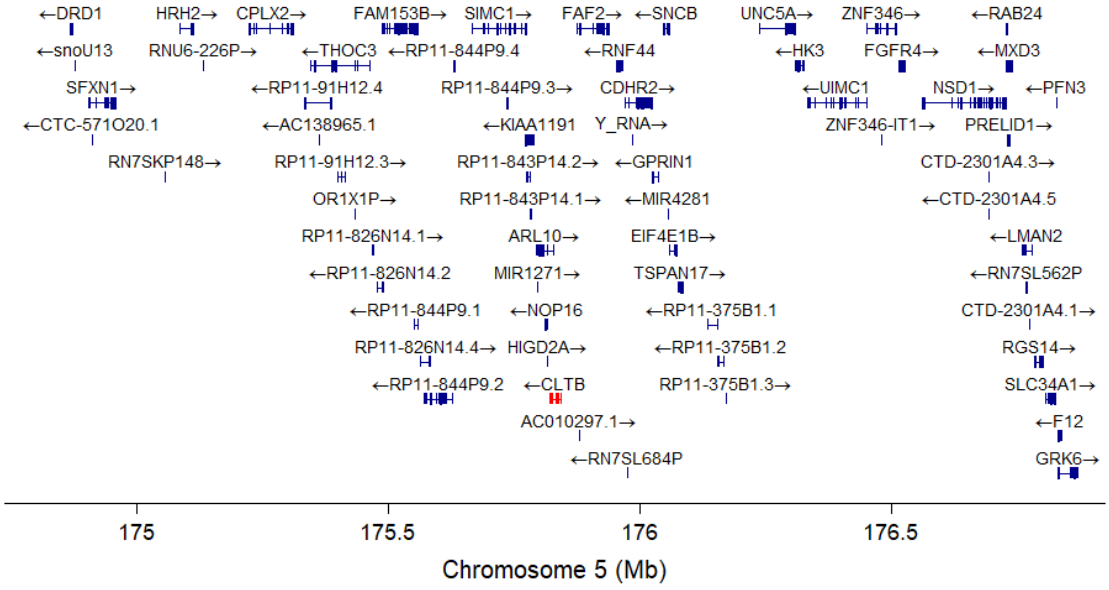


Appendix Figure C-2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways significantly enriched when comparing differentially expressed genes between control and immune checkpoint inhibitor-exposed endothelial cells.

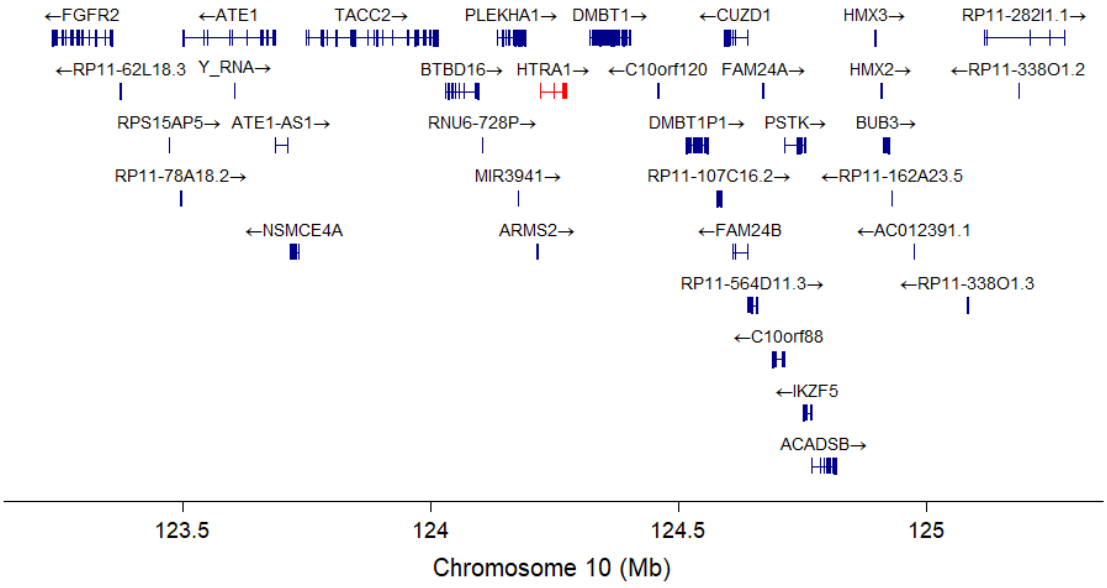
Appendix Figure C-3. Reactome pathways significantly enriched when comparing differentially expressed genes between control and immune checkpoint inhibitor-exposed endothelial cells.



