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NEW PERSPECTIVES IN COMPUTED TOMOGRAPHY BASED CARDIOVASCULAR RISK ASSESSMENT

PhD thesis

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Abbreviations

A: Additive genetic effect

ACC: American College of Cardiology

AHA: American Heart Association

BMI: Body Mass Index

BUDAPEST-GLOBAL: Burden of Atherosclerotic Plaques Study in Twins - Genetic Loci and the Burden of Atherosclerotic Lesions

C: Common environmental factors

CAC: Coronary Artery Calcium

CAD: Coronary Artery Disease

CI: Confidence Interval

CT: Computed Tomography

CTA: Computed Tomography Angiography

CTLA-4: Cytotoxic T Lymphocyte-Associated Protein 4

CVD: Cardiovascular Disease

DZ: Dizygotic

E: Unique environmental factors

HU: Hounsfield Unit

HR: Hazard Ratio

ICI: Immune Checkpoint Inhibitor

IQR: Interquartile Range

LAD: Left Anterior Descending

LCx: Left Circumflex

LM: Left Main

MZ: Monozygotic

NAFLD: Nonalcoholic Fatty Liver Disease

NAFPD: Nonalcoholic Fatty Pancreas Disease

OM1: Obtuse Marginal

PD-1: Programmed Cell Death - 1

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PDA: Posterior Descending Artery

PDL-1: Programmed Cell Death Ligand - 1

PLB: Posterolateral Branch

PROCAM: Prospective Cardiovascular Munster

RCA: Right Coronary Artery

SCORE: European Systematic Coronary Risk Evaluation

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1 Introduction

Cardiovascular disease (CVD) and cancer continue to be the leading cause of death in the developed countries.(1) In Europe, across member states of the European Society of Cardiology, CVD accounted for over 45% of all deaths.(2) Annually 3.8 million casualty, followed by cancer, as the second most common cause; responsible for 1.9 million exits per year.(2) Cancer and CVD commonly co-exist, and these two conditions share many cumulative risk factors, including tobacco use, physical inactivity, obesity, poor diet, hypertension, diabetes, and dyslipidemia.(1, 3, 4) In 2021, with more cancer survivors than ever before, the overlap of cancer survivors and patients with CVD is on the rise.(5) Approximately 26.1 million cancer survivors are predicted to be alive in 2040 only within the United States.(6) In addition, cancer survivors are at increased CVD risk not only due to the overlap of risk factors but also due to the wide range of received cardiotoxic cancer therapies.(7) As cancer survivors live longer, modifiable risk factors that affect both cancer and CVD have to gain more attention.(6, 8) Prevention strategies are classified as primary and secondary; however due to the overlap of CVD and cancer, a more holistic view has been suggested dividing preventative measures into “prevention at the population level” and “prevention in subjects with high CVD risk”.(1, 9) Identifying those at risk for CVD is at utmost importance to target effective dietary, lifestyle, and drug interventions. Whether the presence of a risk factor is determined by genetic or environmental factors, is modifiable or not modifiable, has a significant part in finding the appropriate preventative approaches.

1.1 Risk assessment in cardiovascular diseases

Traditional cardiovascular risk factors have been combined to estimate an individual’s risk for having a CVD event.(10) CVD risk scores allow clinicians to combine information from several CVD risk factors and quantitatively estimate an individual’s absolute risk for having a CVD event during a defined period of time.(10) CVD risk assessment is crucial to many treatment guidelines and may also help individuals to modify their lifestyle and improve adherence to medications.(11) Several risk prediction models have been developed to identify those asymptomatic subjects at higher CVD risk. However, the

accuracy of these risk prediction models is not perfect, and neither is their generalizability.(11, 12) The most commonly used cardiovascular risk assessment scores are the Framingham, Prospective Cardiovascular Munster (PROCAM), European Systematic Coronary Risk Evaluation (SCORE) and the American College of Cardiology (ACC)/American Heart Association (AHA) 2013 Pooled Cohort risk equations.(13-16) These models have been developed from a small set of the population and may work differently in various risk groups or populations.(11) These risk prediction scores and systems cannot identify one-third of the future cardiovascular-related deaths; they overscore the high-risk patients and underscore the low-risk patients.(12) Therefore, a more precise and personalized risk prediction system is needed. These risk prediction systems could be improved by adding nontraditional risk factors such as more metabolic, anthropometric, imaging-based or genetic data.(17)

1.2 Coronary plaque assessment with Computed Tomography

Coronary Computed Tomography Angiography (CTA) is a robust diagnostic tool suitable for the non-invasive visualization of coronary vessels and quantification of coronary artery plaques.(18) Coronary CTA, with its ability to characterize and quantify features of both individual plaques and overall coronary atherosclerosis, has emerged as an important diagnostic modality in patients with chronic coronary syndrome.(19) Several approaches are used to quantify the overall extent of CAD on both non-contrast-enhanced coronary CT images and coronary CTA. Visualization of calcified plaques is possible on non-contrast-enhanced coronary Computed Tomography (CT) images, and both calcified and non-calcified coronary plaques can be visualized on coronary CTA studies.(20) With qualitative plaque assessment using coronary CTA, coronary plaques can be classified based on calcium content as non-calcified, partially calcified, or calcified plaques (***Figure 1***). Detailed assessment of the extent, location, severity, and features of coronary atherosclerosis has prognostic value in patients with chest pain.(21)

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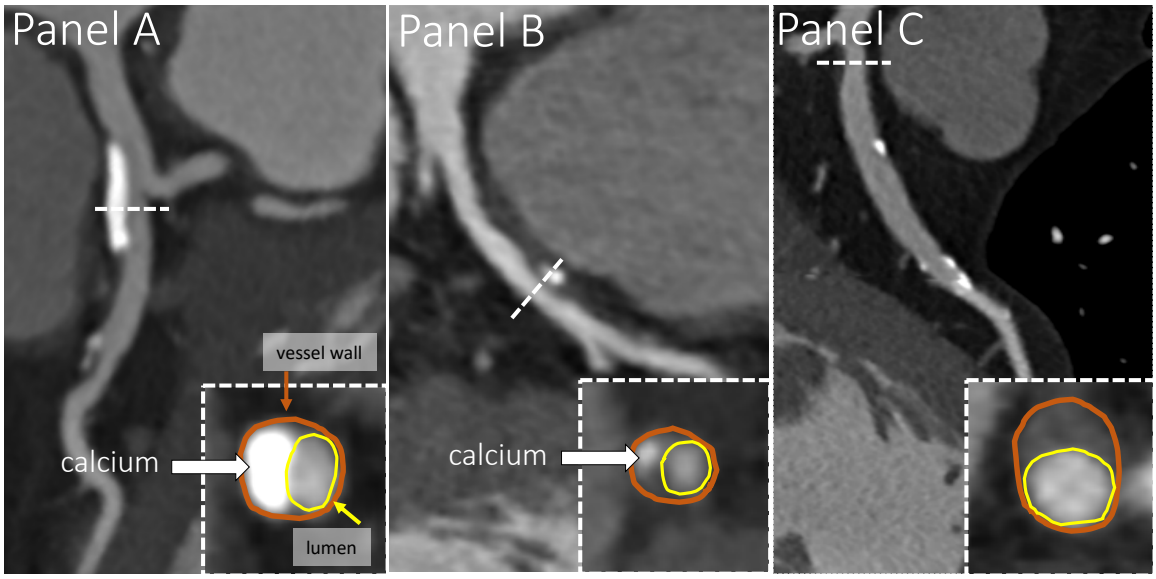

Figure 1 *Coronary CTA images showing curved multiplanar views of coronary arteries. Panel A demonstrates a calcified plaque. Panel B shows a partially calcified plaque, and Panel C shows a non-calcified coronary plaque. Orange contours follow the vessel wall and yellow contours show the vessel lumen contour. (Own figure)*

Coronary artery calcium (CAC) score is an imaging-based marker of coronary artery disease (CAD). Ca-scoring is a well-established method for quantifying calcified coronary plaques. Ca-score is usually quantified on ECG gated non-contrast-enhanced coronary CT studies using the Agatston method.(22) This method quantifies the pixel areas with greater than 130 Hounsfield Units (HU) within the coronary arteries, which are then weighted based on density and volume. CAC score has been shown to be significantly associated with morbidity and mortality.(23, 24) Incorporating CAC data into risk prediction models have improved risk prediction and reclassified individuals towards higher or lower risk for CVD events.(17, 24) Ca-score correlates with overall coronary atherosclerotic plaque burden, therefore it can be used as a surrogate marker of overall CAD. Ca-scoring provides additional value in predicting mortality in asymptomatic patients and is also linked to cardiovascular events. (25, 26)

Calcified plaques are less prone to cause events as compared to non-calcified plaques. Coronary plaques with high-risk plaque features, prone to rupture, are usually partially or

non-calcified plaques.(20, 27) Assessment of high-risk plaque features such as positive remodeling, spotty calcification, napkin-ring sign, or low attenuation is only feasible on contrast-enhanced images.(20) Since the above coronary plaque features carry a higher risk for plaque rupture, the assessment of high-risk plaque features is strongly encouraged by current guidelines.(28, 29)

A generally accepted hypothesis suggests that non-calcified plaques represent an earlier stage in atherosclerotic plaque development, and calcification may only occur in later stages.(20, 27) The detailed genetic background of CAD is unknown. CAD is considered to be a multifactorial disease influenced by the interplay of several environmental and genetic factors.(30) Heritability of CAD has been estimated to be 40-60%, suggesting that genetics play a vital role in its development.(31) Non-contrast CT-based CAC assessment has shown a substantial genetic component, ranging from 30% to 45%.(32-36) A robust familial aggregation has also been observed regarding non-calcified plaques; however it has only been addressed in a handful of family studies.(37-39) Subjects with a family history of early-onset CAD have a higher prevalence of subclinical coronary atherosclerosis, composed primarily of non-calcified plaques.(37) Non-calcified plaques are also more prevalent in younger patients with a family history of CAD, compared to patients with no family history of CAD or even compared to symptomatic patients.(38, 39)

1.2.1 CTA-based plaque quantification

Coronary CTA-based plaque assessment has an important role in cardiovascular risk estimation. Coronary CTA data sets with submillimetre isotropic spatial resolution and attenuation-based tissue characterization carry the potential to quantify total coronary atherosclerotic plaque volume and its components, separated based on HU units (***Figure 2***).(20)

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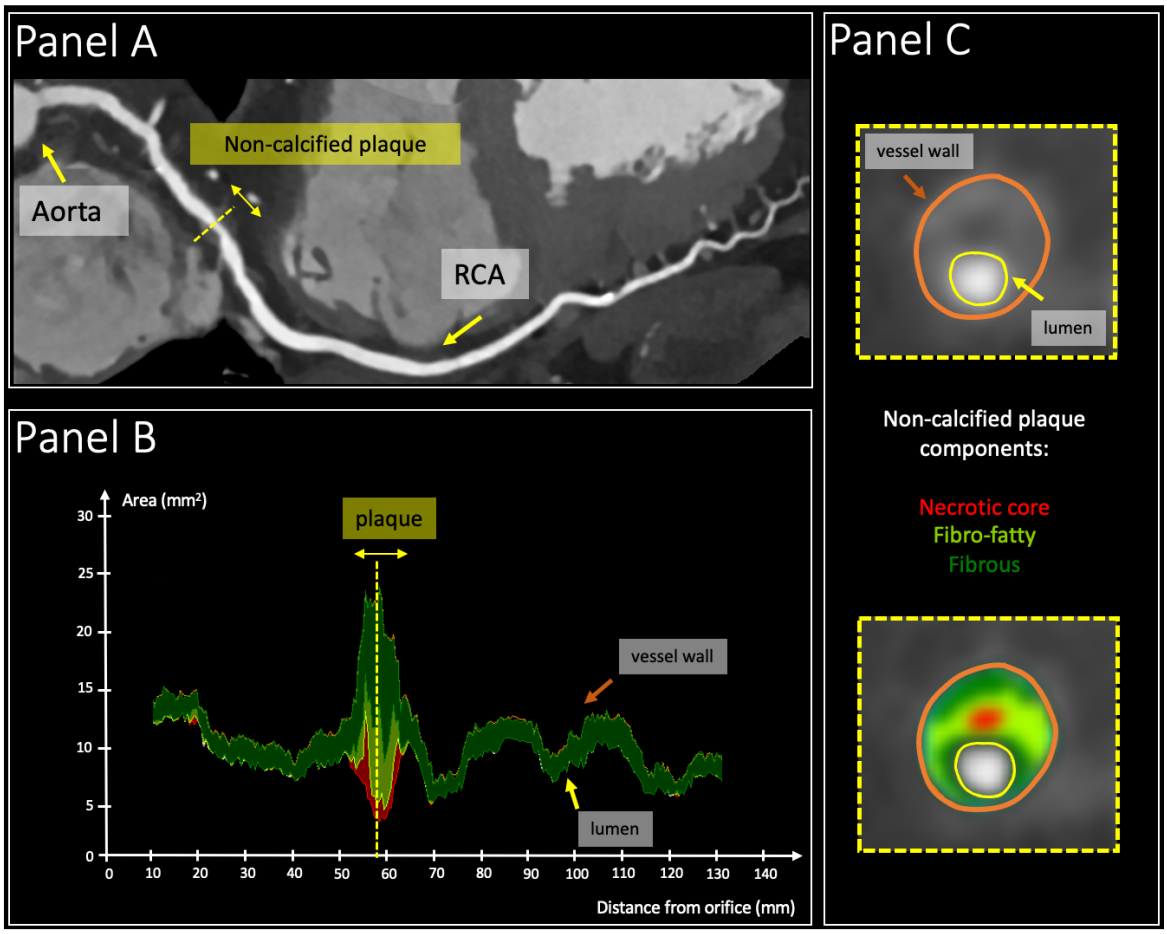

Figure 2 *Demonstrative figure on coronary plaque quantification. Panel A shows a curved multiplanar reconstruction of the right coronary artery with an atherosclerotic plaque at the mid-segment. Panel B shows the corresponding vessel wall and lumen graphs. Panel C shows a cross-sectional image of the plaque with and without color-coding the plaque components. (Own figure)*

Automated software tools are available to quantify and assess coronary plaques. The reproducibility of automated plaque quantification has shown to be excellent, and accuracy has also been validated against greyscale intravascular ultrasound and virtual histology intravascular ultrasound.(40) Volumetric analyses provide the volume of plaque components and have additional clinical value since the two-dimensional analysis based on mean HU does not inform about the extent of the specific component.(41) The usual processing time ranges between 20 and 45 minutes for a complete coronary tree analysis, depending on image

quality and the extent of the disease. For both clinical and research purposes, a complete coronary tree plaque quantification includes the main branches (left main [LM], left anterior descending [LAD], left circumflex [LCx], obtuse marginal [OM1], right coronary artery [RCA], posterolateral branch [PLB] and posterior descending artery [PDA]) with a ≥ 2 mm diameter. After the software auto-contours the vessel wall and lumen, these tracings need to be checked and manually corrected when necessary. Volumes are reported in mm^3 based on the pre-defined HU thresholds. The software calculates plaque volumes of different plaque components based on HU thresholds. Non-calcified plaque components are usually defined as between (-100 and 350 HU), and calcified components are defined as between (351 and 2000 HU). Based on HU, non-calcified plaque components can be further separated into fibrous, fibro-fatty, or necrotic core components (***Figure 2***). Results can be exported as an excel file based on plaques, coronary segments, vessels, or total plaque volumes.

1.3 Ectopic fat depots and cardiovascular diseases

Obesity is a well-known traditional cardiovascular risk factor. The prevalence of obesity has doubled between 1980 and 2008.(42) Obesity, similarly to CAD, is a heterogeneous disease; both genetics, and environmental factors play a significant role in its development. The most commonly used anthropometric measure to diagnose obesity is Body Mass Index (BMI). BMI is derived from height and weight, and obesity is defined as a BMI greater than 30 kg/m^2. However, BMI does not correlate well with the location of fat depots.(43) Several studies have shown that people with the same BMI may have different CVD risk.(44) Part of this phenomenon is caused by the so-called ectopic fat depots. In addition to overall obesity, ectopic fat has been found to contribute to CVD, therefore treated as a nontraditional risk factor.(45)

Ectopic fat is fat accumulation in cells that are histologically not adipose tissue cells; triglyceride accumulates in cells in which generally there is minimal triglyceride content and usually occurs in or around specific organs or body compartments.(46, 47) Ectopic fat depots create an atherogenic environment through both local and systemic effects, inducing inflammation as one of the main effects.(48) The Framingham Heart Study has shown that irrespective of the subcutaneous fat volume, ectopic fat depots carry a high CVD risk.(49)

Incorporating ectopic fat depots into the CVD risk prediction models could improve their accuracy.(50) These ectopic fat depots can be visualized and quantified using CT imaging.(43)

Fat accumulation in the liver and the pancreas are both considered ectopic fat depots. Fat content in the liver and the pancreas are visualized on chest and abdominal CT examinations and can be quantified on non-contrast-enhanced images.

1.3.1 Nonalcoholic fatty liver disease

Hepatic lipid accumulation in the absence of significant alcohol intake (<20g/d), viral infections, toxins, autoimmune disease, or congenital metabolic syndromes is termed nonalcoholic fatty liver disease (NAFLD).(51) In recent years, NAFLD has become increasingly prevalent, affecting about 25% of adult people worldwide and carrying severe vascular and hepatic outcomes.(52-54) NAFLD carries a poor long-term hepatic prognosis and increases cardiovascular morbidity and mortality.(55) NAFLD is often associated with known cardiovascular risk factors, and a high-risk metabolic profile usually characterizes subjects with NAFLD.(56, 57) In the Framingham Heart Study, hepatic lipid accumulation was associated with several cardiovascular risk factors even after adjustment for other fat depots.(57, 58) Clinical studies found that NAFLD is an independent predictor of cardiovascular events, both in patients with type 2 diabetes and non-diabetic subjects.(59-61)

The pathomechanisms responsible for the relationship between NAFLD and cardiovascular risk are still not fully understood. The liver plays a vital role in glucose and lipid metabolism.(62) NAFLD may lead to hepatic insulin resistance, resulting in increased very-low-density lipoprotein production.(63) NAFLD may also induce a pro-inflammatory and pro-atherogenic state by producing inflammatory, hemostatic, and oxidative stress cytokines and mediators.(53) Data about the role of genetic and environmental factors in hepatic lipid accumulation are limited.

1.3.2 Nonalcoholic fatty pancreas disease

Pancreatic lipid accumulation in the absence of significant alcohol intake (<20g/d), viral infections, toxins, or congenital metabolic syndromes is termed nonalcoholic fatty

pancreas disease (NAFPD).(51) The prevalence of NAFPD is estimated to be about 35% in adults.(64) The relationship between obesity and NAFPD has been first demonstrated in 1926.(65) However, the clinical significance of this observation remained unknown for a long time. NAFPD has gained much scientific interest in the last few years. Recently, research has highlighted the association between NAFPD and altered glucose metabolism, which may also contribute to cardiovascular risk.(66) An increasing number of publications have shown the association between NAFPD and increasing age, BMI, metabolic syndrome, cardiovascular and cerebrovascular diseases.(67, 68)

Similarly to hepatic lipid accumulation, the mechanism responsible for the relationship between pancreatic lipid accumulation and cardiovascular risk is still not fully understood. Moreover, data about the role of genetic and environmental factors in pancreatic lipid accumulation are limited.

1.3.3 Assessment of hepatic and pancreatic lipid accumulation

In the clinical setting, various imaging modalities, including ultrasonography, CT, and magnetic resonance imaging, have been used to investigate and quantify hepatic and pancreatic lipid accumulations.(69-71) These measurement methodologies differ in availability, cost, radiation dose, and reproducibility.(72, 73)

Currently, CT-imaging for the measurement of ectopic lipid accumulation has become popular due to its short acquisition time, availability, and widespread clinical use. A variety of CT-based techniques have been applied in the literature, including calculating hepatic and pancreatic attenuations on unenhanced images using three regions of interest placed on the pancreatic head, body, and tail or, in case of hepatic lipid quantification, placed on three different hepatic segments avoiding vascular structures (***Figure 3***).(74, 75)

Hepatic and pancreatic steatosis present with decreased attenuation values of the parenchyma on non-enhanced CT-images.(75, 76) Studies have documented that CT attenuation absolute values and indices can be used to quantify pancreatic and liver fat volumes and the attenuation values were validated by histological measurements.(74, 76)

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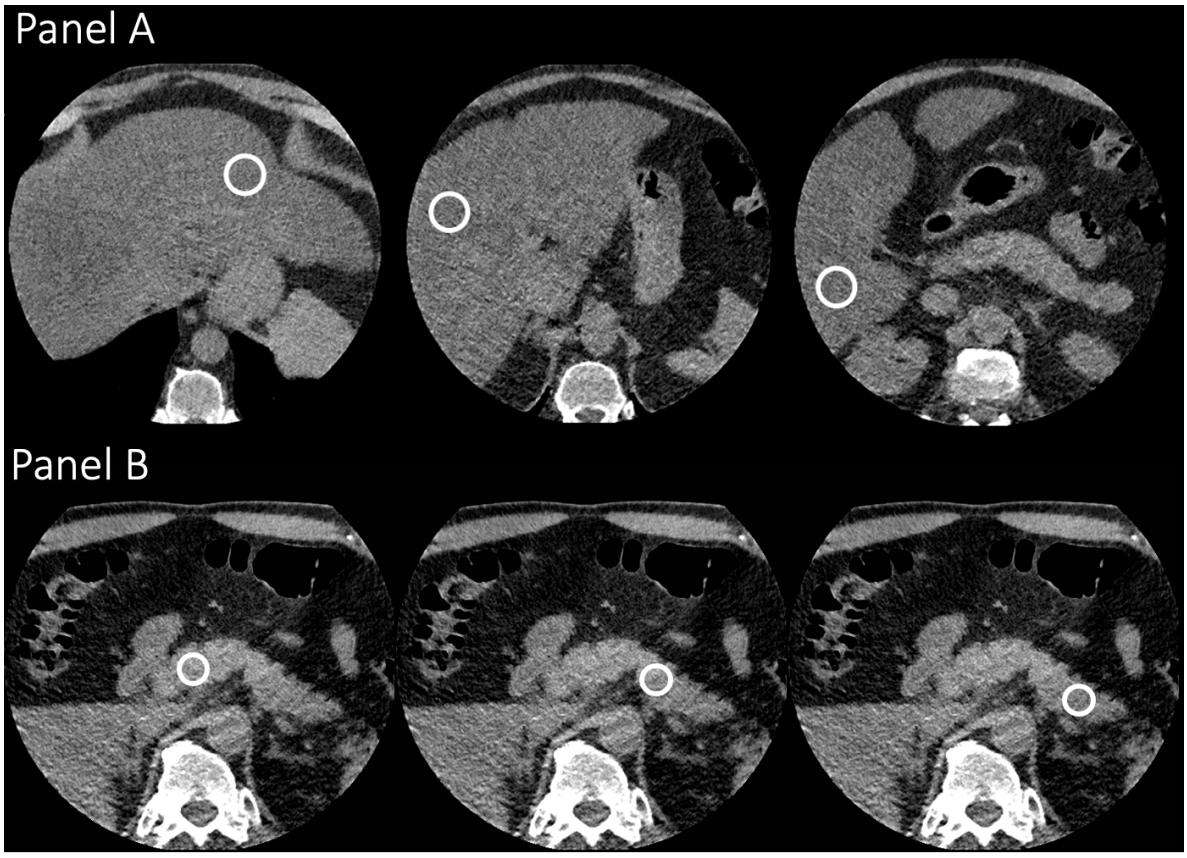

Figure 3 *Measurement of hepatic (Panel A) and pancreatic (Panel B) attenuations on non-enhanced computed tomography images. White circles represent the region of interest, where attenuation is measured. (77) (Own figure)*

1.4 The role of twin studies in risk assessment

Family studies and twin studies have been an excellent starting point for decades to estimate the importance of genetic and environmental backgrounds on a complex trait. Family studies can estimate familial aggregation of a disease; however familial aggregation does not equal genetic contribution since the potential to distinguish between common, shared environmental, and genetic factors is limited in this design.(78) Twin studies can overcome this issue since twins, with their precisely matching age, represent a unique cohort among family studies.(78) Twin siblings also share a wide range of environmental and socioeconomic variables that may influence the expression of complex traits.(79) These unique characteristics of twin studies provide a powerful tool, which has been used for

decades to estimate the degree of genetic and environmental influences on complex traits. (79, 80)

In a classical twin study design, the phenotypic variation between monozygotic (MZ) and dizygotic (DZ) twin pairs is compared. More precisely, the covariance between MZ twins is compared to the covariance between DZ twins.(81, 82) Classical twin studies demonstrate that MZ siblings share almost 100% of their segregating genes, in contrast to DZ pairs, who share on average 50% of their segregating genes. Moreover, both groups share 100% of the common environmental (e.g., parenting, early lifestyle diet) and 0% of the non-shared, unique environmental factors (e.g., unique experiences at school).(81, 83, 84) A similarly high correlation between MZ and DZ twins provides evidence of shared environmental effects and a negative correlation is suggestive of non-shared, unique environmental effects. The stronger correlation among MZ twins in comparison to DZ twins is suggestive of genetic effects.(81)

Based on these twin study principles, Falconer-based broad sense heritability can be calculated, and genetic structural equation models can be built to quantify the proportion of genetic and environmental factors contributing to any phenotype in question.(82) These genetic structural equation models provide the ability to decompose the variation between the twins, which was modeled to be caused by genetic factors, shared environmental factors, and non-shared environmental factors.(81)

1.5 Cancer therapies and cardiovascular diseases

Cancer treatments have evolved over the past thirty years, significantly improving patients' outcomes and increasing the number and nature of treatment-related cardiovascular toxicities.(7) In 2021, with more cancer survivors than previously, the overlap of cancer survivors and patients with CVD is growing.(5) Traditional CVD risk prediction models are not only inaccurate in a general population but also in cancer survivors who are at increased CVD risk due to the wide range of received cardiotoxic cancer therapies.

This increasing cluster led to a new sub-specialty called cardio-oncology. Optimizing cancer therapy at the lowest cardiovascular risk is the most essential goal of cardio-

oncology.(85) Many aspects of treatment-related cardiovascular toxicities and consequences are still unknown. Moreover, in CVD risk scores, cancer therapies are usually not included. Therefore, using traditional risk scores to predict the long-term consequences of cancer treatment-associated cardiovascular side effects could lead to an underdiagnosis of excess CVD risk. This inaccuracy may result in the failure to prevent adverse events or an inappropriate interruption of a potential lifesaving cancer treatment.(7)

1.5.1 Immune checkpoint inhibitors

In recent years, immunotherapy evolved as the fifth pillar of cancer care and has revolutionized cancer treatment.(86) Immune checkpoint inhibitors (ICI) are a type of immunotherapy. Immune checkpoints are negative regulators of immune activation. They play a key role in maintaining immune homeostasis and preventing autoimmunity.(87) Immune checkpoints can also limit the immune system's antitumor response. ICIs release these negative regulators and leverage the immune system to identify and target cancer cells. This can be achieved by antibodies blocking two main T-cell pathways; the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or the programmed cell death 1 (PD-1) pathway.(86) In 2011, ipilimumab, the first antibody blocking CTLA-4, was authorized, rapidly followed by the development of monoclonal antibodies targeting PD1 (pembrolizumab and nivolumab) and programmed cell death ligand 1 (PDL-1) (atezolizumab and durvalumab).(88) The use of ICI has resulted in long-lasting tumor responses in patients with various cancers, and their use is rapidly expanding. For example, in 2014, ICIs were approved for three cancer indications.(89) By 2020, ICIs are used as single agents or in combination with chemotherapies as first or second-line therapies in more than 50 cancer types.(88) Moreover, the percentage of patients with cancer eligible for an ICI has increased from 1.5% in 2011 to greater than 36%.(90) The benefit of ICIs has expanded to the adjuvant setting in some malignancies. The number of active ongoing clinical trials testing the effect of ICI in combination with traditional cancer therapies is estimated to be more than 4,400 as of September 2019. (91, 92)

It is well known that inflammatory pathways drive atherogenesis. The role of inflammation and immune modulation in CVD is well established.(93) Immune cells are an

important part of the atheroma, and animal and cellular studies have demonstrated that these immune checkpoints are critical negative regulators of atherosclerosis.(94, 95) Whether inhibiting the CTLA-4 and PD-1 pathways in atherosclerosis leads to an increase in atherosclerotic plaque and atherosclerosis-related cardiovascular events is unclear.(96, 97)

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2 Objectives

2.1 To assess the heritability of coronary plaque volumes and ectopic fat depots

1) To investigate the magnitude of genetic and environmental impact on calcified and non-calcified coronary plaque volumes.
2) To evaluate the magnitude of the genetic and environmental impact on hepatic lipid accumulation.
3) To evaluate the magnitude of the genetic and environmental impact on pancreatic lipid accumulation.

2.2 To assess the degree of cardiotoxic effects of cancer immunotherapy

1) To test whether ICIs are associated with an increase in atherosclerotic plaque progression in an imaging study.
2) To test whether the use of ICIs leads to an increase in cardiovascular events in a matched cohort study.

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3 Patients and Methods

Methods not described in associated co-authored or first-authored publications are detailed in this section. Methods already published with my authorship are briefly described or excluded from this thesis, following the guidance of the Doctoral School.

3.1 BUDAPEST Twin Study

A prospective, single-center, classical twin study was conducted under the name of BUDAPEST-GLOBAL (Burden of Atherosclerotic Plaques Study in Twins - Genetic Loci and the Burden of Atherosclerotic Lesions) study; participants had been co-enrolled within the large, international, multicenter GLOBAL clinical study (http//:www.ClinicalTrials.gov: NCT01738828).(98) The study was approved by the National Scientific and Ethics Committee (institutional review board number: ETT TUKEB 58401/2012/EKU [828/PI/12], Amendment-1: 12292/2013/EKU [165/2013] and all subjects provided written informed consent.

3.1.1 Study designs and study populations

Detailed study description and enrollment criteria were published previously.(77, 99) The final cohort for the coronary plaque analysis included 196 twin subjects (98 twin pairs; 60 MZ and 38 DZ same-gender twin pairs), with three twin pairs excluded due to insufficient image quality.

3.1.2 Data collection, image acquisition and analysis

A detailed CT scanning protocol was previously published.(77, 99) A detailed description of image analysis was previously published for the hepatic and pancreatic measurements.(77, 99)

For coronary plaque assessment, a quantitative CT analysis was performed using a dedicated software tool (QAngio CT; Medis BV, Leiden, The Netherlands). Coronary atherosclerotic plaques were defined as any visible structure in at least two independent planes which had a CT density below the contrast-enhanced coronary lumen but above the

surrounding connective tissue. The software calculated plaque volumes for different plaque components. Non-calcified components were defined based on HU thresholds of (-100–350 HU), and calcified components were defined as (351–2000 HU). For each sibling, the coronary segments were co-registered in three steps.

The vessel and lumen border segmentation was performed for the main epicardial vessels and for the side branches, with ≥2 mm in luminal diameter. Both the vessel wall and luminal tracings were checked and manually corrected, if necessary, for all the vessels in each patient. Coronary plaques were marked manually by their proximal and distal slices using multiplanar coronary CTA images. As the last step, twin-based co-registration was performed. The same length of coronary arteries needed to be analyzed for each sibling to reduce the healthy segments induced noise. Three scenarios were possible for the twin-based co-registration. 1) If none of the siblings had a coronary atherosclerotic plaque, then the proximal parts of the coronaries were analyzed for both siblings (80 mm for LM and LAD, RCA, and 50 mm from the LCx or for the OM1) (***Example 1 in Figure 4***). In case these vessels reached a minimum of 2 mm in luminal diameter more proximal than the pre-specified lengths as described above, arterial segments from the twin pairs were co-registered based on the shortest vessel length. 2) In case one of the twins had plaques but the other did not, then the plaques were matched in location (using the distance from the orifice or bifurcation). Same length segments in the same location were analyzed for the healthy sibling as for the diseased one (***Example 2 in Figure 4***). 3) If both siblings had CAD, we matched all of their plaques in location and length (***Example 3 in Figure 4***).

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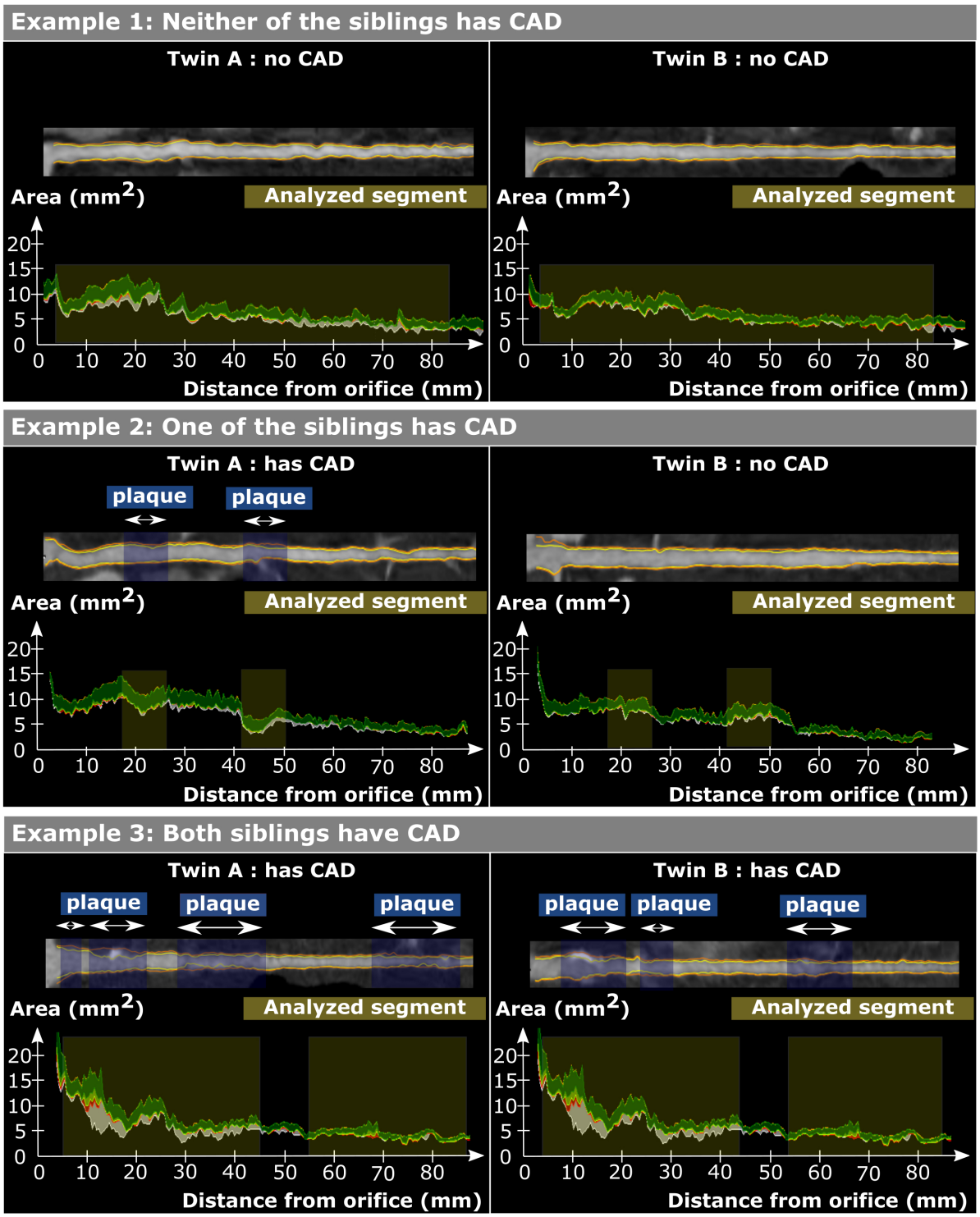

Figure 4 *Demonstration of the plaque analysis method. Stretched multiplanar reconstruction of the left anterior descending (LAD) artery for each subject and the quantification graph of the LAD. Yellow: analyzed segments. Blue: plaque. (Based on a manuscript under review.)*

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3.1.3 Statistical analysis

To assess the heritability of calcified plaque, non-calcified plaque volumes, hepatic, and pancreatic lipid accumulation, two types of heritability estimates were used. To assess the heritability of calcified plaque and non-calcified plaque volumes, the Falconer method for broad-sense heritability (H^2) was used. To assess the heritability of hepatic and pancreatic lipid accumulation, genetic structural equation models were used.

Therefore, the Falconer method for broad-sense heritability was used to assess the heritability of calcified plaque and non-calcified plaque volumes. This method is based on the correlation values between MZ (r_{MZ}) and DZ (r_{DZ}) twin pairs. Correlations were calculated using the Pearson correlation coefficient. H^2 was calculated as follows; $H^2 = 2 \times (r_{MZ} - r_{DZ})$.(82, 100). High within-pair correlation values in the MZ group, when associated with lower within-pair correlation values in the DZ group, confers high heritability. Therefore, if phenotypic similarities occur more often in the MZ twins than in the DZ twins, it indicates genetic influence on the trait. On the contrary, if co-twin analysis shows similar correlation values among the MZ and the DZ siblings, the influence on the trait is environmental (98).

A detailed description of the statistical analysis and the genetic structural equation models were previously published for the heritability of hepatic and pancreatic lipid accumulation.(77, 99) Briefly, using the structural equations model, it is possible to break down the variation between the twins. As mentioned in the Introduction, the variation between twins can be broken down to additive genetic factors (A), common environmental factors (C), and unique environmental factors (E), therefore the acronym “ACE model”.(81)

“A” represents genetic alleles whose effects are additive with regards to a given phenotype; in our study, hepatic and pancreatic lipid accumulation. “C” are circumstances shared by the twin pairs during their lifetime, such as same early childhood, education in the same school, living in the same town, sharing similar socioeconomic status even in adulthood, etc.(81) “E” are conditions to which only one of the siblings was exposed.(84) All calculations were adjusted for age and sex. Log likelihood-based 95% confidence intervals (CI) were calculated for all estimated parameters. All analyses were performed using R

version 3.5.2.(101) Twin modeling was performed using OpenMx version 2.12.2. A *P*-value of <0.05 was considered statistically significant.

3.2 Immune Checkpoint Inhibitor treated population

The Methods for this study have been previously published, and detailed information on the study design, study populations, data collection, image acquisition, and analysis can be found in the publication.(102) Briefly, to evaluate whether the use of ICI leads to an increase in atherosclerotic plaque progression, an imaging study was performed. Then, as a second step, we aimed to determine if the changes observed in the imaging study translate to clinical events or not; therefore a matched cohort study was performed.

In a retrospective database, all individuals treated with an ICI through the end of March 2019 at Massachusetts General Hospital, Boston, MA, USA, were included. Patients with melanoma and at least three CT scans in our system were included in the imaging study from this database All patients from this ICI database were included as cases for the matched cohort study, and historical controls were selected from all patients treated for cancer at the same center between January 1st 2008 and December 31st 2012. For this historical control group, the use of an ICI at any time point was an exclusion criterion. This resulted in a cohort of 8543 patients. From these, we selected controls in a 1:1 ratio matching cases for age, a history of cardiovascular events, and cancer type (***Figure 5***). The study entry for the controls was their first visit after January 1st, 2008. Covariates were derived from the Institutional Research Patient Data Registry. The Partners Human Research Committee approved the study, and no informed consent was required.

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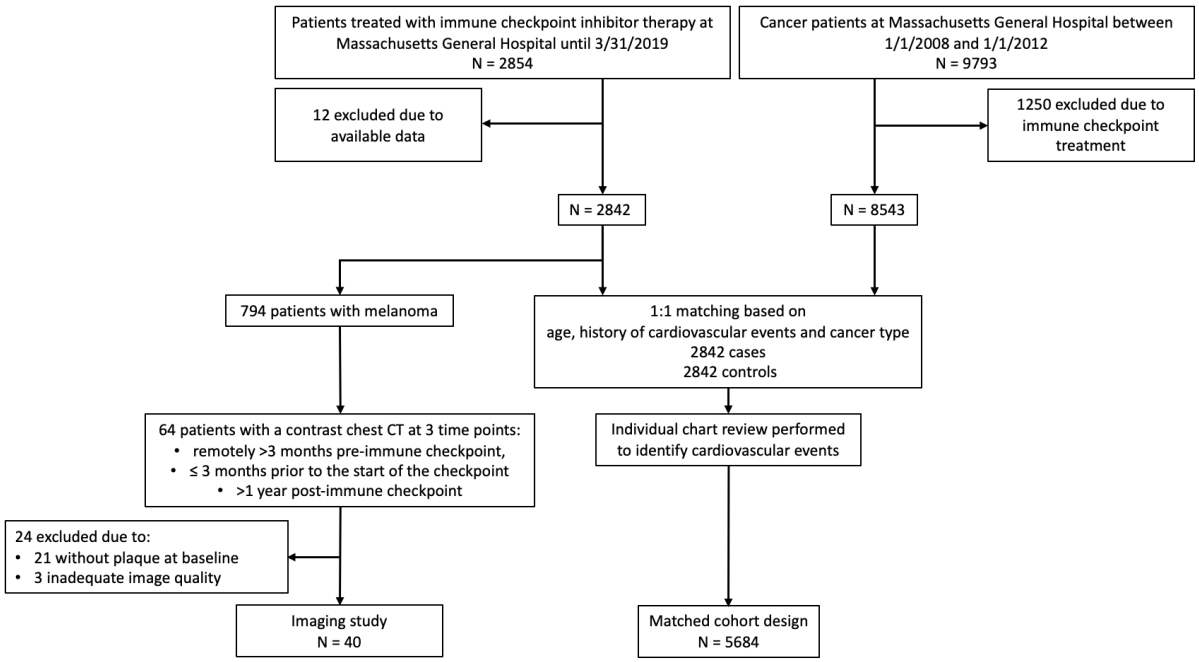

Figure 5 *Flowchart of patient selection for the immune checkpoint inhibitor-treated population and for the controls. The left side shows the inclusion for the imaging study, and the right side demonstrates the selection for the matched cohort design. (Modified based on previous publication.(102))*

In the imaging study, thoracic plaque volumes were measured over time on all three contrast-enhanced CT scans using a dedicated software (QAngioCT, version 3.1.4.2, Medis Medical Imaging Systems, Leiden, the Netherlands).(103) Plaque change was calculated as the difference in plaque volume measured on two consecutive scans (i.e., from scan one to scan two and from scan zero to scan one). Annualized plaque progression rate was computed as plaque change per year given in absolute and relative rates (mm^3 and %).

In the matched cohort study, the primary outcome was the occurrence of a cardiovascular event, defined as a composite of myocardial infarction, coronary revascularization, and ischemic stroke. These events were chart reviewed and blindly adjudicated. Cox proportional hazard regression analysis was performed to calculate hazard ratios (HR) with 95% CIs, counting only the first cardiovascular event. Further information on methods can be found in the publication.(102)

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4 Results

4.1 Results from the BUDAPEST Twin Study

Baseline demographics and clinical characteristics of the twin subjects are summarized in ***Table 1***, showing the different sample sizes in each sub-study. The study population represented a moderately overweight, middle-aged Caucasian population with a slight female predominance. The mean age in the coronary plaque study was 56 ± 9 years, similar to the hepatic and pancreatic lipid accumulation studies. More female subjects were analyzed in all three studies, reaching more than 63%. The most prevalent cardiovascular risk factor in the total cohort was hypertension (83/196, 42.3%, 75/182, 41.2% and 67/136, 49.3%). Both total cholesterol (5.56 ± 1.09 mmol/L) and LDL-cholesterol levels (3.49 ± 0.99 mmol/L) were slightly elevated among participants in all sub-studies.

Table 1 *Baseline demographics and laboratory parameters for the twin subjects.*

Parameters	**Coronary plaque study n = 196**	**Hepatic lipid study n = 182**	**Pancreatic lipid study n = 136**
Demographics and clinical data			
Female, n (%)	124 (63.3)	120 (65.9)	88 (64.7)
Age, mean ± SD, y	56 ± 9	56 ± 10.0	58 ± 9
Body mass index, mean ± SD, kg/m^{-2}	27.7 ± 5.1	27.5 ± 5.0	28.0 ± 4.4
Hypertension, n (%)	83 (42.3)	75 (41.2)	67 (49.3)
Diabetes mellitus, n (%)	17 (8.7)	16 (8.8)	11 (8.0)
Current smoker, n (%)	31 (15.8)	31 (17.0)	21 (15.4)
Laboratory parameters			
Fasting blood glucose, mean ± SD, mmol/L	5.34 ± 1.29	5.31 ± 1.27	5.23 ± 0.87
Serum total cholesterol, mean ± SD, mmol/L	5.56 ± 1.09	5.54 ± 1.08	5.57 ± 1.10
Serum LDL-cholesterol, mean ± SD, mmol/L	3.49 ± 0.99	3.47 ± 0.99	3.56 ± 0.49

Serum HDL-cholesterol, mean ± SD, mmol/L	1.61 ± 0.39	1.60±0.38	1.59 ± 0.35
Triglycerides, mean ± SD, mmol/L	137.7 ± 91.2	1.57±1.06	134.5±74.0

4.1.1 Coronary plaque volumes

A total of 196 twin subjects had adequate image quality to participate in the coronary plaque sub-study. Subjects in the MZ group were older than the DZ group (54 ± 10 vs. 58 ± 8 years, P = 0.005). 16.8% (33/196) of the participants were on primary preventive statin therapy. The 10-year Atherosclerotic Cardiovascular Disease Risk Score was 7.9 ± 7.7 for the total cohort. Significant differences were observed in the Haemoglobin A1C levels between the MZ and DZ groups (MZ: 5.6 ± 1.0 % vs. DZ: 5.3 ± 0.8 %, P = 0.01). Otherwise, there were no significant differences between the groups.