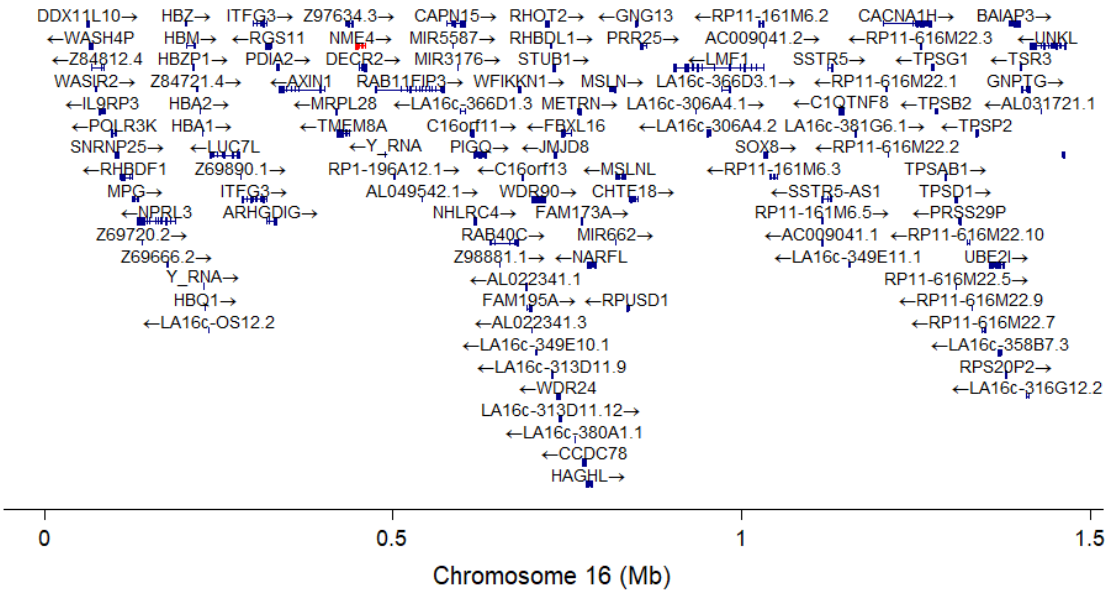
Appendix Figure C-4. Gene track depicting the location of the *Cd55* gene.



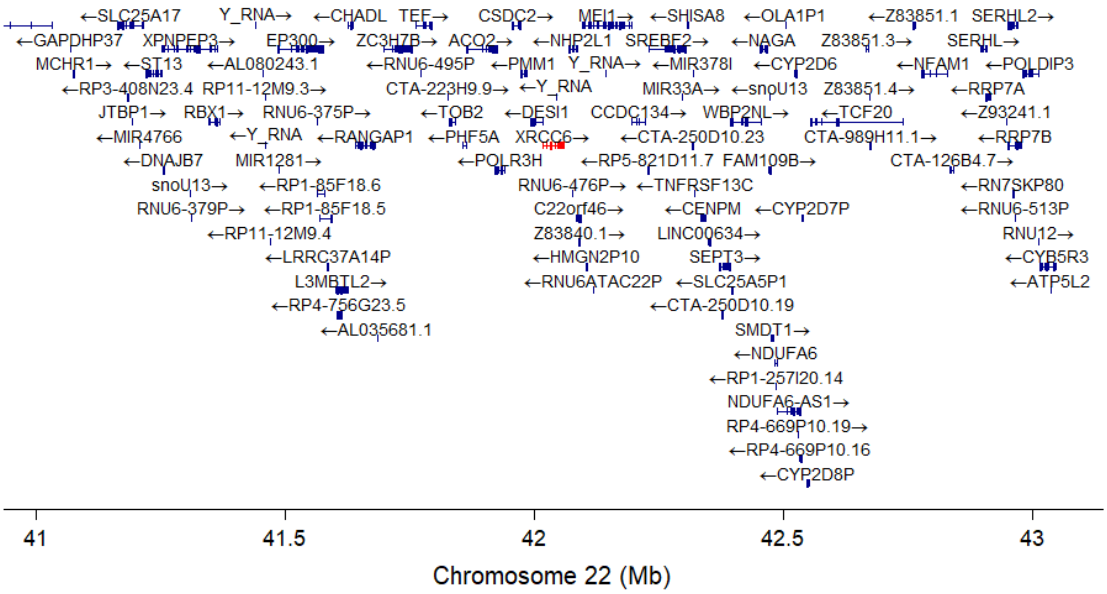
Appendix Figure C-5. Gene track depicting the location of the *Cltb* gene.