Of the 196 twins, 102 (52.0 %) had coronary plaques, 42 were DZ, and 60 were MZ. The prevalence of any CAD was not different among the groups. (DZ: 55.3% [42/76] vs MZ: 50.0% [60/120] group P=0.56). The prevalence of discordant twin pairs, meaning one sibling had CAD the other did not was similar among the groups (DZ: 26.3% [20/76] vs. MZ: 20% [24/120] P=0.38). The prevalence of concordant twin pairs with no CAD was also similar among the groups (DZ: 31.6% [24/76] vs. MZ: 40% [48/120] P=0.29). The prevalence of concordant twin pairs with CAD was not different among the groups (DZ: 42.1% [32/76] vs. MZ: 40% [48/120], P=0.88). Comparing those MZ and DZ twins who had CAD, we found no differences regarding non-calcified plaque volume (DZ: 107 [52 - 178] mm3 vs MZ: 79 [36 - 175] mm3; P=0.5) and calcified plaque volume (DZ: 43 [7 - 65] mm3 vs MZ: 18 [5 - 84] mm3, P=0.4).

Calcified plaque volume showed a strong correlation between both the MZ and DZ twin pairs with an r_{MZ} of 0.96 and an r_{DZ} of 0.87. However, non-calcified plaque volume showed stronger correlations between the MZ as compared to the DZ twins (r_{MZ}=0.73 and r_{DZ}=0.44). Based on these correlation values, the broad heritability of calcified plaque volume was moderate (H^2=0.59), whereas the heritability of non-calcified plaque volume was found to be weak, yielding an H^2 of 0.17 (***Table 2***).

Table 2 *Correlation values and Falconer-based heritability index of non-calcified and calcified plaque in 196 twins.*

	r_{MZ}	r_{DZ}	H^2
Parameteres			
Non-calcified plaque volume	0.96	0.87	0.17
Calcified plaque volume	0.73	0.44	0.59

4.1.2 Hepatic and pancreatic lipid accumulation

A total of 182 twin subjects had sufficient data to measure hepatic lipid accumulation, and 136 twin subjects had sufficient data to measure pancreatic lipid accumulation. Baseline characteristics for both cohorts are shown in ***Table 1***. There was no significant difference between MZ and DZ twin subjects regarding hepatic CT attenuation (57.9 ± 12.6 HU and 59.3 ± 11.7 HU, $P = 0.75$) or regarding pancreatic CT attenuation (48.9 ± 11.9 HU and 49.0 ± 13.0 HU, $P = 0.93$). Using the structural equation model, the best fitting models were the AE models both for the hepatic and pancreatic lipid accumulation. A greater unique environmental influence (E: 62% [95% CI 15-58%]) and a moderate additive genetic dependence (A: 38% [95% CI 5-58%]) was found for hepatic lipid accumulation. Similarly, for pancreatic lipid accumulation, a greater unique environmental influence (E: 54% [95% CI 19-66%]) and a moderate additive genetic dependence (A: 46% [95% CI 34-81%]) was found.

4.2 Results from the Immune Checkpoint Inhibitor treated population

4.2.1 Aortic plaque progression after Immune Checkpoint Inhibitor therapy

In the imaging study of melanoma patients receiving ICI therapy, the mean age was 67 ± 7 years, with 55% male subjects. Patients received ICI monotherapy in most of the cases (87.5%), and the median number of ICI cycles received was 8.5 (4.5–23.5). The clinical characteristics of the patients in the imaging study remained constant over time and, apart from cancer type, were not different from the matched cohort (***Table 3***).

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Table 3 *Comparison between baseline and follow-up characteristics of the patients with melanoma included in the CT study.*

	At scan 0	**At scan 1**
Cardiovascular risk factors – no. (%)		
Hypertension	47.5%	52.5%
Diabetes	7.5%	10.0%
Never smoker	52.5%	55.0%
History of myocardial infarction	7.5%	10.0%
History of coronary revascularization	10.0%	12.5%
Cardiovascular medications – no (%)		
Aspirin	17.5%	27.5%
Statin	42.5%	42.5%

There was an increase in the total plaque volume over the duration of the three CT scans (median and interquartile range [IQR] total plaque volume at baseline 1438 [703–2690] mm^3 to 1567 [703–2676] mm^3 at ICI start to 2183 [923–4150] mm^3 during follow up). Similarly, there was an increase in the non-calcified plaque volume over the duration of the three scans (median [IQR] non-calcified plaque volume at baseline 1285 [643–2193] mm^3 to 1130 [592–1986] mm^3 at ICI start to 1725 [733–3584] mm^3 during follow up). The progression rate, adjusted for the study interval, was greater in the period after ICI as compared with prior, for both total (P=0.02) and non-calcified plaque (P = 0.02, ***Table 4***). Specifically, the rate of total plaque volume progression increased 3-fold from 2.1% per year pre- to 6.7% per year post-ICI.

Table 4 *Absolute and relative change in thoracic atherosclerotic plaque volume from before starting an immune checkpoint inhibitor (Baseline - ICI start) to after starting an immune checkpoint inhibitor (ICI start - follow up).*

	Baseline – ICI start **Scan 0 – Scan 1**	**ICI start – follow up** **Scan 1 – Scan 2**	***P*** **Value**
Absolute change (mm^3/year) – median [IQR]			
Total plaque volume	13.8 [-240-122]	103 [0-511]	0.02
Non-calcified plaque volume	-18.2 [-274-57]	53 [0-382]	0.02
Relative change (%/year) – median [IQR]			
Total plaque volume	2.1 [-13.0-18.6]	6.7% [2.2-28.1]	0.17
Non-calcified plaque volume	-2.3 [-14.0-12.7]	5.3% [1.4-40.1]	0.14

4.2.2 Cardiovascular events after Immune Checkpoint Inhibitor therapy

Baseline demographics and clinical characteristics for the matched cohort study population are summarized in ***Table 5***.

Overall, cases and controls were not different with respect to the type of cancer and a history of any cardiovascular event. Non-small cell lung cancer (28.8%) and melanoma (27.9%) were the most common type of cancer. Controls were more likely to be female (46.9 vs. 42.6%, $P = 0.001$) and had higher rates of hypertension (53.5 vs. 49.2%, $P = 0.001$) and diabetes mellitus (18.2 vs. 15.7%, $P = 0.014$). Among patients receiving ICI therapy, PD-1 inhibitor therapy was the most commonly prescribed (75.3%), and a median of five cycles of ICI were administered.

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Table 5 *Baseline characteristics of patients treated with immune checkpoint inhibitor and control patients.*

	Cases	Controls	*P* Value
Demographic			
Number of Patients	2842	2842	
Sex – no. (%)			
Male	1631 (57.4)	1509 (53.1)	0.001
Female	1211 (42.6)	1333 (46.9)	0.001
Age – years, mean. (SD)	64 (13)	64 (13)	0.14
Race or ethnic group – no. (%)			<0.001
White	2479/2704 (91.7)	2851/2748 (93.9)	
Asian	96/2704 (3.6)	43/2748 (1.6)	
Black or African American	57/2704 (2.1)	64/2748 (2.3)	
Hispanic	29/2704 (1.1)	40/2748 (1.5)	
Clinical variables – mean. (SD)			
Body mass index - (kg/m^2)	27.0 (6.4)	27.6 (5.7)	<0.001
Systolic blood pressure (mmHg)	127.6 (18.6)	127.6 (16.9)	0.93
Cardiovascular risk factors – no (%)			
Hypertension	1356/2756 (49.2)	1518/2837 (53.5)	0.001
Diabetes mellitus	433/2756 (15.7)	517/2837 (18.2)	0.014
Smoking current or prior	429/2756 (15.6)	405/2837 (14.3)	0.19
Hyperlipidemia	840/2756 (30.5)	1048/2837 (36.9)	<0.001
Cardiovascular diagnoses – no (%)			
History of any cardiovascular event	322/2842 (11.3)	357/2842 (12.6)	0.16
History of myocardial infarction	136/2842 (4.8)	167/2842 (5.9)	0.077
History of coronary revascularization	195/2842 (6.9)	230/2842 (8.1)	0.078
History of ischemic stroke	82/2842 (2.9)	101/2842 (3.6)	0.18
Cardiovascular medications – no. (%)			

Angiotensin converting enzyme inhibitor or angiotensin II receptor blocker	612/2704 (22.6)	647/2423 (26.7)	<0.001
Beta-blockers	628/2704 (23.2)	798/2423 (32.9)	<0.001
Calcium channel blockers	396/2704 (14.6)	360/2423 (14.9)	0.86
Statins	704/2704 (26.0)	672/2423 (27.7)	0.17
Aspirin	578/2704 (21.4)	603/2423 (24.9)	0.003
Cancer types – no. (%)			
Non-small cell lung	819/2842 (28.8)	819/2842 (28.8)	
Melanoma	794/2842 (27.9)	794/2842 (27.9)	
Head and neck	344/2842 (12.1)	344/2842 (12.1)	
Renal and genitourinary	182/2842 (6.4)	182/2842 (6.4)	
Breast	119/2842 (4.2)	119/2842 (4.2)	
Gastrointestinal	116/2842 (4.1)	116/2842 (4.1)	
Gynecologic	110/2842 (3.9)	110/2842 (3.9)	
Lymphoma	82/2842 (2.9)	82/2842 (2.9)	
Hepatobiliary	101/2842 (3.6)	101/2842 (3.6)	
Pancreatic	37/2842 (1.3)	37/2842 (1.3)	
Immune checkpoint inhibitor type – no. (%)			
Monotherapy			
Programmed death-ligand-1	283/2842 (10.0)		
Cytotoxic-T-Lymphocyte associated protein 4	221/2842 (7.8)		
Programmed death-protein 1	2141/2842 (75.3)		
Cytotoxic-T-Lymphocyte associated protein 4 or programmed death protein 1	2/2842 (0.1)		
Combination therapy			
Cytotoxic-T-Lymphocyte associated protein 4/Programmed death protein 1	195/2842 (6.9)		
Number of cycles of ICI – no, (IQR)	5 (2-11)		

In univariable Cox proportional hazard model, the use of an ICI was associated with a >4-fold increase in the risk for a composite cardiovascular event (HR, 4.7 [95% CI, 3.5-6.2]; $P < 0.001$). For the individual outcomes, similar results were found (***Table 6*** and **Figure 6**), where the use of an ICI was associated with a higher risk for myocardial infarction (HR, 7.2 [95% CI, 4.5-11.5;] $P < 0.001$), a 3-fold increase in the risk for coronary revascularization (HR, 3.0 [95% CI, 1.9-4.8]; $P < 0.001$), and a 4-fold increase in the risk for ischemic stroke (HR, 4.6 [95% CI, 2.9-7.2]; $P < 0.001$).

In a parsimonious multivariable model, which included known cardiovascular risk factors (male sex, age, body mass index, hypertension, diabetes mellitus, chronic kidney disease, smoking, prior history of a cardiovascular event, statin use, aspirin use, hemoglobin, and low-density lipoprotein), the use of an ICI was associated with a 3-fold increase in the risk for a composite cardiovascular event (multivariable HR, 3.3 [95% CI 2.0-5.5]; $P < 0.001$, ***Table 6, Model 1***).

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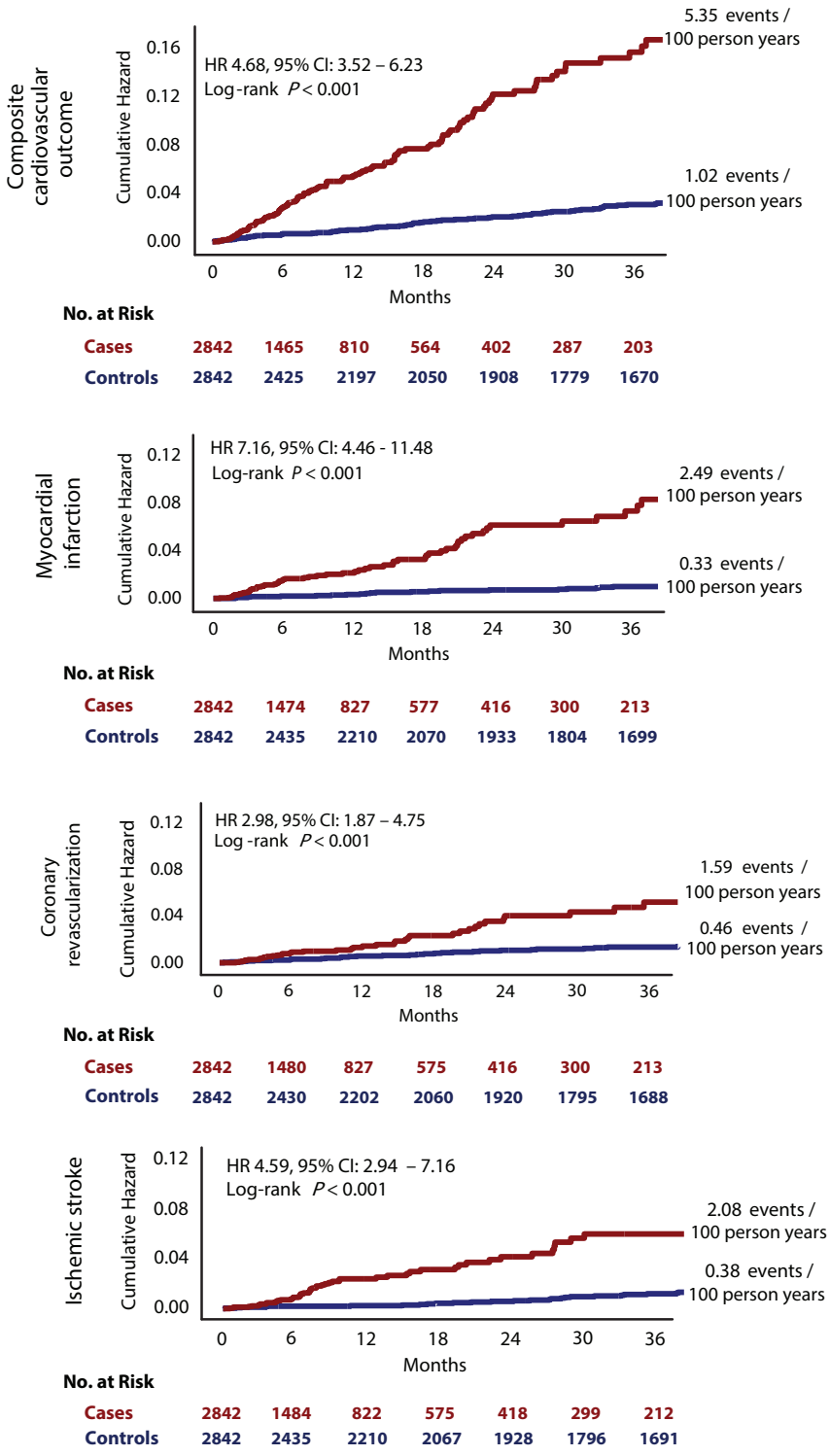

Figure 6 *Kaplan-Meier curves of the cumulative hazard in cases (red) and controls (blue) of the composite and individual component outcomes and the event rates at three years.****(102)***

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Table 6 *Multivariable Cox proportional hazard model results of the composite cardiovascular outcome (myocardial infarction, revascularization, ischemic stroke).*

	Hazard Ratio	**95% CI**		***P* Value**
Multivariable model 1.				
Immune checkpoint inhibitors	3.31	1.99	5.51	<0.001
Male sex	1.71	1.14	2.54	0.009
Age	1.04	1.02	1.06	<0.001
Body mass index	1.03	1.00	1.06	0.076
Hypertension	0.89	0.53	1.51	0.67
Diabetes mellitus	1.41	0.96	2.07	0.082
Chronic kidney disease	0.93	0.60	1.44	0.75
Smoking current or prior	1.27	0.83	1.95	0.27
History of any cardiovascular event	2.14	1.39	3.29	0.001
Statins	0.72	0.48	1.09	0.12
Aspirin	1.14	0.76	1.69	0.53
Hemoglobin	0.88	0.79	0.98	0.023
Low-density lipoprotein	1.00	0.99	1.00	0.68
Multivariable model 2.				
Immune checkpoint inhibitors	4.50	3.30	6.13	<0.001
Age	1.03	1.02	1.04	<0.001
History of any cardiovascular event	2.19	1.63	2.94	<0.001
Diabetes mellitus	1.42	1.07	1.87	0.01
Systolic blood pressure	1.01	1.00	1.02	0.01
Non-small cell lung cancer	1.54	1.19	2.01	<0.001
Prior radiation therapy	1.54	1.13	2.09	0.01
Male sex	1.29	1.00	1.66	0.05

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5 Discussion

Among the BUDAPEST Twin study participants using coronary CTA, we found a weak heritability of non-calcified plaque volume, while calcified plaque volume had a more substantial genetic background.

Positive family history of CAD is considered to be an independent risk factor for future cardiovascular events.(104) Healthy first-degree relatives of patients with early-onset CAD have an approximately 5-fold increase of total coronary plaque volume compared to symptomatic patients.(39) In the Swedish twin registry, among 20,000 twins, if a twin sibling had died from early-onset CAD, the sibling's relative hazard of death due to CAD was roughly double in male MZ twins as compared to male DZ twins, and nearly six-times higher in females.(30)

It has been demonstrated that calcified coronary plaque quantity measured on nonenhanced CT images has a substantial genetic component. (32-36) In a community-based study from Rochester, investigators reported that >40% of the interindividual variation in CAC quantity is attributed to genetic factors. These findings are in congruence with our results, implying a strong hereditary component of coronary calcification.

On the other hand, we observed that environmental factors might play a more important role than genetic effects in determining non-calcified plaque volumes in individuals without known CAD. Based on these results, our findings are seemingly in contradiction to the literature, which describes the importance of familial aggregation and genetics in CAD development.

However, the fact that a trait "runs in families" does not fully imply a strong genetic background since families also share a wide range of shared, common environmental factors (like socioeconomic status). In a family study design, separating genetic and common environmental effects can be challenging, but twin studies can overcome this limitation. With a twin study design, the magnitude of genetic and common environmental components can be estimated separately.(78, 80, 105) Twin studies may be more powerful to examine the heritability of a complex trait than family aggregation studies for several reasons. For example, twin subjects have matching age, and twins share maternal factors and a range of early environmental factors, which might potentially bias the associations. Twins are exposed

to a higher degree of family environment compared with non-twin sib pairs, and they also share a range of environmental variables and various socioeconomic statuses even in adulthood, which do contribute to the expression of complex traits.

It is important to note that our findings do not explain the underlying pathophysiological background of CAD and plaque formation. Our results indicate that early development of coronary plaques is less influenced by genetic factors, therefore primarily influenced by environmental factors such as socioeconomic status, in contrast to plaque calcification which is more dependent on genetics.

In the same prospective classical twin study, we found a moderate additive genetic and a greater unique environmental influence on hepatic and pancreatic lipid accumulations. These results may indicate that development of hepatic and pancreatic fat accumulations, as ectopic fat depots are mainly driven by lifestyle factors. Therefore, both hepatic and pancreatic lipid accumulations could be treated as a nontraditional and modifiable cardiovascular risk factor.

We used non-enhanced CT images to evaluate hepatic and pancreatic lipid accumulations, using methods published in the literature. However, there are several methods and criteria to define hepatic and pancreatic fat accumulations. Using absolute attenuation values or derived ratios or differences (spleen-to-liver attenuation ratio or difference in attenuation values between liver/pancreas and spleen) are both accepted. In this study, due to the twin heritability analysis, we decided to use absolute numbers of HUs instead of derived numbers since absolute values are proposed to be a better approach for twin statistics. Our current CT-based findings of hepatic lipid accumulation reflect similar results found from ultrasonography-based studies.(106) The results of ACE models have few numerical differences, which could be attributed to the difference in study methodology and study participants.

Our findings indicate that hepatic and pancreatic fat accumulations as a marker of ectopic fat depots may be helpful as a tool for cardiovascular risk assessment and improve clinical practice. The development of hepatic and pancreatic lipid accumulation is predominantly influenced by environmental factors. Therefore, lifestyle changes are

importance for prevention at the population level and especially for patients with high cardiovascular risk. Lifestyle changes and weight management should be considered as an essential element for preventing or decreasing hepatic and pancreatic lipid accumulation.

In an imaging study of patients receiving ICI therapy, there was a >3-fold increase in the rate of atherosclerotic plaque progression after initiation of ICI treatment. In a matched cohort study, ICI therapy was associated with a 3-fold higher risk for atherosclerotic cardiovascular events as compared with cancer patients who did not receive ICI.

Progression of atherosclerotic plaque measured on contrast-enhanced CT images is a robust predictor of atherosclerotic cardiovascular events and an established outcome measure for randomized clinical trials.(107-109) Our CT imaging subüstudy demonstrates the association between ICI use with accelerated progression of atherosclerosis. The rate of plaque progression in our study (annually 6.7%) is nearly 3-times higher than reported in patients with subclinical (annually 2.4%) and clinical CVD (annually 0.5-1.3%).(110, 111) Basic science data strongly support the hypothesis that immune checkpoint inhibition may accelerate atherosclerosis. Animal data have shown that the same immune checkpoints are established negative regulators of atherosclerosis.(94, 95, 112, 113) For example, the PD-1/PD-L1 pathway downregulates the proatherogenic T-cell response, and mice lacking PD-L1 had a 3-fold increase in atherosclerotic plaque with an associated increase in T-cells and macrophages.(95, 112) In a hyperlipidemic mice model short term CTLA-4 blockade resulted in the formation of plaques with large necrotic cores, accelerated predominantly by T-cell driven inflammation and increased endothelial activation was also observed.(114)

So far, research on the cardiac toxicities of ICIs has focused on the development of myocarditis, suggesting that myocarditis is an uncommon but potentially fatal complication.(115-122) Since the middle of 2020, a few cohort studies have been published which tested the association between ICIs and cardiotoxicity.(123, 124) It is becoming clear that ICI-associated cardiotoxicity is not only ICI-associated myocarditis, but ICI therapy also increases the risk of future heart failure and cardiovascular events.(123-126) In a pooled analysis of 59 oncological trials submitted to the FDA for approval (sample size: 21,664), among patients on an ICI compared to patients receiving traditional cytotoxic

chemotherapies, there was a 35% increase in coronary ischemia over six months of follow-up.(127) Similarly, in a large retrospective meta-analysis including >20,000 immune checkpoint-treated patients, 9.8% of treatment-related deaths were from cardiovascular events, including; heart failure, myocardial infarction, and the development of a cardiomyopathy.(128) In a nationwide Danish study, patients with lung cancer and melanoma receiving ICI therapy had an increased risk for a cardiac event, defined as arrhythmia, peri- or myocarditis, and heart failure or cardiovascular death. Lung cancer patients receiving ICI therapy had a hazard of 2.14 (95% CI: 1.50-3.05), and melanoma patients had a hazard of 4.30 (95% CI: 1.38-13.42) for a composite event as compared to controls who did not receive ICI therapy.(123) Consistent with prior studies in patients with cancer, in a multivariable model, we also found that older age, diabetes mellitus, ICI use, higher blood pressure, male sex, prior radiation treatment, and a history of a cardiovascular event all increased the risk for a composite cardiovascular event.(129) In addition to our data, these studies also suggest a higher rate of atherosclerotic cardiovascular events with ICIs.

5.1 Limitations

In the BUDAPEST Twin study, our sample size is relatively modest but comparable with other clinical studies in twins.(130) Our results were derived from a healthy white twin population; therefore, the generalizability of our findings is limited. The aim was to balance the overall population for 50% females and ≥50% DZ twins, however, 63% of the twins are female, and 39% are DZ twins. This might be since females, and MZ twins are more willing to participate in research than are males.(131) In our study, the zygosity was classified according to validated questionnaires, but this method is widely used in clinical studies.(132, 133) The DZ pairs' age was somewhat higher than MZ pairs, but all models were corrected for age and sex in the genetic structured equation models. In the coronary plaque twin study, statins were used in 16.8% of the patients, which is relatively low; however, still can influence the presence and phenotype of CAD.(134) Measurement error may appear as part of the unique environmental component as it is uncorrelated across measurements. Due to the cross-sectional nature of our study, we had no data about the plaque, liver, and pancreatic

fat development over time in the study subjects. We used non-enhanced CT images for measuring hepatic and pancreatic lipid accumulation without histological validation. Nevertheless, non-enhanced CT-based evaluation of the hepatic and pancreatic fat accumulation is accepted, and histopathological correlations have already been published by others.(74, 75) Visualization of the liver and the pancreas is often challenging due to inadequate coverage or image quality on coronary CT images. In all the twin sub-studies, we excluded twin subjects (and their siblings) from the analysis due to poor image quality; therefore, the sample size differed for each sub-study. In our study, we refrained from using derived ratios or differences as we aimed to assess genetic and environmental dependence of hepatic and pancreatic lipid accumulation, and crude but not derived numbers should be considered more appropriate for assessing the phenotype in statistical analysis of a classical twin study.

The primary limitations of our ICI matched cohort study are the retrospective nature of the study at a single center and the presence of missing data. However, our cohort of patients on ICI is over 20 times larger than any prior publication, the number of events was substantial, and the directionality of our findings is supported by prior smaller studies. These factors overall provide much improved statistical power and thus confidence in our findings. This was a retrospective study, and it is possible that there remain several unmeasured residual confounders that may have influenced the association between ICI use and vascular events. An significant limitation is that it is difficult to control for other variables which may change over time in a patient with cancer and which may also impact cardiovascular risk; however, we did not find significant changes over the study period in clinical variables (e.g., blood pressure) or cardiovascular medication use in either the clinical or the imaging cohort. Another limitation of this study design would be whether a previous cardiovascular event altered the exposure to an ICI. However, prior CVD is not a contraindication to ICI therapy, is not an exclusion from most clinical trials testing the efficacy of ICI.(91, 135-138) ICIs are associated with an increase in inflammation; however, routine measures of inflammation such as measures of cytokines and C-Reactive Protein were not performed. We measured

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other related markers such as the white blood cell count, neutrophil count, and lymphocyte count and found no difference between those with and without events.

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6 Conclusions

In conclusion, we observed that non-calcified plaque volume is less determined by genetic factors, predominantly by environmental factors, while calcified plaque volume is influenced mainly by genetics. These findings suggest that lifestyle may have an essential role in the initiation of CAD since non-calcified plaques were more determined by environmental factors, and genetic factors showed a more substantial effect on the presence of calcified plaque. We have also found that unique environmental influences outweighed additive genetic effects on the phenotypic appearance of both hepatic and pancreatic lipid accumulations.

In a retrospective study of patients receiving ICI therapy, a greater atherosclerotic plaque progression was found after starting the therapy. Moreover, as compared to cancer patients who did not receive ICI therapy, there was a higher rate of cardiovascular events associated with the therapy.

Our results underline the importance of optimal cardiovascular risk factor management early in life and prior to therapy with ICIs. Favorable changes of modifiable environmental factors are of great importance in preventing and treating non-calcified coronary plaques and ectopic fat depots such as hepatic and pancreatic fat accumulations. ICI therapy should be considered as a modifier of cardiovascular risk. Patients eligible for ICI therapy should undergo a comprehensive cardiovascular risk evaluation and optimization of preventive measures with close monitoring thereafter.

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7 Summary

Cardiovascular diseases and cancer are the leading cause of death worldwide. With the rising number of cancer survivors, the number of patients with both cardiovascular disease and cancer is increasing. Therefore, identifying those at high cardiovascular risk has utmost importance, especially in patients receiving potentially cardiotoxic therapies. During my PhD studies, my research has focused on cardiovascular risk prediction using computed tomography-based imaging of atherosclerosis and ectopic fat.

Our study aimed to investigate the magnitude of genetic and environmental impact on calcified and non-calcified coronary plaque volumes, hepatic lipid accumulation and pancreatic lipid accumulation among subjects enrolled in a prospective classical twin study. Moreover, in a retrospective matched cohort study, our goal was to test whether a novel cancer therapy, immune checkpoint inhibitors, were associated with an increase in atherosclerotic plaque progression and cardiovascular events.

We have observed that non-calcified plaque volume was predominantly determined by environmental factors, while calcified plaque volume was influenced mainly by genetics. The presence of both hepatic and pancreatic lipid accumulation was more determined by environmental factors than by genetic influences. A 3-fold greater rate of atherosclerotic plaque progression and a 3-fold higher risk for cardiovascular events was observed in patients receiving immune checkpoint inhibitor therapy.

These findings suggest that lifestyle may have an important role in the initiation of coronary artery disease, and genetics may have a stronger effect on calcified plaque formation. Our results also highlight the importance of environmental factors in hepatic and pancreatic lipid accumulation. Our work underlines the importance of optimal risk factor management early in life and provides a rationale to consider treatment with immune checkpoint therapy as a modifier of cardiovascular risk.

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ORIGINAL RESEARCH ARTICLE

Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque

Editorial, see p 2312; Article, see p 2396

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BACKGROUND: Immune checkpoint inhibitors (ICIs) treat an expanding range of cancers. Consistent basic data suggest that these same checkpoints are critical negative regulators of atherosclerosis. Therefore, our objectives were to test whether ICIs were associated with accelerated atherosclerosis and a higher risk of atherosclerosis-related cardiovascular events.

METHODS: The study was situated in a single academic medical center. The primary analysis evaluated whether exposure to an ICI was associated with atherosclerotic cardiovascular events in 2842 patients and 2842 controls matched by age, a history of cardiovascular events, and cancer type. In a second design, a case-crossover analysis was performed with an at-risk period defined as the 2-year period after and the control period as the 2-year period before treatment. The primary outcome was a composite of atherosclerotic cardiovascular events (myocardial infarction, coronary revascularization, and ischemic stroke). Secondary outcomes included the individual components of the primary outcome. In addition, in an imaging substudy (n=40), the rate of atherosclerotic plaque progression was compared from before to after the ICI was started. All study measures and outcomes were blindly adjudicated.

RESULTS: In the matched cohort study, there was a 3-fold higher risk for cardiovascular events after starting an ICI (hazard ratio, 3.3 [95% CI, 2.0–5.5]; *P*<0.001). There was a similar increase in each of the individual components of the primary outcome. In the case-crossover, there was also an increase in cardiovascular events from 1.37 to 6.55 per 100 person-years at 2 years (adjusted hazard ratio, 4.8 [95% CI, 3.5–6.5]; *P*<0.001). In the imaging study, the rate of progression of total aortic plaque volume was >3-fold higher with ICIs (from 2.1%/y before 6.7%/y after). This association between ICI use and increased atherosclerotic plaque progression was attenuated with concomitant use of statins or corticosteroids.

CONCLUSIONS: Cardiovascular events were higher after initiation of ICIs, potentially mediated by accelerated progression of atherosclerosis. Optimization of cardiovascular risk factors and increased awareness of cardiovascular risk before, during, and after treatment should be considered among patients on an ICI.

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ORIGINAL RESEARCH ARTICLE

Clinical Perspective

What Is New?

- Immune checkpoint inhibitors are associated with a 3-fold higher risk for atherosclerotic cardiovascular events, including myocardial infarction, coronary revascularization, and ischemic stroke.
- Immune checkpoint inhibitors are associated with a >3-fold higher rate of aortic plaque progression.
- The increase in aortic atherosclerotic plaque was modified by concomitant statin and corticosteroid use.

What Are the Clinical Implications?

- Optimization of cardiovascular risk factors before, during, and after treatment with immune checkpoint inhibitors is warranted.
- Increased awareness of atherosclerotic cardiovascular risk during and after treatment with immune checkpoint inhibitors is needed.

Immune checkpoint inhibitors (ICIs) represent a paradigm shift in cancer care, leveraging the immune system to identify and target cancer cells.[1] The use of ICIs is rapidly expanding. For example, in 2014, ICIs were approved for 3 cancer indications.[2] By 2020, this number had increased to >50, and the percentage of patients with cancer eligible for an ICI has increased from 1.5% in 2011 to >43.6%.[3] The benefit of ICIs has expanded to the adjuvant setting in some malignancies[4,5] and will continue to expand to patients with a much longer anticipated survival.[4]

Consistent animal and cellular studies have demonstrated that these immune checkpoints, currently targeted in approved indications, are critical negative regulators of atherosclerosis: PD-1 (programmed cell death protein 1), programmed death ligand 1, and CTLA-4 (cytotoxic T-lymphocyte–associated protein 4).[6–8] However, there are conflicting clinical and imaging data testing whether ICIs, by inhibiting these key pathways in atherosclerosis, lead to an increase in atherosclerotic plaque and atherosclerosis-related cardiovascular events.[9–12] Given the potentially significant impact on public health, we performed both a matched cohort study and a case-crossover study to determine whether the use of ICIs leads to an increase in cardiovascular events. To provide further insights, we also tested whether ICIs were associated with accelerated atherosclerotic plaque in a subsample.

METHODS

The data, analytical methods, and study materials will be made available from the corresponding author on reasonable request after institutional approval and following institutional process.

Study Design, Setting, and Population

We chose 2 study designs to examine the association between ICIs and cardiovascular events: a matched cohort study and a case-crossover study. All individuals treated with an ICI through the end of March 2019 at a single academic institution (Massachusetts General Hospital, Boston) were included. The use of an ICI was derived from a pharmacy database. The study entry date for the cases was defined as the first date an ICI was administered. For the matched cohort study, controls were selected from all patients treated for cancer at our center between January 1, 2008, and December 31, 2012. For the control group, the use of an ICI at any time point was an exclusion criterion. There were 9793 individual patients with cancer treated at our institution during that period. Of these, 1250 were excluded because they were treated with an ICI subsequently. This resulted in a cohort of 8543 patients. From these, we randomly selected controls in a 1:1 ratio to match cases for age, a history of cardiovascular events, and cancer type (Figure 1). The study entry for the controls was their first visit after January 1, 2008. For the case-crossover design, we defined the observation period as the interval from 2 years before to the start of the ICI. We defined the at-risk period as the 2-year interval after the start of the ICI (Figure I in the Data Supplement). Covariates were derived from the Research Patient Data Registry. The study was approved by the Partners Human Research Committee, and no informed consent was required. The authors vouch for the completeness and accuracy of the data and all analyses.

Procedures

Covariates of interest obtained included patient demographics, medications, and standard cardiovascular risk factors (eg, diabetes, hypertension, smoking). Data relevant to cancer included the cancer type, previous potentially cardiotoxic cancer therapies (radiation therapy, 5-fluorouracil, anthracyclines, and tyrosine kinase inhibitors), and the specific ICI treatments, including the use of combined immune checkpoint therapy. Data specific to the ICI cohort also included the number of ICI cycles, the occurrence of any immune-related adverse event, and the use of corticosteroids.

Clinical Outcomes

The primary outcome was the occurrence of a cardiovascular event, defined as a composite of myocardial infarction, coronary revascularization, and ischemic stroke. The individual components of these were prespecified as key separate secondary outcomes. Events were initially identified from individual chart review of all records with a broad key word search, and then all potential clinical events were independently adjudicated by a study team blinded to all other data and using standard definitions (Document I in the Data Supplement, Key Words and Definitions Used for Each of the Adjudicated Clinical Events).[13–15]

Imaging Study

We performed an imaging substudy in which we measured the thoracic atherosclerotic plaque burden over time among patients with melanoma who were treated with an ICI.

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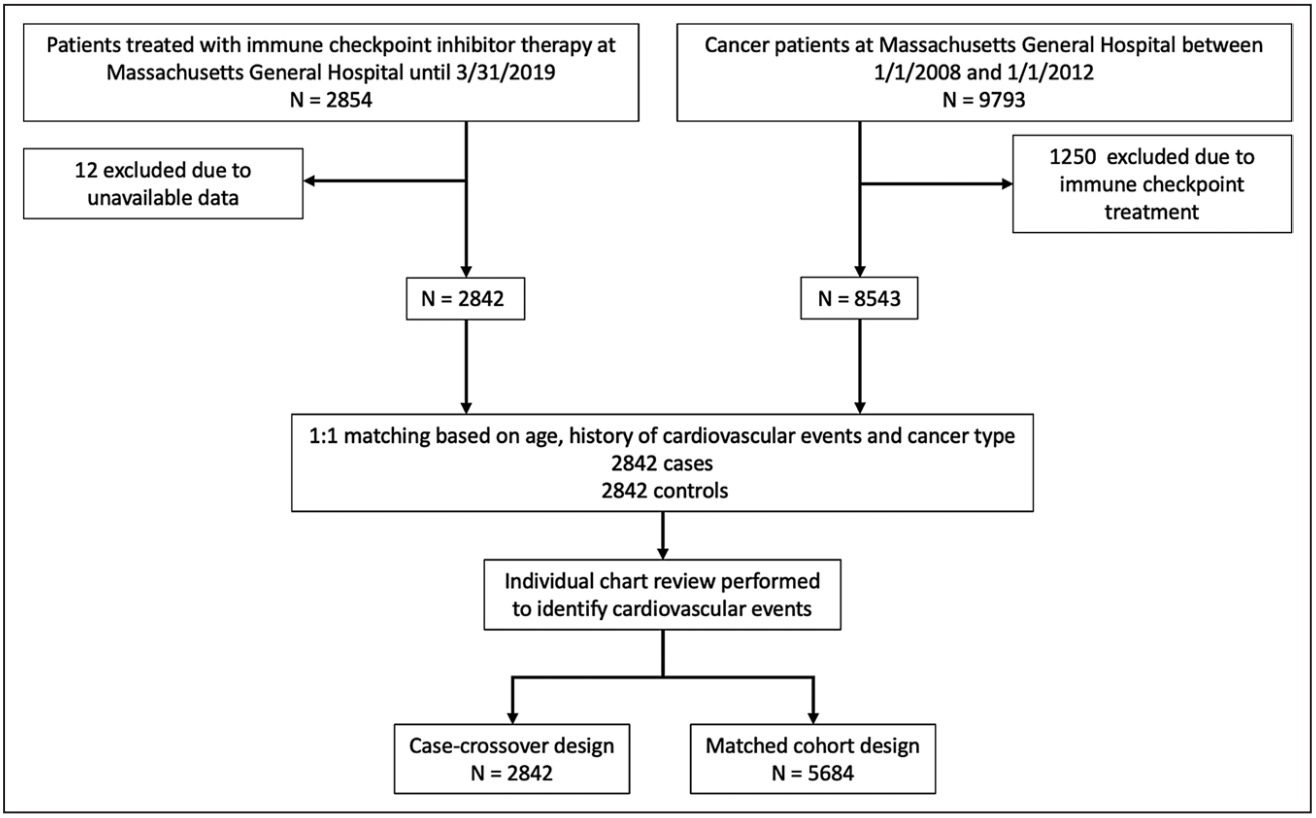

Figure 1. **Flow diagram.**

Melanoma was chosen as the population for the substudy as it was one of the most common cancer seen in our study, ICIs are frequently used,[16] and these therapies have had a marked impact on cancer outcomes.[4,16] Studies were performed as part of their routine clinical care for cancer staging. Thoracic aortic plaque volume was measured from these studies in a standardized fashion in a core laboratory by investigators blinded to all other study variables, including treatment status and sequence of imaging studies. The plaque volume was assessed on a limited field of view that excluded the surrounding nonvascular structures. The full analysis protocol, accuracy, and reproducibility of these methods have been reported by our group previously (Figure II and III and Document II in the Data Supplement).[13,14,17] This volumetric plaque assessment technique has demonstrated excellent intraobserver and interobserver, as well as interscan, reproducibilities.[18–20] In brief, total and noncalcified thoracic aortic plaque volumes were measured on all 3 contrast computed tomography scans with dedicated software (QAngioCT, version 3.1.4.2, Medis Medical Imaging Systems, Leiden, the Netherlands).[21] Relative plaque volume measures were assessed as percent of total segment volume. Plaque change was calculated as the difference in plaque volume measured on 2 consecutive scans (ie, scan 2–scan 1 and scan 1–scan 0). Annualized plaque progression rate was computed as plaque change per year given in absolute and relative rates (cubic millimeters and percent).

Statistical Analysis

Descriptive statistics were used to assess the distribution of variables; continuous variables were summarized as mean with SD or medians with interquartile ranges, and categorical variables were summarized as counts and percentages. In the matched cohort study, controls were matched 1:1 on the basis of age, a history of cardiovascular events, and cancer type. In the matched cohort and case-crossover designs, Cox proportional hazard regression analysis was performed to calculate hazard ratios (HRs) with 95% CIs, counting only the first cardiovascular event. Two approaches were applied. In the first, a parsimonious multivariable Cox proportional hazard model was performed, including known cardiovascular risk factors (model 1). In a second approach, a forward stepwise selection was used; clinically relevant unique predictor variables with a value of $P<0.10$ in univariable analysis were entered into the final multivariable model (model 2). The incremental value between steps was measured by the likelihood-ratio test. The proportional hazard assumption was tested with the use of log-log plots and examination of Schoenfeld residuals. We performed subgroup analyses of HRs by sex, age (<65 years versus ≥65 years), body mass index (<30 kg/m^2 versus ≥30 kg/m^2), a history of cardiovascular events, hypertension, diabetes, statin use, melanoma, and lung cancer. We evaluated the presence of interactions in these subgroups, and HRs stratified by these subgroups were compared with the χ^2 test. In the case-crossover analysis,[22,23] Cox proportional hazard regression analyses were performed with calculation of 100 person-years and an HR adjusted for age. We compared atherosclerotic cardiovascular events in the 2-year period before and the 2-year period after the start of the ICI. We used Poisson regression during the 2-year periods before and after ICI and calculated incidence rate ratio with the outcome variable as a count variable including all events (first event and the

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events that occurred subsequently after the first event during the follow-up period). In addition, we tested a narrower risk period (1 year before and 1 year after) and performed sensitivity analyses excluding patients who died within 60 days of the cardiovascular event. In the imaging substudy, the primary outcome of interest was the change in total plaque volume over time in patients from before to after ICI. The secondary imaging outcome was the change in noncalcified plaque volume. The annualized rate of change in plaque volume was compared from before to after ICI using the Wilcoxon signed-rank test. We performed analyses of plaque progression in prespecified subgroups defined by statin use and the use of corticosteroids during ICI therapy. All statistical tests were 2 tailed, and values of $P<0.05$ were considered to indicate statistical significance. Analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC) and STATA software, version 15.1 (StataCorp, College Station, Texas).

RESULTS

Patient Demographics, Comorbidities, and Cancer Data

Baseline demographics and clinical characteristics are summarized in Table 1. Baseline laboratory values are summarized in Table I in the Data Supplement. Overall, cases and controls were not different with respect to age, type of cancer, and history of any cardiovascular event. Non–small cell lung cancer (28.8%) and melanoma (27.9%) were the most common types of cancer. Controls had higher rates of hypertension (53.5% versus 49.2%; $P=0.001$) and diabetes (18.2% versus 15.7%; $P=0.014$). Controls were more likely female (46.9% versus 42.6%; $P=0.001$). The use of statins was not different between cases and controls (26.0% versus 27.7%; $P=0.17$). Among the cases, PD-1 inhibitor therapy was the most commonly prescribed (75.3%), and cases had a median of 5 cycles of the ICI administered. Overall, 43.2% of the cases had an immune-related adverse event, and 26.9% were treated with corticosteroids, 62.2% of those with immune-related adverse events.

Primary and Secondary Outcomes

Demographic, clinical, and cancer-related variables were included in a univariable Cox proportional hazard model (Table II in the Data Supplement). The use of an ICI was associated with a >4-fold increase in the risk for a composite cardiovascular event (univariable HR, 4.7 [95% CI, 3.5–6.2]; $P<0.001$). For the individual outcomes, similar results were found (Figure 2) in which the use of an ICI was associated with a higher risk for myocardial infarction (univariable HR, 7.2 [95% CI, 4.5–11.5;] $P<0.001$), a 3-fold increase in the risk for coronary revascularization (univariable HR, 3.0 [95% CI, 1.9–4.8]; $P<0.001$), and a 4-fold increase in the risk for ischemic stroke (univariable HR, 4.6 [95% CI, 2.9–7.2]; $P<0.001$). Kaplan-Meier curves of the cumulative hazard in cases and controls of the composite and individual component outcomes and the event rates at 3 years are shown in Figure 2.

In a parsimonious multivariable model, which included known cardiovascular risk factors (male sex, age, body mass index, hypertension, diabetes, chronic kidney disease, smoking, history of a cardiovascular event, statin use, aspirin use, hemoglobin, and low-density lipoprotein), the use of an ICI was associated with a 3-fold increase in the risk for a composite cardiovascular event (multivariable HR, 3.3 [95% CI 2.0–5.5]; $P<0.001$; Table 2, model 1). In a second approach, the variables, identified as $P<0.1$ in the univariable Cox model, were entered into a multivariable model. In this model, the use of an ICI was associated with a 4-fold increase in the risk for a composite cardiovascular event (multivariable HR, 4.5 [95% CI, 3.3–6.1]; $P<0.001$; Table 2, model 2).

In the case-crossover study, the number of patients who had an event and the cumulative number of cardiovascular events were compared only among the 2842 patients who were treated with an ICI. Overall, among the 2842 patients who were treated with an ICI, 119 patients had a cardiovascular event during the 2-year period after starting an ICI compared with 66 patients in the 2-year period before starting an ICI, a 4-fold increase from 1.37 to 6.55 per 100 person-years (adjusted HR, 4.8 [95% CI, 3.5–6.5]; $P<0.001$; Table 3). In the case-crossover study, there was also an increase in each of the individual components of the primary outcome (Figure 3 and Table 3). The total numbers of events in the risk and control periods in the case-crossover study were also compared. Among the 2842 patients treated with an ICI, there were 139 events among the 119 patients during the 2-year period after ICI. In comparison, in the same cohort of 2842 patients, who subsequently were treated with an ICI, there were 78 events among the 66 patients during the 2-year period before ICI (incidence rate ratio, 1.8 [95% CI, 1.4–2.4]; $P<0.001$). Similar findings were also noted when the risk period and control period were restricted to 1 year before and 1 year after ICI (Figure 3 and Table III in the Data Supplement), and findings of a higher risk for atherosclerotic cardiovascular event with an ICI persisted after the exclusion of individuals who died within 60 days of the event (Table IV in the Data Supplement).