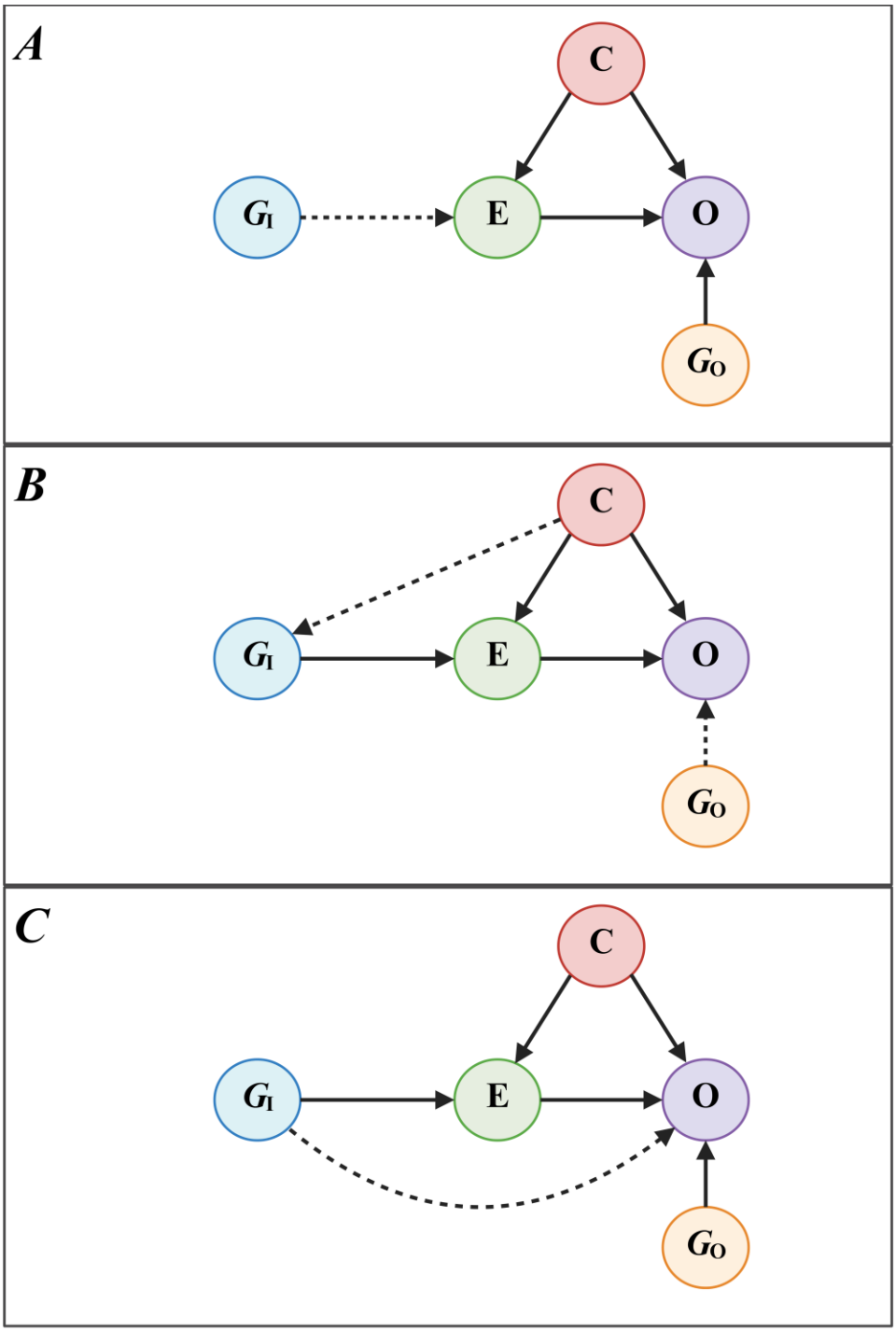
Appendix Figure C-6. Gene track depicting the location of the *Htra1* gene.