Subgroup Analyses

In the subgroup analyses, a significant interaction was noted between baseline hypertension and ICI use ($P=0.003$; Figure IV in the Data Supplement) in which the relative risk for a cardiovascular event was higher among patients without hypertension compared with

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Table 1. Baseline Characteristics of Patients Treated With ICIs and Controls

	Cases	Controls	*P* value
Demographics			
Patients, n	2842	2842	
Sex, n (%)			
Male	1631 (57.4)	1509 (53.1)	0.001
Female	1211 (42.6)	1333 (46.9)	0.001
Age, mean (SD), y	64 (13)	64 (13)	0.14
Age, median. (IQR), y	66 (57–74)	65 (55–74)	0.11
Race or ethnic group, n (%)			<0.001
White	2479/2704 (91.7)	2851/2748 (93.9)	
Asian	96/2704 (3.6)	43/2748 (1.6)	
Black or African American	57/2704 (2.1)	64/2748 (2.3)	
Hispanic	29/2704 (1.1)	40/2748 (1.5)	
Other	43/2704 (1.6)	20/2748 (0.7)	
Clinical variables, mean (SD)			
Body mass index, kg/m^2	27.0 (6.4)	27.6 (5.7)	<0.001
Systolic blood pressure, mm Hg	127.6 (18.6)	127.6 (16.9)	0.93
Cardiovascular risk factors, n (%)			
Hypertension	1356/2756 (49.2)	1518/2837 (53.5)	0.001
Diabetes	433/2756 (15.7)	517/2837 (18.2)	0.014
Smoking, current or previous	429/2756 (15.6)	405/2837 (14.3)	0.19
Hyperlipidemia	840/2756 (30.5)	1048/2837 (36.9)	<0.001
Cardiovascular diagnoses, n (%)			
History of any cardiovascular event	322/2842 (11.3)	357/2842 (12.6)	0.16
History of myocardial infarction	136/2842 (4.8)	167/2842 (5.9)	0.077
History of coronary revascularization	195/2842 (6.9)	230/2842 (8.1)	0.078
History of ischemic stroke	82/2842 (2.9)	101/2842 (3.6)	0.18
Cardiovascular medications, n (%)			
Angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker	612/2704 (22.6)	647/2423 (26.7)	<0.001
β-Blockers	628/2704 (23.2)	798/2423 (32.9)	<0.001
Calcium channel blockers	396/2704 (14.6)	360/2423 (14.9)	0.86
Statins	704/2704 (26.0)	672/2423 (27.7)	0.17
Nonstatin dyslipidemia therapies	65/2704 (2.4)	122/2423 (5.0)	<0.001
Aspirin	578/2704 (21.4)	603/2423 (24.9)	0.003
Other antiplatelet therapies	66/2704 (2.4)	98/2423 (4.0)	0.001
Other medical comorbidities, n (%)			
Chronic obstructive pulmonary disease	285/2756 (10.3)	169/2837 (6.0)	<0.001
Chronic kidney disease	327/2756 (11.9)	326/2837 (11.5)	0.69
Cancer types, n (%)			
Non–small cell lung	819/2842 (28.8)	819/2842 (28.8)	
Melanoma	794/2842 (27.9)	794/2842 (27.9)	
Head and neck	344/2842 (12.1)	344/2842 (12.1)	
Renal and genitourinary	182/2842 (6.4)	182/2842 (6.4)	
Breast	119/2842 (4.2)	119/2842 (4.2)	
Gastrointestinal	116/2842 (4.1)	116/2842 (4.1)	
Gynecological	110/2842 (3.9)	110/2842 (3.9)	

(*Continued*)

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Table 1. Continued

	Cases	Controls	*P* value
Lymphoma	82/2842 (2.9)	82/2842 (2.9)	
Hepatobiliary	101/2842 (3.6)	101/2842 (3.6)	
Pancreatic	37/2842 (1.3)	37/2842 (1.3)	
Other	138/2842 (4.9)	138/2842 (4.9)	
Previous potentially cardiotoxic cancer therapies, n (%)			
Radiation therapy	572/2756 (20.8)	287/2837 (10.1)	<0.001
5-Fluorouracil	284/2723 (10.4)	151/2710 (5.6)	<0.001
Anthracyclines	151/2723 (5.5)	153/2710 (5.6)	0.92
Tyrosine kinase inhibitors	61/2723 (2.2)	59/2710 (2.2)	0.95
ICI type, n (%)			
Monotherapy			
Programmed death ligand-1	283/2842 (10.0)		
CTLA-4	221/2842 (7.8)		
PD-1	2141/2842 (75.3)		
CTLA-4 or PD-1	2/2842 (0.1)		
Combination therapy			
CTLA-4/PD-1	195/2842 (6.9)		
Cycles of ICI, n (IQR)	5 (2–11)		
Immune-mediated adverse events after ICI start			
Gastrointestinal	500/2748 (18.2)		
Skin	429/2748 (15.6)		
Pulmonary	189/2748 (6.9)		
Hepatic	179/2748 (6.5)		
Endocrine	175/2748 (6.4)		
Renal	120/2748 (4.4)		
Neuromuscular	98/2748 (3.6)		
Pancreas	61/2748 (2.2)		
Any of the above adverse events	1186/2748 (43.2)		
Immune-mediated adverse events treated with steroids, n (%)			
Among the entire cohort	738/2748 (26.9)		
Among those with immune-mediated adverse events	738/1186 (62.2)		

CTLA-4 indicates cytotoxic-T-lymphocyte–associated protein 4; ICI, immune checkpoint inhibitor; IQR, interquartile range; and PD-1, programmed cell death protein 1.

patients with hypertension (HR, 10.7 [95% CI, 6.1–18.8] versus HR, 3.4 [95% CI, 2.4–4.9]). There was no relative difference in the risk for a cardiovascular event between males and females, those <65 years versus ≥65 years of age, and those with a body mass index <30 versus ≥30 kg/m^2, a history of cardiovascular events, baseline diabetes, statin use, or a diagnosis of melanoma or lung cancer.

Imaging Substudy

The imaging study cohort included 40 patients with melanoma with computed tomography performed at 3 time points (Figure III in the Data Supplement). The clinical characteristics of the patients in the imaging substudy, apart from cancer type, were not different from those of the main study cohort (Table V in the Data Supplement). The presence of cardiovascular risk factors except for age, clinical variables, and the use of cardiac medications remained relatively constant throughout the study period (Table VI in the Data Supplement). There was an increase in the total and noncalcified plaque volumes over the duration of the 3 scans (Table VII in the Data Supplement). The progression rate, adjusted for the study interval, was greater in the period after ICI compared with before ICI for both total (*P*=0.02) and noncalcified plaque (*P*=0.02; Table 4). Specifically, the rate of total plaque volume progression

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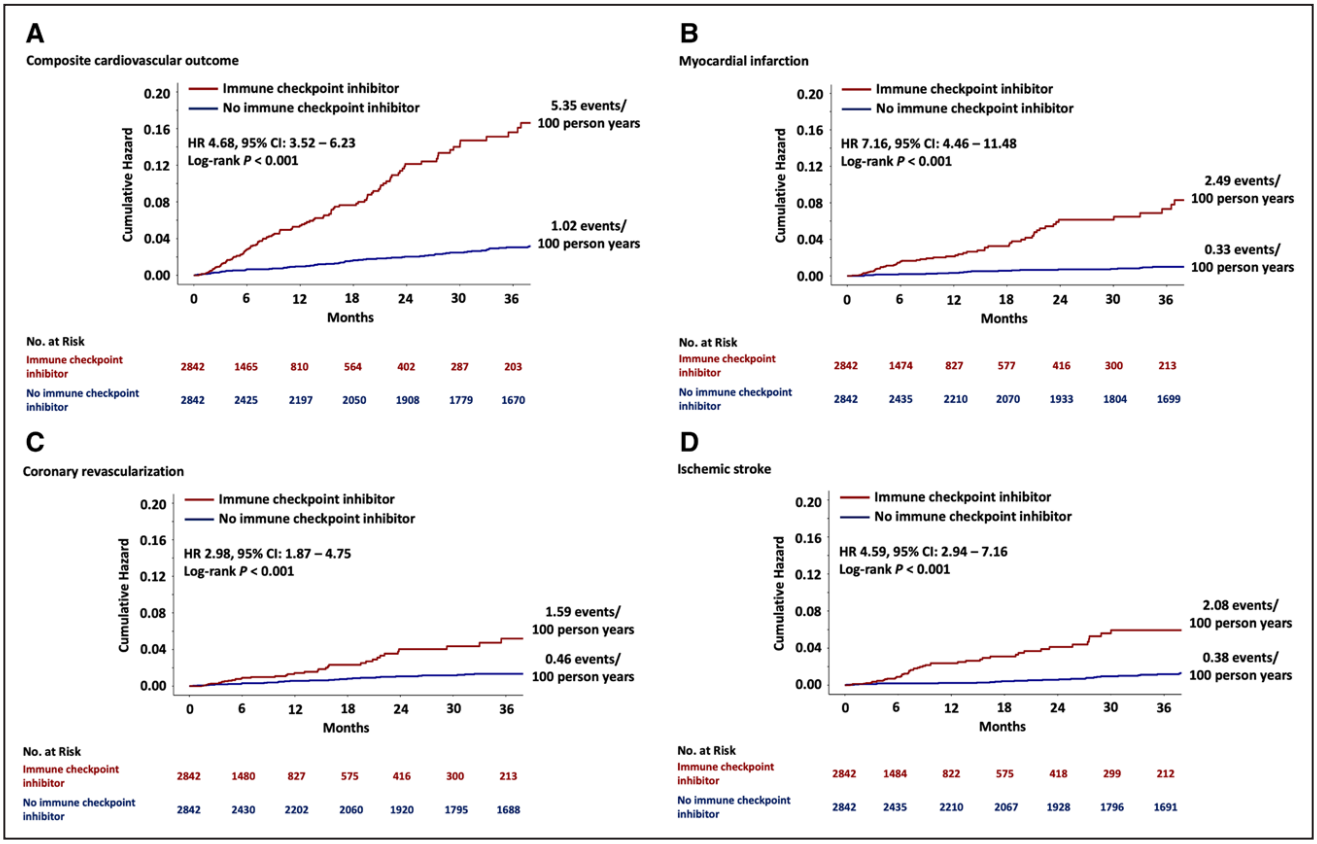

Figure 2. Kaplan-Meier curves of the cumulative hazard for atherosclerotic cardiovascular events.
A, Cumulative hazard for the composite cardiovascular outcome. **B**–**D**, Individual components of the primary outcome. Cases (those treated with an immune checkpoint inhibitor [ICI]) are marked with red; controls (not treated with an ICI) are marked with blue. HR indicates hazard ratio.

increased 3-fold from 2.1%/y before to 6.7%/y after ICI. The rate of noncalcified plaque also increased after ICIs (Table VII in the Data Supplement). In stratified analysis, compared with statin nonusers, those on statins (n=18) showed a 3.1% absolute lower rate of plaque progression each year of total aortic plaque volume (5.2% versus 8.3%, *P*=0.04) and a 3.9% absolute lower yearly rate of noncalcified plaque progression (3.1% versus 7.0%; *P*=0.04; Table 5). Similarly, among patients who were prescribed corticosteroids during checkpoint therapy, there was a lower rate of plaque progression among those on corticosteroids (Table 5); specifically, the rate of noncalcified plaque progression was 3.5%/y among those prescribed a corticosteroid compared with a rate of progression of 6.9%/y among those not prescribed a corticosteroid (total plaque volume, *P*=0.04).

DISCUSSION

The rate of atherosclerotic cardiovascular events was higher after an ICI was started. In a matched cohort study, ICI treatment was associated with a 3-fold higher risk for atherosclerotic cardiovascular events compared with cancer patients who did not have ICI. Similar findings of a higher risk for atherosclerotic cardiovascular events were noted in a case-crossover study. In an imaging substudy, there was a >3-fold increase in the rate of atherosclerotic plaque progression after the initiation of ICI therapy. The association with increased atherosclerotic plaque was attenuated in patients with concomitant use of statins or corticosteroids, who had an ≈50% reduction in plaque progression compared with those not on statins or corticosteroids. Overall, these data suggest that patients treated with an ICI are at a higher risk for atherosclerotic cardiovascular events and that this risk is potentially mediated through accelerated atherosclerosis progression but may be modifiable. Our findings are important both for patients for whom ICIs are currently indicated and perhaps more so for the expanding pool of patients who are candidates for adjuvant and neoadjuvant therapy.

Data on the cardiac toxicities of ICIs have related principally to the development of myocarditis[24–26]; small cohort studies have suggested that myocarditis is an uncommon but potentially fatal complication.[27–31] A limited number of previous studies have tested the association between ICIs and atherosclerotic cardiovascular disease. In a single-center case-control studies with 135 subjects, a single cancer type (non–small cell lung cancer), and a 6-month follow-up period, there were no increases in cardiovascular death, nonfatal

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Table 2. Multivariable Cox Proportional Hazard Model Results of the Composite Cardiovascular Outcome (Myocardial Infarction, Revascularization, Ischemic Stroke)

	HR	95% CI		Wald test *P* value
Multivariable model 1				
ICIs	3.31	1.99	5.51	<0.001
Male sex	1.71	1.14	2.54	0.009
Age	1.04	1.02	1.06	<0.001
Body mass index	1.03	1.00	1.06	0.076
Hypertension	0.89	0.53	1.51	0.67
Diabetes	1.41	0.96	2.07	0.082
Chronic kidney disease	0.93	0.60	1.44	0.75
Smoking, current or previous	1.27	0.83	1.95	0.27
History of any cardiovascular event	2.14	1.39	3.29	0.001
Statins	0.72	0.48	1.09	0.12
Aspirin	1.14	0.76	1.69	0.53
Hemoglobin	0.88	0.79	0.98	0.023
Low-density lipoprotein	1.00	0.99	1.00	0.68
Multivariable model 2				
ICIs	4.50	3.30	6.13	<0.001
Age	1.03	1.02	1.04	<0.001
History of any cardiovascular event	2.19	1.63	2.94	<0.001
Diabetes	1.42	1.07	1.87	0.01
Systolic blood pressure	1.01	1.00	1.02	0.01
Non–small cell lung cancer	1.54	1.19	2.01	<0.001
Previous radiation therapy	1.54	1.13	2.09	0.01
Male sex	1.29	1.00	1.66	0.05

ICI indicates immune checkpoint inhibitor; and HR, hazard ratio.

myocardial infarction, nonfatal stroke, and hospitalization for heart failure with ICIs (HR, 1.2 [95% CI 0.6–2.4]; *P*=0.66).[12] Similarly, in a study of 92 patients with non–small cell lung cancer, there was no increase in venous and arterial vascular events (pulmonary emboli, deep vein thrombosis, cerebrovascular accident, transient ischemic attack, and acute coronary syndrome) compared with patients being treated with cytotoxic chemotherapy.[10] In contrast, in a pooled analysis of 59 oncological trials submitted to the US Food and Drug Administration for approval (sample size, 21 664), compared with traditional cytotoxic chemotherapies, there was a 35% (95% CI, 0.76–2.4) increase in coronary ischemia (defined with Medical Dictionary for Regulatory Activities Terminology) over 6 months of follow-up among patients on an ICI.[11] Similarly, in a

Table 3. Number of Patients With an Event and Number of Events, Rate per 100 Person-Years From Our Cohort of 2842 Cases, and HR for Cardiovascular Events

	Before treatment		After treatment			
Outcome, n (%)	Events, n (%)	Rate per 100 person-y	Events, n (%)	Rate per 100 person-y	Hazard ratio* (95% CI)	*P* value
Patients with cardiovascular events	66 (2.32)	1.37	119 (4.2)	6.55	4.78 (3.50–6.53)	<0.001
Myocardial infarction	27 (0.95)	0.48	58 (2.04)	2.73	4.84 (2.76–8.09)	<0.001
Coronary revascularization	25 (0.87)	0.44	36 (1.26)	1.70	3.18 (1.46–6.10)	<0.001
Ischemic stroke	26 (0.91)	0.46	45 (1.58)	2.12	2.97 (1.41–5.53)	<0.001

Cardiovascular events are compared for the 2-year period before immune checkpoint inhibitor and 2-year period after immune checkpoint inhibitor. ICI indicates immune checkpoint inhibitor; and HR, hazard ratio.

*Cox proportional hazard model

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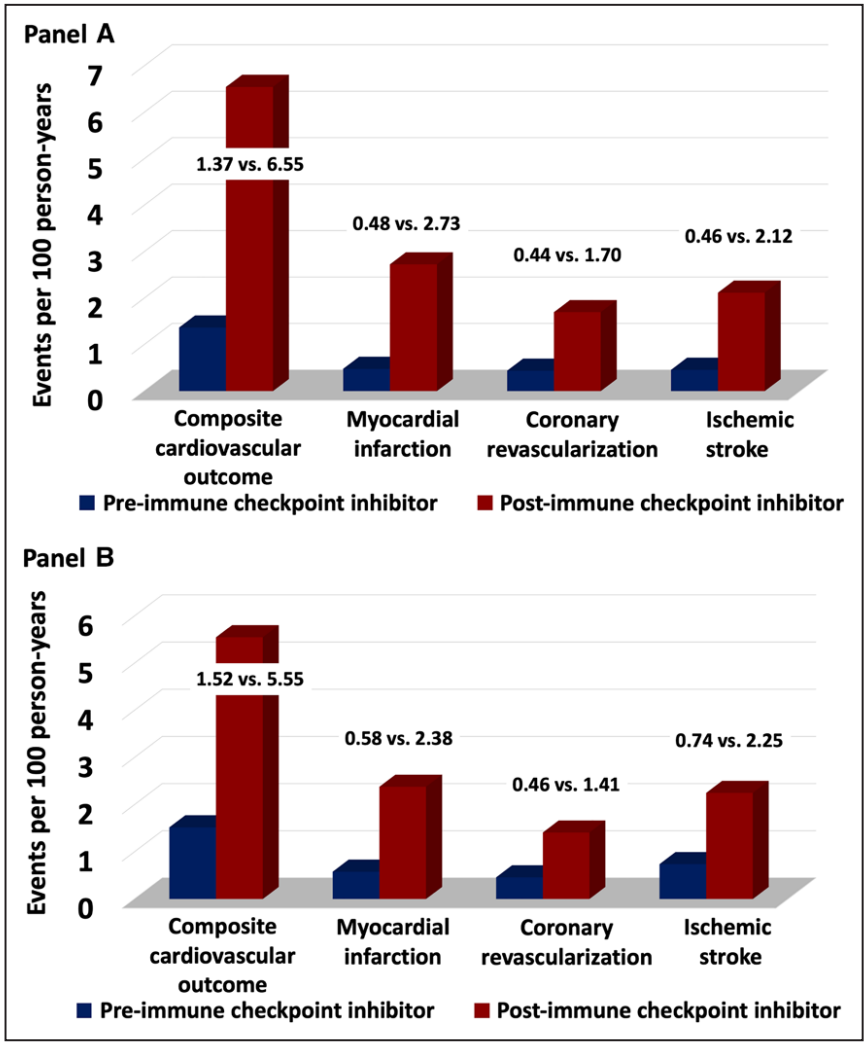

Figure 3. Cardiovascular events in the case-crossover study.
A, Composite cardiovascular outcomes in the 2-year periods before and after immune checkpoint inhibitor (ICI). Cardiovascular event rates per 100 person-years from the 2 years before the start of an ICI to 2 years after starting an ICI are included. Individual components of the primary outcome are also shown. **B**, Composite cardiovascular outcomes in the 1-year period before and after ICI.

large retrospective meta-analysis including >20 000 ICI-treated patients, 9.8% of treatment-related deaths were from cardiovascular events, including heart failure, myocardial infarction, and the development of a cardiomyopathy.[32] Consistent with previous studies in patients with cancer,[33] we also found that older age, diabetes, ICI use, higher blood pressure, male sex, previous radiation treatment, and a history of a cardiovascular event all increased the risk for a composite cardiovascular event. Combined with our data, these studies suggest a higher rate of atherosclerotic cardiovascular events with ICIs. For comparison, the event rate noted in this study (5%/y) is higher than the event rate noted in patients presenting with chest pain (≈0.7%/y),[13] in patients at risk of cardiovascular events (≈0.3%/y),[34] and in other at-risk populations in whom immune activation and inflammation play a key role (eg, individuals with HIV, ≈0.5%/y).[35]

Progression of atherosclerotic plaque is a robust predictor of atherosclerotic cardiovascular events and an established outcome measure for randomized clinical trials.[36–38] Our imaging substudy supports the biological plausibility of our clinical observations by demonstrating an association between ICI use and accelerated progression

Table 4. Absolute and Relative Change in Thoracic Atherosclerotic Plaque Volume From Before Starting an ICI (Scan 0-Scan 1) to After Starting an ICI (Scan 1 to Scan 2)

Change	Indexed change per year	Plaque volume	Scan 0–scan 1	Scan 1–scan 2	*P* value*
Absolute change	Indexed change per year, mm^3/y	Total plaque volume	13.8 (–240 to 122)	103 (0 to 511)	0.02
		Noncalcified plaque volume	–18.2 (–274 to 57)	53 (0 to 382)	0.02
Relative change	Indexed change per year, %/y	Total plaque volume	2.1 (–13.0 to 18.6)	6.7 (2.2 to 28.1)	0.17
		Noncalcified plaque volume	–2.3 (–14.0 to 12.7)	5.3 (1.4 to 40.1)	0.14

Values are median (interquartile range). ICI indicates immune checkpoint inhibitor.
*Wilcoxon signed-rank test comparing annual rate of progression in plaque volume from scan 0 to scan 1 and from scan 1 to scan 2. Relative change is the change in plaque volume per year.

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Table 5. Subgroup Analysis of the Change in Plaque Volume After Starting an ICI by Statin and Corticosteroid Use

Plaque measure, median (IQR)	Drug, yes	Drug, no	*P* value
Statin			
Total aortic plaque volume			
Before ICI, mm^3	1903 (1038 to 2661)	1281 (358 to 2691)	0.38
After ICI, mm^3	2214 (1730 to 4090)	1644 (588 to 4211)	0.32
Absolute change in total plaque, mm^3/y	79.2 (0 to 524)	115 (0 to 509)	0.001
Relative change in total plaque volume, %/y	5.2 (0.6 to 23.7)	8.3 (4.7 to 42.5)	0.04
Noncalcified aortic plaque volume			
Before ICI, mm^3	1233 (956 to 1835)	998 (353 to 2663)	0.68
After ICI, mm^3	1781 (1180 to 3517)	1631 (576 to 3652)	0.62
Absolute change in noncalcified plaque, mm^3/y	45.3 (–38 to 387)	69.5 (0 to 377)	0.002
Relative change in noncalcified plaque volume, %/y	3.1% (–2.3 to 30.4)	7.0% (2.6 to 43.6)	0.04
Corticosteroid			
Total aortic plaque volume			
Before ICI, mm^3	1687 (751 to 2661)	1281 (655, 2691)	0.65
After ICI, mm^3	2161 (690 to 4090)	2214 (1193, 6165)	0.77
Absolute change in plaque, mm^3/y	61.8 (–52.8 to 451)	278 (38.0 to 524)	0.02
Relative change in total plaque volume, %/y	5.9% (–2.2 to 30.2)	7.4% (4.7 to 21.0)	0.04
Noncalcified aortic plaque volume			
Before ICI, mm^3	998 (530 to 1835)	1278 (654 to 2663)	0.71
After ICI, mm^3	1548 (576 to 2750)	1968 (1180 to 5029)	0.28
Absolute change in noncalcified plaque volume, mm^3/y	42.9 (–84.0 to 290)	80.3 (37.5 to 494)	0.02
Relative change in noncalcified plaque volume, %/y	3.5 (–11.3 to 43.4)	6.8 (3.1 to 22.3)	0.04

ICI indicates immune checkpoint inhibitor; and IQR, interquartile range.

of atherosclerosis. The rate of plaque progression in our study (annually 6.7%) is nearly 3 times higher than that reported in patients with subclinical (2.4%/y)[39] and clinical (0.5–1.3%/y) cardiovascular disease.[40] Thus, the acceleration in atherosclerosis is substantial after an ICI and may be one mechanism by which there is an increase in incident cardiovascular events. However, there are other potential mechanisms by which ICIs can accelerate atherosclerosis. These other mechanisms in particular include vasculitis and focal myocarditis misdiagnosed as acute myocardial infarction.[41] All diagnosed myocarditis cases were not included in the analysis, but myocarditis remains a difficult diagnosis,[42,43] and not all patients underwent a coronary angiogram, so vasculitis remains a possibility. However, the potential for ICI to accelerate atherosclerosis is strongly supported by animal and cellular models, in which the same immune checkpoints being targeted for cancer are established negative regulators of atherosclerosis.[6,8,44,45] For example, the PD-1/programmed death ligand 1 pathway downregulates the proatherogenic T-cell response, and mice lacking programmed death ligand 1 had a 3-fold increase in atherosclerotic plaque with an associated increase in T cells and macrophages.[8,44] In addition, PD-1–deficient myeloid progenitors upregulate genes involved in cholesterol synthesis and uptake and downregulate genes promoting cholesterol metabolism, cumulatively leading to markedly increased cellular cholesterol levels.[7] This latter finding is of particular relevance because statin use in our study was associated with reduced progression of atherosclerotic plaque after ICIs (annual progression rate of total plaque volume, 5.2% on statin versus 8.3% not on statin; *P*=0.04). However, we did not find an association between statin use and cardiovascular events in our clinical study. This analysis testing the association with statin therapy on clinical outcomes may have been confounded by indication, with patients on a statin being at a higher baseline risk for events. We observed a similar trend for reduced atherosclerotic plaque in patients receiving corticosteroids. However, these latter findings should be interpreted with caution because the mechanisms involved are less clear; corticosteroids may increase blood sugar and blood pressure and lead to lipid abnormalities, and the association between corticosteroids and overall cancer outcomes is unclear.[46] Moreover, although this observation may be related to the potential anti-inflammatory association with corticosteroids, it may also be cofounded by the indication for corticosteroids (immune mediated adverse events) for which an ICI may be held or stopped if the adverse event is severe.

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The primary limitation of our study is the retrospective nature of the study at a single center and the presence of missing data. However, our cohort of patients on ICI is >20 times larger than in any previous publication, the number of events was substantial, and the directionality of our findings is supported by previous smaller studies, overall providing much improved statistical power and thus confidence in our findings. Advantages and limitations relate to the use of the matched cohort and case-crossover designs,[47,48] and using these 2 designs together may remove the potential fixed and time-varying confounding effects of specific cardiovascular risk factors or age. In addition, the risk of a cardiovascular event would not be expected to change 3-fold over a period of 2 to 4 years, and our results were consistent regardless of the analytical strategy. This was a retrospective study, and it is possible that several unmeasured residual confounders remain that may have influenced the association between ICI use and vascular events. These include physical activity, family history, and other active inflammatory ICI-related diseases such as a thyroid disease. An important limitation is that it is difficult to control for other variables that may change over time in a patient with cancer and that may also affect cardiovascular risk; however, we did not find significant changes over the study period in clinical variables (eg, blood pressure) or cardiovascular medication use in either the clinical or the imaging cohort. A limitation of this study design is whether the exposure to an ICI was altered by a previous cardiovascular event. However, previous cardiovascular disease is not a contraindication to ICI use[49] and is not an exclusion from most of clinical trials testing the efficacy of ICI,[4,16,50,51] and until this publication, the potential for an association between cardiovascular events and ICIs was not established. In addition, it should be noted that the median number of cycles of ICIs was between 4 and 5, and cycles are administered every 2 to 3 weeks, whereas the risk period was longer at 2 years for the primary analysis and 1 year for the secondary analysis. Combination ICI therapy has been associated with a higher risk for myocarditis. In this study, there was no association between combination ICI use and atherosclerotic cardiovascular events; however, only 6.9% of the patients were treated with combination ICIs, thus limiting the interpretation of this negative finding. ICIs are associated with an increase in inflammation. However, routine measures of inflammation such as measures of cytokines and C-reactive protein were not performed and would be affected by the presence and trajectory of cancer; thus, we are unable to test the association between inflammation secondary to ICIs and atherosclerosis or atherosclerosis-related events. We did measure other related markers such as the white blood cell count, neutrophil count, and lymphocyte count and found no difference between those with and those without events and no change over time. We also considered whether the increase in the event rate may have reflected a change in the goals of treatment after a major vascular event among patients with predominately late-stage cancer, specifically whether late-stage cancer influenced the treatment decisions after a major vascular event and led to a shorter follow-up period and a higher rate of events. For example, there was a significantly higher rate of myocardial infarction compared with the modest increase in coronary revascularization. Whether the relative risk of an event would be as high in patients with early-stage cancer with a longer cancer-related survival is less clear and will need to be studied in future cohorts.

Conclusions

In this study, there was a higher rate of cardiovascular events after an ICI was started. The study provides additional biological plausibility of the clinical findings by finding greater atherosclerotic plaque progression after an ICI was started, and we provide initial data suggesting that this effect can be modified. Taken together, these data provide a rationale to consider an approach treating immune checkpoint therapy as a modifier of cardiovascular risk and suggest that candidates for ICI therapy should undergo a comprehensive cardiovascular risk evaluation and optimization of preventive medical therapy with close monitoring thereafter.

ARTICLE INFORMATION

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Supplemental Materials

Supplemental Methods; Documents I and II
Data Supplement Tables I–VII
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A koronária-CT-angiográfia jelentősége a mindennapi gyakorlatban stabil anginás betegek körében

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A kardiovaszkuláris megbetegedések világszerte vezetik a mortalitási és morbiditási statisztikákat. A nagy kockázatú betegek korai azonosítása kiemelt jelentőséggel bír.
A koronária-CT-angiográfia (CTA) egyre jelentősebb szerepet tölt be a stabil anginás betegek kivizsgálásában. Kiváló negatív prediktív értékének köszönhetően fő indikációs területe a kis és közepes kardiovaszkuláris rizikóval rendelkező betegek esetén a koszorúér-betegség jelenlétének kizárása.
A szív-CT-vizsgálat során, a natív felvételeken meghatározhatjuk a kalcium-score értékét, amely kiváló független prediktora a kardiovaszkuláris eseményeknek. A koronária-CTA-felvételek pedig lehetővé teszik a lumen és a koronária plakkok ábrázolását, amely segítségével a lumenszűkületek és a koszorúérplakkok mennyisége nagy pontossággal meghatározhatók.
A betegség jelentlétének kizárása, vagy a koronária-betegség kiterjedtségének meghatározása fontos szereppel bír a mellkasi panaszokkal rendelkező betegek vizsgálatában.
Az összefoglalóban kitérünk a különböző nemzetközi ajánlásokban fellelhető különbségekre. Az elkövetkező néhány évben a koronária-CTA-vizsgálatok száma nagy valószínűséggel jelentősen emelkedik majd és tovább erősödik az invazív angiográfia vizsgálatot megelőző „kapuőr” szerepe.

Kulcsszavak: koronária-CT-angiográfia, stabil angina, kalcium-score, ateroszklerotikus plakk

The role of Coronary CT angiography in patients with stable angina, in the rutine clinical practice
Cardiovascular diseases are the leading cause of mortality and morbidity worldwide. Coronary CT angiography (coronary CTA) is a robust non-invasive diagnostic tool in the work up of patients with stable angina. With its high negative predictive value, coronary CTA is and excellent tool to rule out the presence of coronary artery disease in patients with low to intermediate risk.
Ca-score can be assesed on ECG-gated native cardiac scans and it provides an excellent independent tool for prognostication. The coronary lumen and wall can be visualized by coronary CTA, which allows for plaque characterization and quantification.
Coronary CTA has an important role to guide management strategies of patients with chest pain. The European and American guidelines give slightly different recommendations for the use of coronary calcium score and coronary CTA in patients with chest pain. The number of coronary CTA exams will increase during the upcoming years, and this imaging modality will play an imprtant role as a gatekeeper of catheterisation in individuals with suspected coronary artery disease.

Keywords: Coronary CT angiography, stable angina, calcium-score, coronary plaque

A koronária-CT-angiográfia (koronária-CTA) fő indikációs területe a koszorúér-betegség kizárása kis és közepes rizikójú betegek körében (1). Az Európai Kardiológus Társaság ajánlása alapján a koronária-CTA a stabil anginás, 15-50% pre-teszt probabilitással (PTP) rendelkező betegek számára ajánlott *(1. táblázat)* (2). A vizsgálat magas negatív prediktív értékkel rendelkezik, tehát amennyiben a koronária-CTA során koszorúér-betegség nem ábrázolódik, a mellkasi panaszok hátterében koszorúér-betegség nagy biztonsággal kizárható (3). A koronária-CTA a kis és közepes rizikóval rendelkező betegeknél az iszkémia-provokációs tesztek alternatívája lehet.

Kalcium-score vizsgálat

EKG-kapuzott natív szív-CT-vizsgálat segítségével meghatározható a koszorúerek plakk-terheltségét leíró kalcium-score (Ca-score) érték. A natív vizsgálat során a koszorúerekben lévő meszes plakkok mennyiségéről kapunk információt, amelyet az Agatston-féle Ca-score metódussal számszerűsíthetünk szemiautomatikus szoftver segítségével (4). A meszesedések területét a CT-denzitással való súlyozást követően a szoftver Ca-score értékké alakítja, amely jó korrelációt mutat a koszorúerekben jelen lévő teljes plakktömeggel. A Ca-score a nemkívánt kardiovaszkuláris események független prediktora *(2. táblázat)* (5, 6). Az utóbbi évek során több munkacsoport is igazolta, hogy panaszmentes betegek körében a Ca-score=0 kitűnő negatív kardiovaszkuláris rizikómarker, segít azonosítani azokat a betegeket, akiknél nem, vagy kevésbé indokolt a gyógyszeres prevenciós kezelés (7). Megemlítendő továbbá, hogy a Ca-score mérése növeli a statinterápiával kapcsolatos compliance-t (8). A közepes rizikóval rendelkező egyének körében a Ca-score erősebb prognosztikai értékkel rendelkezik, mint a carotis intima-media vastagság, boka-kar index, a CRP-érték vagy a pozitív családi anamnézis (9, 10).

A több mint 5000 randomizált beteget számláló Multi-Ethnic Study of Atherosclerosis (MESA) vizsgálatban statint korábban nem kapó betegeket követtek medián 7,6 évig és vizsgálták a kardiovaszkuláris események alakulását. Azon betegeknél, akiknél a Ca-score ≥100 volt (21%), a kardiovaszkuláris események gyakorisága 22,7-29,5/1000 fő volt. Azon betegeknél pedig, akiknél maximálisan egy lipidparaméterben volt eltérés, az események gyakorisága alacsonyabb, 2,7-5,9/1000 fő volt. Azon egyéneknél, akiknél egyik lipidparaméterben sem volt eltérés, de a Ca-score ≥100 volt, több esemény történt, mint abban a csoportban, ahol mindhárom lipidparaméter (LDL ≥3,36 mmol/l, HDL <1,03 mmol/l férfiaknál vagy HDL <1,29 mmol/l nőknél, triglicerid ≥1,69 mmol/l) emelkedett volt, de a Ca-score=0 volt. Mindezek alapján felmerül a Ca-score szerepe a személyre szabott statinterápia beállításában, azonban ennek megerősítése további klinikai vizsgálatokat igényel (11).

Kiemelendő azonban, hogy a Ca-score értéke a koszorúér-szűkület mértékével nem mutat szoros összefüggést, tehát magas Ca-score nem jár feltétlenül obstruktív koronáriabetegséggel (legalább egy szegmentumon >50% lumenszűkület), ugyanakkor az alacsony Ca-score sem zárja ki az obstruktív betegséget. A Ca-score=0 értékkel rendelkező betegek 12%-nál azonosítható koszorúér-betegség, és ezen betegek közül minden tizedik esetben obstruktív koronáriabetegség igazolható (12). Az utóbbi esetekben a nem kalcifikált ateroszklerotikus plakkok állnak a háttérben, ezért azok a natív felvételen nem ábrázolódnak *(1. ábra)*. Ezt tükrözi az Európai Kardiológus Társaság stabil koszorúér-betegség vizsgálatáról szóló ajánlása (2013), amely a Ca-score mérését nem ajánlja (III C) a koszorúér-szűkület diagnosztizálására (1).

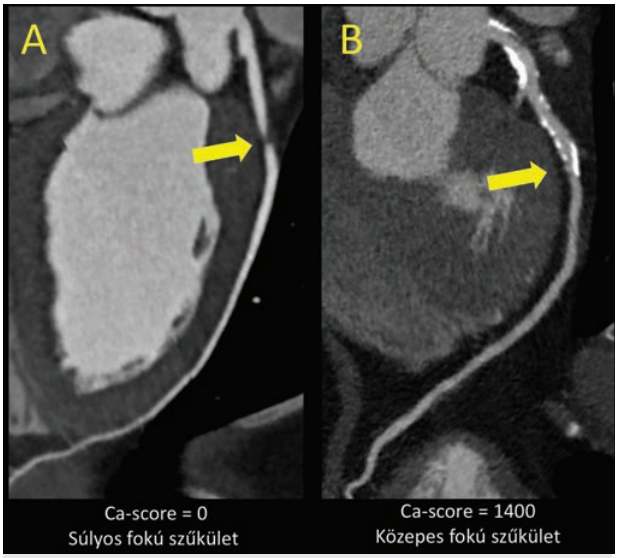
Amennyiben a beteg anamnézisében beszűkült vesefunk-

1. TÁBLÁZAT. Klinikai preteszt probabilitás (PTP) a stabil mellkasi fájdalommal jelentkező betegeknél az Európai Kardiológiai Társaság (ESC) ajánlása alapján. A fehér színnel jelölt pre-teszt probabilitással rendelkező betegek esetében a stabil koszorúér-betegség valószínűsége kisebb mint 15%, ezért további vizsgálatok nélkül kezelhetők. A kék színnel jelölt betegek esetében, terheléses EKG-vizsgálat ajánlott kiindulásként, illetve noninvazív iszkémia-tesztek jönnek szóba, ha azok elérhetők. A sárga színnel jelölt kategóriába tartozó betegeknél noninvazív funkcionális vizsgálat ajánlott. A piros, magas pre-teszt probabilitással rendelkező betegek esetében stabil koszorúér-betegség jelenléte igen valószínű, rizikóbecslés szükséges.

	Típusos angina		Atípusos angina		Nem specifikus fájdalom	
Életkor	**Férfi**	**Nő**	**Férfi**	**Nő**	**Férfi**	**Nő**
30-39	59	28	29	10	18	5
40-49	69	37	38	14	25	8
50-59	77	47	49	29	34	12
60-69	84	58	59	28	44	17
70-79	**89**	68	69	37	54	24
>80	**93**	76	78	47	65	32

2. TÁBLÁZAT. Ca-score értékek és a hozzájuk tartozó rizikókategóriák, valamint a relatív halálozási rizikók (5, 6)

Ca-score érték	Meszes koszorúér-plakkok	Szívinfarktus kockázat	Relatív halálozási rizikó
0	Nem mutatható ki		1,31
1-10	Kis mennyiségben	Kicsi	1,48
11-100	Több	Enyéh emelkedett	3,61
101-400	Közepes mennyiségben	Közepes-magas	3,84
>400	Nagy mennyiségű	Magas	5,78



1. ÁBRA. „A" panelen Ca-score = 0 értékkel rendelkező beteg, súlyos fokú szűkülettel. „B" panelen egy magas, Ca-score=1400 értékkel rendelkező beteg, akinél súlyos fokú lumenszűkület nem ábrázolódott a kontrasztanyag adás után végzett CT-angiográfián

ció (GFR<60 ml/perc/1,73 m^2) vagy kontrasztanyag-allergia szerepel, a kalcium-score vizsgálatot követően a koronária-CT-angiográfia elvégzését mérlegelni szükséges.

Koronária-CT-angiográfia

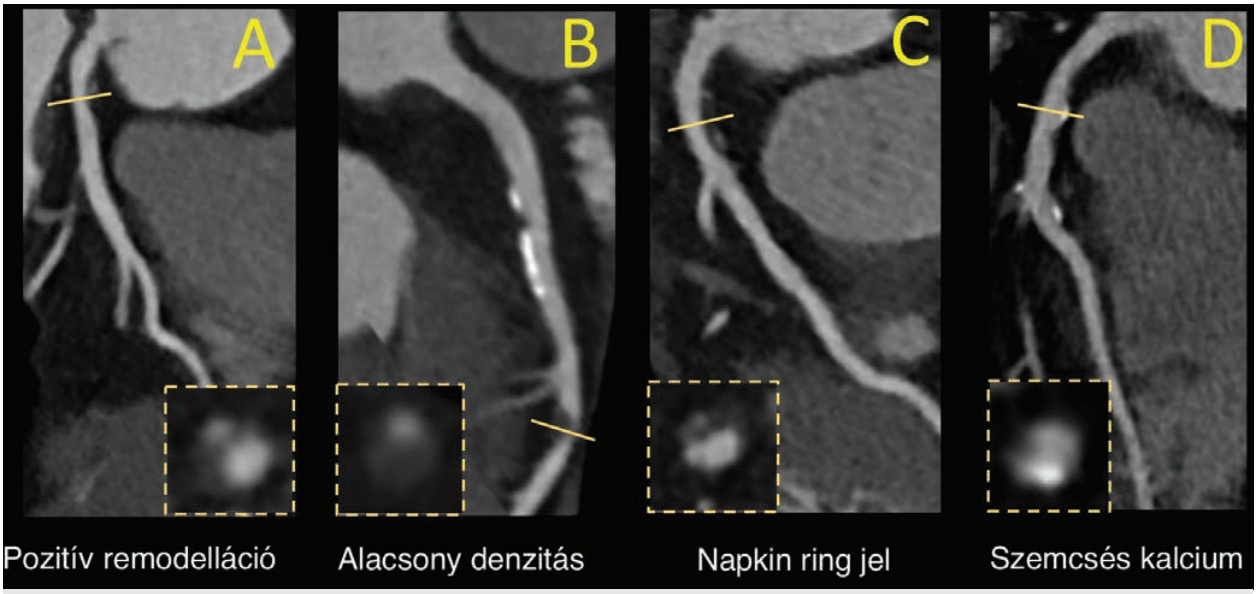
A koronária-CTA során nem csupán a meszesedések ábrázolódnak, hanem a koronáriák lumene, az érfal és a szívüregek is megítélhetővé válnak. A vizsgálat magas szenzitivitása (95-99%) és magas negatív prediktív értéke (97-99%), kitűnő vizsgálati módszerré teszi a kis és közepes rizikójú betegek körében a koszorúér-betegség kizárása terén (3, 13). Fontos azonban figyelembe venni, hogy a koronária-CTA ionizáló sugárterheléssel és jódos kontrasztanyag adásával jár. A vizsgálat megfelelő indikáció alapján végezhető csak el. Széles körben a Diamond–Forrester-módszer szerint kerül meghatározásra a preteszt probabilitás. Továbbá az esetleges relatív vagy abszolút kontraindikációk (veseelégtelenség GFR<60 ml/perc/1,73 m^2, kontrasztanyag-allergia) kizárását követően a diagnosztikus felvételt befolyásoló tényezők (obesitas, légzéstartási nehézség, ritmuszavarok) ismeretében mérlegelni szükséges a vizsgálat indokoltságát és megfontolni más diagnosztikai eljárás alkalmazását (14).
A koronária-CTA inkonklúzív stressz-tesztet követően IIa osztályú C-szintű ajánlással javasolt, tehát a vizsgálat elvégzése megfontolandó. Csakúgy, mint azoknál a betegeknél, akiknél a stressz-teszt kontraindikált. Szintén IIa, C-indikáció vonatkozik azokra az egyénekre, akiknél kis-közepes preteszt probabilitás áll fenn és valószínűsíthetően diagnosztikus CT-képminőség érhető el, így ebben a populációban a koronária-CTA a stressz-teszt alternatívája lehet. A koronária-CTA elvégzése panaszmentes egyénekben, koszorúér-betegség gyanúja nélkül, szűrés jelleggel nem ajánlott (III. C). Emellett magas (>50%) PTP esetén, illetve sztentek vizsgálata esetén a koronária-CTA egyéni mérlegelés tárgyát képezi (1).

Plakkmorfológia

A koronária-CTA során ábrázolódott koszorúér-plakkokat morfológiájuk és mennyiségük alapján is jellemezhetjük. A vizsgálat során az obstruktív (>50% szűkület) és nem obstruktív (<50% szűkület) plakkok is nagy pontossággal ábrázolódnak (15). A koronária-CTA szubmilliméteres térbeli felbontóképessége, valamint a CT-denzitáson alapuló szöveti karakterizálás lehetővé teszi az egyes plakk-komponensek jellemzését és így megkülönböztethetünk kalcifikált, részben kalcifikált és nem kalcifikált plakkokat (16). Ezen csoportokon belül azonosíthatunk nagy rizikóra utaló plakkjellegzetességeket, komponenseket (17). Ilyen komponens a szemcsés kalcium, az alacsony CT-denzitás, a napkin ring jel, valamint a pozitív remodelláció *(2. ábra)* (18, 19).
A szemcsés kalcium definíciója alatt a 3 mm-nél kisebb átmérőjű, >130 HU denzitást mutató plakk-komponenseket értjük, amelyeket nemkalcifikált komponens vesz körül. Alacsony CT-denzitású komponensekről (<60 HU), ex vivo vizsgálatokkal bizonyították, hogy azok zsírdús plakkokat jelölnek, továbbá a vékonysapkás fibroatheromákban is jelen van alacsony CT-denzitású komponens (20). A napkin ring jel a nem kalcifikált plakkokra jellemző mintázat. Ekkor a plakk középső részén alacsony attenuációjú terület figyelhető meg, amelyet gyűrűszerűen magasabb attenuációjú terület határol. A napkin ring jel specificitása kiváló a magas kockázatú plakkok és a vékonysapkás fibroatheromák azonosítása terén (21). Pozitív remodelláción az érfal plakkokat tartalmazó szakaszának kompenzatorikus kitágulását értjük, ezáltal a lumenátmérő nem változik, szignifikáns szűkület nem alakul ki, így a betegnél panaszok nem jelentkeznek. Ezeknek a plakk-jellegzetességeknek a jelenléte a nagy kardiovaszkuláris rizikóval társul (15, 22). *Motoyama és munkatársai* 27±10 hónapos után követéses vizsgálatában az akut koronária-szindrómát szenvedett egyének korábban készült koronária-CTA-felvételeit elemezték. Az akut koronária szindróma kockázata nagyobb volt azoknál az egyéneknél, akiknél pozitív remodellációt és alacsony denzitást mutató plakk volt megfigyelhető (23).