Appendix Figure C-7. Gene track depicting the location of the *Nme4* gene.



Appendix Figure C-8. Gene track depicting the location of the *Xrcc6* gene.



Appendix Figure C-9. Schematic demonstration of Mendelian randomization principles, as well as the core assumptions allowing for causal estimates to be derived. Panel A demonstrates the relevance assumption, Panel B depicts the exchangeability assumption, and Panel C shows the exclusion-restriction assumption. G_I, genetic variants used as instrumental variables for the exposure; G_O, genetic variants used as instrumental variables for the outcome; E, exposure variable; O, outcome variable; C, potential unmeasured confounding variables.

Supplementary References

1. The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. 2020;369:1318-1330. doi: 10.1126/science.aaz1776
2. Ongen H, Buil A, Brown AA, Dermitzakis ET, Delaneau O. Fast and efficient QTL mapper for thousands of molecular phenotypes. Bioinformatics. 2016;32:1479-1485. doi: 10.1093/bioinformatics/btv722
3. Relton CL, Gaunt T, McArdle W, Ho K, Duggirala A, Shihab H, Woodward G, Lyttleton O, Evans DM, Reik W, et al. Data Resource Profile: Accessible Resource for Integrated Epigenomic Studies (ARIES). Int J Epidemiol. 2015;44:1181-1190. doi: 10.1093/ije/dyv072
4. Golding J, Pembrey M, Jones R, Team AS. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol. 2001;15:74-87. doi: 10.1046/j.1365-3016.2001.00325.x
5. Cole MB, Risso D, Wagner A, DeTomaso D, Ngai J, Purdom E, Dudoit S, Yosef N. Performance Assessment and Selection of Normalization Procedures for Single-Cell RNA-Seq. Cell Syst. 2019;8:315-328 e318. doi: 10.1016/j.cels.2019.03.010
6. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7. doi: 10.7554/eLife.34408
7. Sanderson E, Glymour MM, Holmes MV, Kang H, Morrison J, Munafo MR, Palmer T, Schooling CM, Wallace C, Zhao Q, et al. Mendelian randomization. Nat Rev Methods Primers. 2022;2. doi: 10.1038/s43586-021-00092-5

8. Zeng P, Zhao Y, Qian C, Zhang L, Zhang R, Gou J, Liu J, Liu L, Chen F. Statistical analysis for genome-wide association study. J Biomed Res. 2015;29:285-297. doi: 10.7555/JBR.29.20140007

9. Brumpton B, Sanderson E, Heilbron K, Hartwig FP, Harrison S, Vie GA, Cho Y, Howe LD, Hughes A, Boomsma DI, et al. Avoiding dynastic, assortative mating, and population stratification biases in Mendelian randomization through within-family analyses. Nat Commun. 2020;11:3519. doi: 10.1038/s41467-020-17117-4

10. Walker VM, Zheng J, Gaunt TR, Smith GD. Phenotypic Causal Inference Using Genome-Wide Association Study Data: Mendelian Randomization and Beyond. Annu Rev Biomed Data Sci. 2022;5:1-17. doi: 10.1146/annurev-biodatasci-122120-024910

11. Glymour MM, Tchetgen Tchetgen EJ, Robins JM. Credible Mendelian randomization studies: approaches for evaluating the instrumental variable assumptions. Am J Epidemiol. 2012;175:332-339. doi: 10.1093/aje/kwr323