Koszorúér-plakkterheltség

A koszorúér-plakkterheltség számításához számos szemikvantitatív és kvantitatív módszer áll rendelkezésünkre. A leggyakrabban alkalmazott plakkterheltséget



2. ÁBRA. Demonstratív ábra a nagy rizikójú plakk-komponensekről

leíró pontrendszer számítása az ateroszklerotikus plakkokkal rendelkező koronária-szegmentumok összegén alapul. Az összeadás során figyelmen kívül hagyjuk, hogy egy adott szegmentumon milyen fokú szűkület ábrázolódik. Az így számított értéket, „szegmentum érintettség pontszám"-nak nevezzük. Következő lépésben, már beszámítjuk a lumenszűkület mértékét, minimális fokú szűkülettől az elzáródott szegmentumig 5 pontos skálát használunk, és megkapjuk a „szegmentumszűkület pontszám"-ot. Ezek az egyszerűen számítható, plakkterheltséget jellemző pontszámok a kardiovaszkuláris események erős, független prediktorai (24). *Bittencourt és munkatársai* több mint háromezer koronária-CTA-vizsgálaton átesett beteget követett négy évig és az ábrázolódott koszorúér-betegség függvényében vizsgálták a kardiovaszkuláris események arányát. Eredményük alapján, azon betegek akiknél a koszorúér-betegség kiterjedtnek bizonyult (több mint 4 szegmentum érintett), de egyik szegmentumban sem volt obstruktív betegség, magasabb kardiovaszkuláris rizikóval rendelkeztek, mint azon egyének akiknél a koszorúér-betegség nem volt kiterjedt (kevesebb mint 4 szegmentum érintett), de valamelyik szegmentumon jelen volt obstuktív betegség. Tehát a lumenszűkület (obstruktív vagy nem obstruktív) mértékétől függetlenül a jövőbeli kardiovaszkuláris események számát nagyban meghatározza a betegséggel érintett koszorúér-szegmentumok száma, a betegség kiterjedtsége (25). Minél több plakk található a koszorúérrendszerben, annál nagyobb eséllyel lesz jelen egy nagy kockázatú plakk is, aminek következtében a jövőbeni kardiovaszkuláris esemény valószínűsége megnő.

A koronária-CTA során a negatív lelet is hordoz információt. Több mint 1300 koronária-CTA-vizsgálaton átesett beteg 4 év 4 hónapos utánkövetése során egyáltalán nem történt kardiovaszkuláris esemény azoknál a betegeknél, akiknél a vizsgálat koszorúér-betegség jelenlétét kizárta. Elmondhatjuk tehát, hogy egy negatív eredményű koronária-CTA minimum négy kardiovaszkuláris eseménytől mentes évet jelent (26).

Koronária-CTA vezérelte terápia

A koronária-CTA-vizsgálatok egyharmadában a lelet alapján a koszorúér-betegség kizárható, a felvételen szűkület vagy plakk nem ábrázolódik. Ilyen esetben további megerősítő vizsgálatok nem szükségesek *(3. táblázat)* (27). A vizsgálati alanyok harmada-fele esetében non-obstruktív szűkületet okozó koszorúér-betegség található. A CT alapján non-obstruktív betegcsoportban a terápia indokoltsága egyelőre nincs alátámasztva randomizált vizsgálatokkal. Olyan non-obstruktív betegek esetében, akiknél a betegség kiterjedt (több mint 4 koronária-szegmentum érintett), statinterápia mellett a kardiovaszkuláris halálozás és a miokardiális infarktusok száma csökkenést mutatott, ezért a mindennapi gyakorlatban ebben a populációban a statinterápia indítását javasoljuk, függetlenül a vér lipidértékektől (28, 29). Amennyiben obstruktív betegséget találtak a koronária-CTA során és a beteg optimális gyógyszeres terápia mellett is típusos panaszokról számol be, invazív kardiológiai konzílium javasolt.

Puri és munkatársai a koszorúér-plakkok változását vizsgálta nagy dózisú, standard dózisú statinnal és statinnal nem kezelt egyénekben. Csupán a nagy dózisú statinnal kezelt csoportban figyelték meg plakktérfogat regresszióját (0,6±0,1%), azonban a plakkok kalciumtartalma mindhárom csoportban növekedett.

3. TÁBLÁZAT: A koronária-CTA lelete alapján javasolt teendők és mérlegelendő beavatkozások (37, 38)

Koronária-CTA lelet	Javasolt teendő, mérlegelendő beavatkozás
Nincs eltérés vagy minimális koszorúér-betegség	Nem koszorúér eredetű etiológia megfontolandó Terápia a primer prevenciós ajánlások alapján
Kiterjedt nem obstruktív (<50% szűkület) koszorúér-betegség	Életmódbeli változtatások, statin, aszpirin
Nem nagy kockázatú obstruktív (>50% szűkület) koszorúér-betegség	Életmódbeli változtatások, statin, aszpirin Iszkémia elleni gyógyszeres terápia a panaszok csökkentése érdekében Funkcionális vizsgálat a hemodinamikai szituáció tisztázása érdekében Terápiarefrakter panaszok és kiterjedt iszkémia esetén intervenció
Nagy kockázatú obstruktív (>50% szűkület) koszorúér-betegség	Életmódbeli változtatások, statin, aszpirin Iszkémia elleni gyógyszeres terápia a panaszok csökkentése érdekében Intervenció mérlegelendő

A kalciumtartalom növekedése egyik csoportban sem mutatott korrelációt a lipoprotein- vagy a CRP-szinttel. A statinterápia tehát hozzájárul – a plakk-regressziót okozó hatásától függetlenül – a koszorúér-plakkok kalcifikációjához, azaz a plakkok stabilizálódásához. Ezen megfigyelés ismeretében a szekunder prevencióban részesülő betegek esetleges után követésére a Ca-score értéke önmagában nem alkalmas (30).

Az ISCHEMIA és DISCHARGE nemzetközi vizsgálatok befejezését kiemelt figyelem kíséri, mindkét vizsgálat fontos eredményeket hozhat a stabil anginával rendelkező betegek kivizsgálását és kezelését illetően. Az ISCHEMIA-vizsgálatba az iszkémia provokációs teszten legalább közepes fokú iszkémiát mutató betegek kerülnek beválasztásra. Minden betegnél történik koronária-CT-angiográfia és amennyiben obstruktív betegség igazolódik, de az nem a bal főtörzset érinti, a betegek randomizációra kerülnek, konzervatív terápia vagy invazív angiográfia ágra.

A DISCHARGE-vizsgálatba olyan közepes rizikójú, stabil anginás betegek kerülnek beválasztásra, akiknél invazív angiográfiára klinikai indikációja áll fenn. A betegek a beválasztás után invazív angiográfia vagy koronária-CT-angiográfia ágra randomizálódnak. A DISCHARGE a koronária-CT-angiográfia „kapuőr” szerepét vizsgálja az invazív vizsgálat előtt.

Az American Heart Association (AHA) ajánlása

Az AHA 2013-ban közzé tett ajánlásában a Ca-score meghatározását a kockázatbesorolást pontosító leghasznosabb vizsgálatnak ítélték azon betegek esetében, akiket a standard rizikóbecslés a közepes kategóriába sorolt (31). A Ca-score alapján történő rizikóbecslés pontosabbnak bizonyult, mint a CRP vagy más biomarkerek fehasználásával végzett rizikóbesorolás (32). Már a 2010-ben publikált AHA-ajánlásban közepes rizikójú, panaszmentes egyének kardiovaszkuláris rizikóbecslésére IIa szintű ajánlásként szerepelt a Ca-score vizsgálata (33). A Ca-score értéke a erős független prediktornak bizonyult az általános populációban, az idősekben és a cukorbetegekben is. Az AHA-ajánlás külön kiemeli, hogy a Ca-score alapján nemcsak az anti-ateroszklerotikus terápiára állítandó betegek azonosíthatók, hanem a kalcifikáció nélküli betegek esetében kiderül az, hogy kiknél nincs szükség statin- és/vagy aszpirinterápiára (34).

A NICE ajánlása

A National Institute for Health and Care Excellences (NICE) a legújabb ajánlását kiegészítette 2017 márciusában, amelyben negatív kardiovaszkuláris anamnézissel rendelkező új keletű anginás betegek nem invazív kivizsgálását elemezték költséghatékonyság szempontjából (27). A stabil anginával jelentkező betegeknél a koronária-CTA-vizsgálatot alacsony ára és magas szenzitivitása, valamint az alacsony szövődményráta miatt elsővonalbeli tesztnek ítélték, és ezért az Egyesült Királyságon belül a következő években a vizsgálatok számának 700%-os növekedését várják (35). Fontos kiemelni, hogy a koronária-CTA anatómiai diagnózist nyújt, funkcionális információt önmagában nem hordoz. A koronária-CTA dobutamin stressz-echokardiográfiával kiegészítve azonban a költséghatékonyság terén a második volt a sorban az Egyesült Királyságban (35). A NICE irányelve alapján a koronária-CTA-vizsgálatnak „kapuőr” szerepet kellene betölteni az invazív angiográfiára kerülők betegek esetében.

Jövőbeni fejlesztési irányok

A koronária-CTA több mint tizenöt éves múlttal rendelkezik a koszorúér-betegség azonosításában és a szűkület mértékének meghatározásában. A koronária-CTA legújabb fejlesztési irányai nagy hangsúlyt fektetnek arra, hogy a vizsgálatból funkcionális információ is kinyerhető legyen. A koronária-CTA által ábrázolt anatómiai információt computational fluid dinamikával kombinálva Frakcionális Flow Rezerv (FFR) értékhez juthatunk. A CT-alapú FFR-értékek invazív vizsgálat nélkül segítenek azonosítani a lézióspecifikus iszkémiát, mindezt három dimenzióban, a teljes koszorúér-rendszerre vonatkozóan. A mérés elvégzéséhez elegendő a koronária-CTA-felvétel önmagában. Az FFR-CT-vizsgálattal tovább erősödne a koronária-CTA „kapuőr” szerepe az invazív angiográfia előtt (36).

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Szív-CT szerepe a cardiovascularis rizikóbecslésben

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A cardiovascularis rizikóbecslést leggyakrabban nagy statisztikákon alapuló pontrendszerek alapján végezzük. Ezen pontrendszerek azonban túlbecslik a kis rizikóval rendelkező betegek cardiovascularis kockázatát és alulértékelik a nagy rizikójú betegek kockázatát. Fontos körülmény továbbá, hogy a cardiovascularis események egyharmadát nem képesek előre jelezni. A coronariák komputer tomográfiás (CT) vizsgálata pontosíthatja a cardiovascularis rizikóbecslést és lehetővé teheti a személyre szabott rizikócsökkentést és a prevenciós terápia beállítását.

Epidemiológia

A szív- és érrendszeri megbetegedések világszerte a vezető halálokok közé tartoznak, az Európai Unióban évente mindegy 4,1 millió életet követelnek. A nagy kockázatú, panaszmentes betegek azonosítása nagy kihívást jelent a klinikusok számára. Az esetek kétharmadában ugyanis a koszorúér-betegség első klinikai manifesztációja a szívinfarktus vagy a hirtelen szívhalál.

Koszorúér betegség kizárása mellkasi fájdalom esetén, alacsony-közepes cardiovascularis rizikó mellett
Ischaemia provokációs teszt (stressz EKG, szívizom perfúzió szcintigráfia) eredménye nem egyértelmű
Akut mellkasi fájdalom alacsony-közepes rizikójú betegeknél, normális/bizonytalan EKG esetén biomarker eltérés nélkül (Triple-rule-out)
Aorto-coronariás bypass graftok ellenőrzése
Coronaria stent beültetés után, amennyiben a stent átmérő >3 mm
Preoperatív vizsgálat nem szíven végzett műtét előtt közepes cardiovascularis rizikó mellett
Új keletű szívelégtelenség etiológiájának tisztázása
Koszorúér anomália alapos gyanúja esetén
Percutan coronaria intervenció tervezése
Az invazív coronaria angiográfia után fennmaradt kérdések tisztázása

1. táblázat: A szív-CT vizsgálat főbb indikációi.

A szívinfarktus hátterében leggyakrabban egy vulnerábilis, atherosclerotikus plakk hirtelen kialakuló megrepedése, majd ennek következtében létrejövő koszorúér-trombózis áll. A koszorúér-atherosclerosis klinikai standard diagnosztikáját az invazív koronarográfia („érfestés") jelenti, mely a súlyos fokú coronaria lumenszűkület azonosítása mellett lehetővé teszi az azonnali beavatkozást stent beültetés formájában. Fontos körülmény azonban, hogy az invazív koronarográfiák 50–60%-ban nem társulnak stent beültetéssel, hanem csupán diagnosztikus célból történnek (1).

Nem-invazív koronarográfia

A szív-CT az egyik legmodernebb nem invazív képalkotó eljárás. A szív-CT-vel a koszorúérszűkület fokának (minimális, enyhe, közepes, súlyos) megítélésén túl információt kapunk az atherosclerotikus plakkok („érfali felrakódások") kiterjedéséről és szerkezetéről, ezáltal diagnosztizálhatjuk a panaszokat még nem okozó elváltozásokat is (2, 3). A korszerű, 256-szeletes CT-berendezésekkel a szív-CT vizsgálat két szívdobbanás alatt elvégezhető, mely a beteg számára minimális sugár- és kontrasztanyag-terheléssel jár.

A mindennapi kardiológiai gyakorlatban a szív-CT legfontosabb indikációs köre a súlyos fokú coronaria-atherosclerosis kizárása mellkasi panaszokkal rendelkező, kis vagy közepes cardiovascularis rizikójú betegek körében. További fontos indikációs kört jelentenek az akut mellkasi fájdalommal rendelkező betegek, akiknél az EKG nem mutat ischaemiára jellemző elváltozást és cardialis enzimemelkedés sem mérhető *(1. táblázat).*

A szív-CT vizsgálat abszolút kontraindikációi közé tartozik a terhesség, az anamnézisben szereplő kontrasztanyag-allergia, haemodinamikai instabilitás és a veseelégtelenség.

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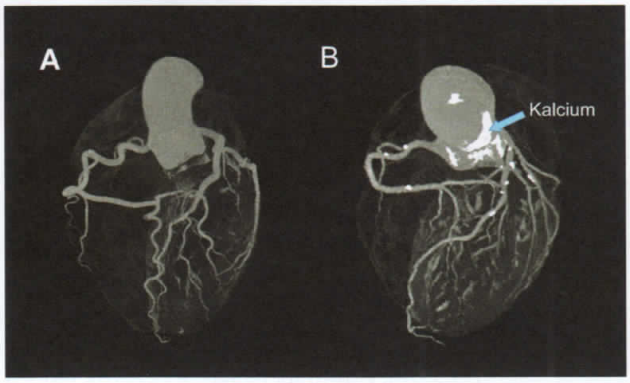


1. ábra: Szív-CT felvételek 3D rekonstrukciós képei. A bal oldali panelen nem ábrázolódik coronaria kalcifikáció a koszorúerek lefutása mentén. A jobb oldali panelen mindhárom koszorúeret érintő kalcifikáció ábrázolódik, valamint az aorta billentyűn is jelentős meszesedés látható.

Kalcium-score vizsgálat

A kalcium-score vizsgálat során igen alacsony sugárdózissal, natív (kontrasztanyag adása nélküli) felvételeket készítünk a koszorúerekről szív-CT segítségével. A natív felvételeken speciális programok segítségével lehetőség nyílik a koszorúérrendszerben jelen lévő kalcium vagy meszesedés mennyiségének mérésére. A meszesedés mértékét pontrendszerek segítségével jellemezzük, így egy összesített pontértéket (score) kapunk. A kalcium-score a klasszikus rizikófaktoroktól független módon képes előre jelezni a cardiovascularis eseményeket (4). A kalcium-score mérése közepes cardiovascularis rizikóval rendelkező panaszmentes egyének körében alkalmazható a rizikó pontosítására és a prevenciós stratégia felállítására. Amennyiben a koszorúerekben meszes plakk ábrázolódik (kalcium-score nagyobb mint nulla), a hagyományos cardiovascularis rizikófaktoroktól függetlenül statin-terápia bevezetése lehet indokolt. Tehát a kalcium-score a személyre szabott statin-terápia beállításában nyújthat fontos segítséget (5) *(1. ábra)*.

2. ábra: Szív-CT plakk-klasszifikáció. Panel A: a bal elülső leszálló ág proximális szakaszán kalcifikált plakk ábrázolódik. Panel B: a coronaria proximális szakaszán részben kalcifikalt plakk látható. Panel C: nem kalcifikalt plakk. A keresztmetszeti képeken a narancssárga vonal a coronariafalat, a citromsárga vonal a lument jelöli.

Plakkmorfológia

Napjainkban használt diagnosztikus vizsgálatok leginkább a koszorúér lumenszűkületének mértékéről, valamint a myocardialis ischaemia jelenlétéről adnak információt. Kórbonctani vizsgálatok feltárták, hogy a szívizominfarktust és/ vagy hirtelen szívhalált szenvedett betegek kétharmadánál a coronaria-thrombosist a korábban érdemi lumenszűkületet nem okozó sérülékeny ún. vulnerábilis plakk rupturája okozza (6).

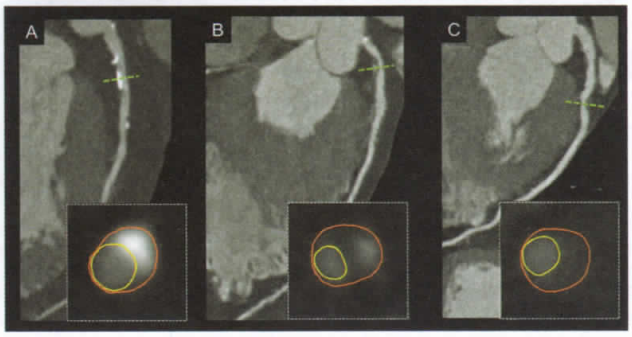
A szív-CT lehetővé teszi az egyes plakk-komponensek jellemzését. Nemzetközi klinikai vizsgálatok alapján a megnövekedett plakktérfogat igen erős összefüggést mutat az szívinfarktus bekövetkeztének valószínűségével. A nagy kockázatú plakkokra jellemző morfológia ismerete lehetővé teheti a pontosabb rizikó stratifikációt, hozzájárulhat a személyre szabott prevenciós terápia beállításához.

Plakk jellemzők

A kalcifikált plakk-komponensek alapján a plakkokat alapvetően három csoportba sorolhatjuk. Kalcifikált, részben kalcifikált, valamint nem kalcifikált plakkok *(2. ábra)* (7). A nagy kockázatú plakkok leggyakrabban a nem kalcifikált csoportból kerülnek ki. A közelmúltban publikált tanulmányok szerint, amennyiben a koszorúerekben nagy kockázatú plakk azonosítható a CT felvételen, a vizsgált betegek egyötödében, 1–3 éven belül cardiovascularis esemény következik be (8, 9). Szövettani vizsgálatok igazolták, hogy a nagyméretű zsírdús magot tartalmazó plakk a szív-CT felvételeken jellemző mintázatot, ún. asztalkendő gyűrű jelet mutat *(3. ábra)*, (10, 11). Az asztalkendő-gyűrű nagy pontossággal jelzi előre a szívinfarktust (12).

A koszorúér plakkterheltség

A plakkok jelenlétét a koszorúér plakkterheltséggel is jellemezhetjük. A koszorúér plakkterheltség összegét megkapjuk, ha az atherosclerotikus plakkot tartalmazó coronaria-szegmentumok számát összeadjuk. A plakkterheltség a klasszikus rizikófaktoroktól független becs-



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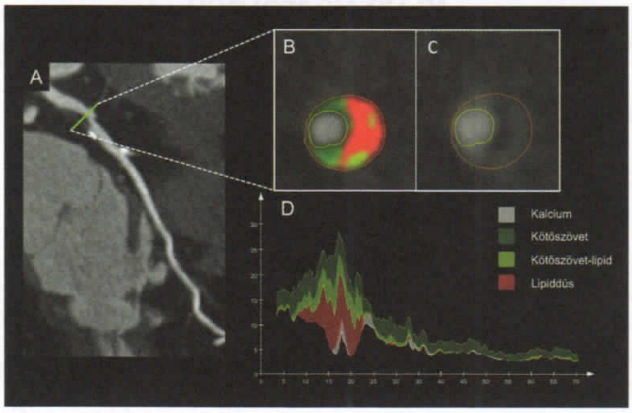

3. ábra: Szemi-automatikus plakk-kvantifikáció speciális szoftver segítségével. Panel A: Bal elülső leszálló ág proximális szegmentumán részben kalcifikált plakk látható. Plakk-keresztmetszet CT-denzitások alapján színkódolva (B panel). A színkódolt és a szürkeárnyalatos (C panel) keresztmetszeteken napkin-ring jel mintázat ábrázolódik. A D panelen látható coronariafal keresztmetszetének denzitástérképe a szájadéktól számított különböző távolságokban.

lést tesz lehetővé a cardiovascularis eseményeket illetően. Nemzetközi vizsgálatok alapján a plakkterheltség növekedésével a szívinfarktusok száma nőtt, viszont az ischaemiás myocardium-tömeg növekedésével kapcsolatban ez nem volt kimutatható (13).

Plakk-regresszió és -stabilizáció

Az ASTEROID (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden) vizsgálat során összesen 349 beteget kezeltek rosuvastatinnal 24 hónapon át, napi 40 mg dózissal. A vizsgálat kezdetekor, valamint a 2 éves utánkövetés végén meghatározták a koszorúér plakkterheltséget. Vizsgálták a koszorúrér plakkterheltség százalékos változását, a vér lipidértékek szintváltozásának függvényében. A gyógyszeres terápia hatására az LDL-értékek megfeleződtek és a koszorúér plakktérfogat megközelítőleg 7%-kal csökkent. Az ASTEROID tanulmány alapján elmondhatjuk, hogy az LDL célérték alá való csökkentésével a koszorúérplakkok progressziója megállítható és számos esetben regresszió is megfigyelhető (14).

A YELLOW-vizsgálat a rövid időtartamú, intenzív statin-kezelés (40 mg rosuvastatin) és a standard statinkezelés plakkösszetételre gyakorolt hatását vizsgálta. A 87 többér-betegségben szenvedő beteg legalább egy súlyos szűkületet okozó koszorúér plakkját 7 hetet követően vizsgálták. Az eredmények alapján az intenzív kezelést kapott csoportban a plakk lipidtartalma szignifikánsan csökkent a standard kezelést kapott csoporthoz képest (15). Elmondható tehát, hogy már rövid ideig tartó, intenzifikált statin-kezeléssel plakkstabilizáció érhető el.

Összefoglalás

Szív-CT-vel lehetőség nyílik a koszorúerek mésztartalmának meghatározására, koszorúér plakkterheltség számítására, valamint a nagy kockázatú plakkok korai azonosítására. A nagy kockázatú, rupturára hajlamos plakkok időben történő azonosítása lehetőséget teremthet az akut coronaria események jövőbeni megelőzésére. Intenzifikált statinterápiával plakkregresszió és plakkstabilizáció érhető el.

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ORIGINAL ARTICLE

Environmental Factors Slightly Outweigh Genetic Influences in the Development of Pancreatic Lipid Accumulation: A Classical Twin Study

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Abstract

Background: Several studies showed that lipid accumulation in the pancreas (NAFPD: nonalcoholic fatty pancreas disease) may lead to different pancreatic disorders, including beta-cell dysfunction. The role of genetic and environmental factors in pancreatic lipid accumulation is unclear. We evaluated the magnitude of genetic and environmental impact on pancreatic lipid content within a cohort of adult twin pairs.

Patients and Methods: We investigated 136 twin subjects [monozygotic (MZ, $n=86$) and dizygotic (DZ, $n=50$) same-gender twins (age 57.7 ± 9.1 years; body mass index [BMI] 28.0 ± 4.4 kg/m^2; females 64.7%)] with a 256-slice computed tomography (CT)-scanner. Using nonenhanced CT images, we calculated the average value of pancreatic attenuation expressed in Hounsfield unit (HU) suggesting pancreatic lipid content. Crude data were adjusted to age, sex, BMI, and hemoglobinA1c values. Intrapair correlations were established, and structural equation models were used for quantifying the contribution of additive genetic (A), common environmental (C), and unique environmental (E) components to the investigated phenotype.

Results: The study cohort represented a moderately overweight, middle-aged Caucasian population. Average pancreatic attenuation was 48.9 ± 11.9 HU in MZ and 49.0 ± 13.0 HU in DZ twins ($P=0.934$). The intrapair correlation between HU values was stronger in MZ compared to DZ twins (rMZ = 0.536, $P<0.001$; rDZ = 0.115, $P=0.580$). Using the structural equation model, a greater unique environmental influence [E: 54%, 95% confidence interval (CI) 19%–66%] and a moderate additive genetic dependence (A: 46%, 95% CI 34%–81%) were found.

Conclusions: The results of our classical twin study indicate that environmental (lifestyle) influences slightly outweigh genetic effects on the phenotypic appearance of pancreatic lipid accumulation known as NAFPD.

Keywords: ectopic fat, heritability, nonalcoholic fatty pancreas disease (NAFPD), pancreas, twins, twin study

Introduction

ALTHOUGH THE FIRST human autopsy investigation about the association between pancreatic fat and obesity was published as early as 1933, the clinical consequences of lipid accumulation in the pancreas remained unrevealed for a long period of time.[1] Nevertheless, fat deposition in the pancreas (fatty pancreas), termed nonalcoholic fatty pancreas disease (NAFPD), has gained much attention in the last years.[2–9] It was due, at least in part, to the development of imaging techniques enabling to perform clinical investigations to characterize the clinical significance of pancreatic lipid accumulation. There is a consensus that NAFPD is associated with increasing age, body mass index (BMI), and other factors of the metabolic syndrome.[3,9–12] Furthermore, lipid accumulation in pancreas may promote the development of

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chronic pancreatitis and exacerbate the clinical picture of acute pancreatitis.[13–15] It was also suggested that pancreatic steatosis promotes dissemination and worsens prognosis of pancreatic cancer.[16]

The potential relationship of pancreatic lipid accumulation with β cell dysfunction is debated.[17] A study with Korean subjects documented with computed tomography (CT) that pancreatic volume and fat deposition might be associated with the development and progression of Type 2 diabetes.[18] Namely, the authors investigated four groups of patients from normal glucose tolerance to overt Type 2 diabetes with different duration and found that decreasing pancreatic volume and increasing lipid accumulation were associated with increasing duration of diabetes.[18] Another clinical investigation documented that pancreatic fat is negatively correlated with insulin secretion in subjects with prediabetes.[19] Nevertheless, others found in a cross-sectional clamp study that pancreatic fat content increased in individuals with prediabetes (compared to subjects with normal glucose tolerance) but without a direct relation with beta-cell function.[20] Notably, NAFPD can frequently be observed; the prevalence rate may vary between 16% and 35% depending on the method used and population investigated.[10,21,22]

The reliable measurement of pancreatic fat accumulation is challenging in the clinical practice. Abdominal ultrasonography, CT, and magnetic resonance imaging techniques were used in different clinical studies; however, these measurement methodologies differ in availability, cost, radiation dose, and reproducibility, which should be considered both in research and clinical settings.[23] A recent clinical study documented that CT attenuation indices can be used to quantify pancreatic fat volume; the results were validated by histological measurements.[24]

Lipid accumulation in different organs may be triggered by genetic and environmental factors. In general, anthropometric parameters such as weight, height, and, consequently, BMI have relatively strong genetic dependence.[25] Importantly, heritability of different adipose tissue compartments and that of ectopic fats may vary.[26] Classical twin studies allow determining the effect of genetics and environmental factors on a certain phenotype. Comparing data of monozygotic (MZ) versus dizygotic (DZ) same-gender subjects, the contribution of genetics and environment to the investigated phenotype (in this case: CT density representing pancreatic lipid content) may be quantified. We could identify in the literature only a small study with eight healthy MZ twin pairs where effects of physical activity on hepatic and pancreatic fat contents were investigated.[27]

The role of genetic and environmental factors in the development of NAFPD is unclear. Therefore, the aim of our study was to evaluate the magnitude of genetic and environmental impact on pancreatic lipid content within a cohort of healthy adult twin pairs using standardized measurements of nonenhanced CT imaging.

Subjects and Methods

Study population

This study was a prospective, single-center classical twin study involving MZ and DZ same-gender twin subjects of self-reported Caucasian ethnicity. The investigation was conducted under the name of BUDAPEST-GLOBAL (Burden of atherosclerotic plaques study in twins—Genetic Loci and the Burden of Atherosclerotic Lesions) clinical study; the participants had been co-enrolled with the large, international, multicenter GLOBAL clinical study (www.ClinicalTrials.gov: NCT01738828).[28] Detailed study description and enrollment criteria were published previously.[29] The study population was recruited from the Hungarian Twin Registry.[30] All subjects provided written informed consent. The study was approved by the National Scientific and Ethics Committee [institutional review board number: ETT TUKEB 58401/2012/EKU (828/PI/12), Amendment-1: 12292/2013/EKU (165/2013)] and was carried out according to the principles stated in the Declaration of Helsinki.

In the current study we included 68 twin pairs (136 twin subjects; 86 women, 50 men); 33 twin pairs from the original cohort were excluded due to inadequate image quality as imaging was tailored for cardiac CT acquisition. We assessed zygosity using a multiple self-reported questionnaire[31] and, accordingly, our study population consisted of 43 MZ and 25 DZ same-gender twin pairs. For evaluating genetic and environmental influences on pancreas lipid accumulation, we investigated CT attenuation of the pancreas to quantitate pancreatic lipid accumulation. This method was earlier validated by histologic assessment and has been accepted for assessing NAFPD in clinical settings.[18,24]

CT scanning protocol

For the original study a noncontrast enhanced CT scan of the heart was performed with a larger coverage to visualize the upper part of the abdomen.[29] Importantly, the native CT image acquisition resulted in a small (<1 mSv) radiation dose.

Measurements of pancreatic CT attenuation were performed in a blinded manner, that is, researchers who performed CT measurements were blinded to zygosity of twin subjects. Pancreatic CT attenuation was assessed in three regions of interests (ROIs), which were placed in the head, body, and tail and were at least 1.5 cm^2. Special attention was taken not to include the peripheral margin of the pancreas or any vasculature structures. For further analysis we used the mean values of CT attenuation measured in three ROIs of the pancreas (Fig. 1). Pancreatic attenuation was measured by two radiologists (Á.L.J., A.P., both with 5 years of experience with CT) in consensus. CT attenuation was expressed in Hounsfield unit (HU).

Anthropometric data, medical history, and laboratory analysis

We recorded basic anthropometric parameters (weight, height, waist circumference). Brachial blood pressure was measured before the CT examination. Questionnaires regarding past medical history and current lifestyle, smoking, and dietary habits were recorded for every participant. Fasting peripheral blood draw was performed before the CT examination.

Laboratory parameters were investigated using standard methods in certified laboratory.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation, whereas categorical variables are expressed as numbers and percentages. MZ and DZ twins were compared

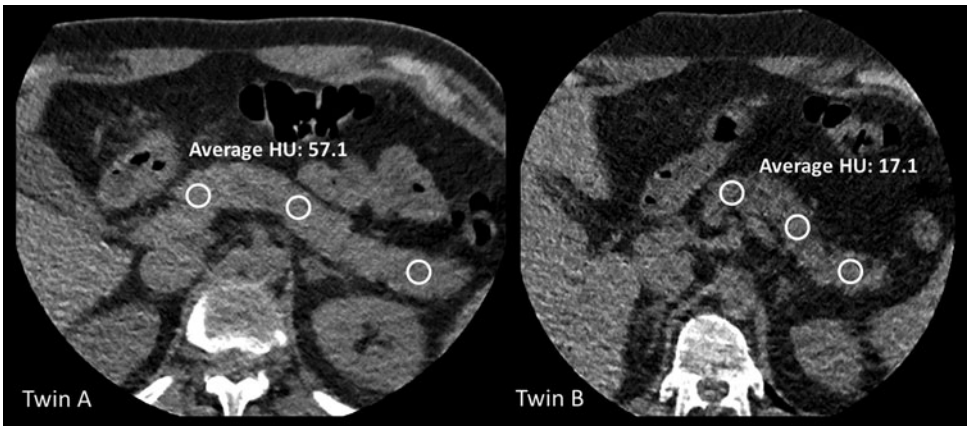

FIG. 1. Evaluation of pancreatic attenuation in a dizygotic twin pair. Measurements were performed in the head, body, and tail of the pancreas with at least 150 mm^2 sized regions of interests (*white circles*), and average values of the three measurements were used for analysis. HU, Hounsfield unit.

using Student's *t*-tests (continuous variables) or chi-squared tests (categorical variables). Correlations were calculated using Pearson correlation coefficients. Descriptive statistics, correlations, and reproducibility measurements were calculated using IBM SPSS Statistics version 23 (IBM, Armonk, NY). The level of significance was set at $P < 0.05$.

For evaluating genetic and environmental influences on NAFPD, first we assessed co-twin correlations of HU in MZ and DZ pairs separately, and then we evaluated the heritability using structural equation models often called ACE models. In the ACE model, the best fit model substantiates the quantification of genetic or environmental effects on phenotype investigated (in our study: pancreas lipid accumulation assessed by CT attenuation, expressed in HU). All models were corrected for age, sex, BMI, and hemoglobinA1c (HbA1c) values. Log likelihood-based 95% confidence intervals (CIs) were calculated for all estimated parameters. All calculations were performed using R version 3.6.0.[32] Twin modeling was performed using OpenMx version 2.12.2.[33] Using the structural equation model, the effect of genetic and environmental influences on a given phenotype can be partitioned into additive genetic effects (A), common (or shared) environmental (C), and unshared (or unique) environmental (E) factors, which drive the

Table 1. Demographic Characteristics, Medical History, and Clinical Laboratory Data of Twin Subjects

Variables	*Twin subjects (total,* n = *136)*	*MZ twin subjects (*n = *86)*	*DZ twin subjects (*n = *50)*	P *value (MZ vs. DZ twins)*
Demographic characteristics, medical history, and clinical data				
Female, *n* (%)	88 (64.7%)	54 (62.8%)	34 (68.0%)	0.540
Age (years)	57.7 ± 9.1	56.3 ± 9.5	60.1 ± 7.8	0.011
BMI (kg/m^2)	28.0 ± 4.4	27.9 ± 4.1	28.2 ± 5.0	0.723
Waist circumference (cm)	97.3 ± 11.5	96.4 ± 11.2	98.9 ± 12.0	0.244
Hypertension, *n* (%)	67 (49.3%)	41 (47.7%)	26 (52.0%)	0.627
Diabetes mellitus, *n* (%)	11 (8.0%)	7 (8.1%)	4 (8.0%)	0.977
Current smoker, *n* (%)	21 (15.4%)	14 (16.3%)	7 (14.0%)	0.764
Laboratory parameters				
Fasting glucose (mg/dL)	94.2 ± 15.7	93.3 ± 15.0	95.9 ± 16.9	0.371
HbA1c (%)	5.5 ± 0.6	5.5 ± 0.7	5.3 ± 0.5	0.236
Total cholesterol (mg/dL)	215.2 ± 42.3	220.0 ± 42.9	207.1 ± 40.3	0.081
LDL-cholesterol (mg/dL)	137.3 ± 18.9	141.5 ± 40.7	130.0 ± 33.9	0.078
HDL-cholesterol (mg/dL)	61.2 ± 13.5[a]	60.5 ± 13.9	62.7 ± 12.7	0.370
Triglycerides (mg/dL)	134.5 ± 74.0	141.6 ± 80.5	122.3 ± 60.0	0.112
Serum creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.877
ALT (U/L)	20.7 ± 12.2	21.7 ± 13.3	21.5 ± 8.9	0.182
AST (U/L)	22.5 ± 11.0	23.1 ± 12.1	21.5 ± 9.0	0.364
GGT (U/L)	37.5 ± 41.8	39.2 ± 40.0	34.7 ± 45.1	0.556
Insulin (μU/mL)	8.4 ± 7.1	8.8 ± 7.4	7.8 ± 6.6	0.404
C-peptide (ng/mL)	2.4 ± 1.4	2.4 ± 1.3	2.5 ± 1.6	0.561
hsCRP (mg/L)	3.0 ± 4.9	2.8 ± 2.8	3.4 ± 7.2	0.568
HOMA-IR	2.17 ± 3.22	2.33 ± 3.71	1.91 ± 2.29	0.340

Continuous variables are presented as mean ± SD, while categorical as *n* (%). MZ and DZ twins were compared using Student's *t*-tests and chi-squared tests, as appropriate. *P* values represent two-sided *P* values for independent *t*-tests (continuous variables) or chi-squared tests (categorical variables) done between the MZ and DZ twin groups.

[a]HDL-cholesterin in males: 72.3 ± 13.3 mg/dL, in females: 63.6 ± 14.3 mg/dL ($P < 0.001$).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DZ, dizygotic; GGT, γ-glutamyl transpeptidase; HbA1c, hemoglobinA1c; HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; MZ, monozygotic; SD, standard deviation.

TABLE 2. DETAILED MODEL INFORMATION REGARDING SINGLE TRAIT CLASSICAL TWIN MODEL OF COMPUTED TOMOGRAPHY-BASED PANCREAS ATTENUATION

Variable	*Full model*	*Estimated parameters*	*A*	*95% CI*	*C*	*95% CI*	*E*	*95% CI*	*Model -2LL*	*AIC*	*BIC*	*Difference to full model -2LL*	*Difference to full model* P
Pancreas (HU)	ACE	ACE	0.46	0.01–0.66	0.00	0.00–0.34	0.54	0.34–0.81	360.46	96.46	364.74		
		AE	**0.46**	**0.19–0.66**			**0.54**	**0.34–0.81**	360.46	94.46	363.67	0.00	1.00
		CE			0.30	0.07–0.50	0.70	0.50–0.93	364.41	98.42	367.63	3.95	0.04
		E					1.00	1.00–1.00	370.91	102.91	373.05	10.45	<0.001

A: additive genetic factor, C: common environmental factor, E: unique environmental factor.
AIC, Akaike information criterion; BIC, Bayesian information criterion; CI, confidence interval; HU, Hounsfield unit; LL, log-likelihood; *Bold*, best fit model.

variance in the phenotype for each twin. Additive genetic effect (A) is perfectly ($r=1.0$) correlated across MZ twins and less ($r=0.5$) correlated across DZ twins. Environmental components are grouped as common factors (C), which equally affect the siblings, and unique factors (E), which cause differences within families. Both MZ and DZ twins shared 100% of their C factors and none of their E factors. Since measurement error in the phenotype is also uncorrelated across measurements, it appears as part of the unique environmental component.

Results

Our study cohort (68 twin pairs, 136 twin subjects) represented a middle-aged, moderately overweight Caucasian population with a slight female predominance (age: 57.7 ± 9.1 years, 64.7% females, BMI: $28.0\pm4.4\,kg/m^2$, waist circumference 97.3 ± 11.5 cm). Overweight (BMI 25.0–29.9 kg/m^2) and obesity (BMI $\geq30.0\,kg/m^2$) were more frequently observed among males (44.4% and 37.5%) than in females (36.9% and 26.9%), respectively ($P=0.024$). However, males and females did not differ significantly regarding prevalence of increased waist circumference (males: >94 cm, 86.9%; females >80 cm, 76.4%, $P=0.056$). In the total cohort, mean values of laboratory parameters indicating glucose and lipid metabolism, as well as those of renal and hepatic function, were in normal or near-normal ranges; homeostasis model assessment of insulin resistance value (2.17 ± 3.22) was slightly elevated (Table 1).

There was no significant difference between MZ and DZ twin subjects regarding pancreatic CT attenuation (48.9 ± 11.9 HU and 49.0 ± 13.0 HU, $P=0.934$, respectively).

Age-, sex-, BMI-, and HbA1c-adjusted co-twin correlations between the siblings showed that MZ twins have stronger correlation of HU values than DZ twins (rMZ = 0.536, $P<0.001$; rDZ = 0.115, $P=0.580$, respectively).

Using the structural equation model, a greater unique environmental influence (E: 54%, 95% CI 19%–66%) and a moderate additive genetic dependence (A: 46%, 95% CI 34%–81%) were found. Common environmental influence was not identified (C: 0%) (Table 2).

Discussion and Conclusion

We found a moderate additive genetic and a greater unique environmental dependence of pancreatic lipid accumulation in our twin cohort indicating that development of NAFPD is mainly driven by environmental factors (lifestyle characteristics).

Importantly, we have enrolled adult twin pairs with the mean age of 57.7 ± 9.1 years. The female predominance (64.7% in our study) has been described in previous twin studies, as female twin subjects are more willing to participate in such clinical studies than males.[34]

We used nonenhanced CT images for measuring pancreatic lipid accumulation; this method was also popular among other researchers in clinical settings.[18,24] Undoubtedly, visualization of pancreas is often challenging due to inadequate coverage or image quality. In our study, we excluded some twin subjects (and their siblings) from the analysis simply due to poor pancreas image quality.

It is noteworthy that there are no consensus criteria for the CT diagnosis of pancreatic lipid accumulation. Although

absolute number of attenuations was proposed for diagnosis,[23] other authors preferred to use ratio of pancreatic to splenic attenuation or difference between pancreatic and splenic attenuation.[18,24] In our study we refrained from using derived ratios or differences as we aimed to assess genetic and environmental dependence of pancreatic lipid accumulation, and crude but not derived numbers should be considered more appropriate for assessing the phenotype in statistical analysis of a classical twin study.

Although pancreatic lipid accumulation was mainly driven by environmental factors in our study, a moderate genetic effect on pancreatic fat was also documented. This observation can be based on the results of structural equation model, although co-twin correlations (rMZ > rDZ) already suggested that genetic dependence should not be considered negligible. To our best knowledge, this is the first systematic clinical observation with twin pairs documenting slightly dissimilar impact of environmental and genetic influence on development of NAFPD.

The greater environmental and a moderate genetic effect on developing NAFPD can translate to clinical practice. As pancreatic lipid accumulation was documented even in children and adolescents with overweight,[35] early and intensive preventive efforts should be implemented to reduce or at least to halt this pathological process. All modifiable lifestyle characteristics should be appropriately treated with medical nutrition therapy, regular physical activity, or behavioral interventions. Clearly, lifestyle changes and weight management should be considered as key element for preventing or decreasing pancreatic lipid accumulation. For example, bariatric surgery resulted in a significant decrease of pancreatic fat within 12 months in obese subjects.[36] In contrast, active lifestyle resulted in a beneficial effect only on hepatic but not on pancreatic lipid contents in a Finnish twin study.[27] In a recent small study, metformin therapy for 4 months did not result in any change of pancreatic CT attenuation among patients with newly diagnosed Type 2 diabetes.[37]

Our results have to be interpreted within the context of their limitations. The sample size (136 twin subjects) is limited but comparable to other classical twin studies.[34] Lipid accumulation in the pancreas was assumed by CT attenuation without any histological validation. Nevertheless, this method is widely accepted, and histopathological correlations have been already published by others.[24] In our study, the zygosity was classified according to validated questionnaires, but this method is widely accepted in clinical studies.[31] The age of DZ pairs was somewhat higher compared to MZ pairs, but all models were corrected for age, sex, BMI, and HbA1c values in our study. Our results were derived from an adult twin Caucasian population; therefore, the generalizability of our findings is limited.

In conclusion, our classical twin study documented that unique environmental influences slightly outweighed additive genetic effects on the phenotypic appearance of pancreatic lipid accumulation known as NAFPD.

Author Disclosure Statement

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Effect of genetic and environmental influences on hepatic steatosis: A classical twin study based on computed tomography

IMAGING

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ABSTRACT

Background and aims: Non-alcoholic fatty liver disease (NAFLD) increases cardiovascular morbidity and mortality, and carries poor long-term hepatic prognosis. Data about the role of genetic and environmental factors in the hepatic lipid accumulation are limited. The aim of the study was to evaluate the genetic and environmental impact on the hepatic lipid accumulation within a cohort of adult twin pairs.

Patients and methods: We investigated 182 twin subjects [monozygotic (MZ, n = 114) and dizygotic (DZ, n = 68) same-gender twins (age 56.0 ± 9.6 years; BMI 27.5 ± 5.0 kg/m^2; females 65.9%)] who underwent computed tomography (CT) with a 256-slice scanner. Using non-enhanced CT-images, we calculated the average value of hepatic attenuation [expressed in Hounsfield unit (HU)] suggesting hepatic lipid content. Crude data were adjusted to age, sex, BMI and HbA1c values. Intra-pair correlations were established, and structural equation models were used for quantifying the contribution of additive genetic (A), common environmental (C) and unique environmental (E) components to the investigated phenotype.

Results: The study cohort represented a moderately overweight, middle-aged Caucasian population. There was no significant difference between MZ and DZ twin subjects regarding hepatic CT-attenuation (57.9 ± 12.6 HU and 59.3 ± 11.7 HU, respectively; p = 0.747). Age, sex, BMI and HbA1c adjusted co-twin correlations between the siblings showed that MZ twins have stronger correlations of HU values than DZ twins (rMZ = 0.592, p < 0.001; rDZ = 0.047, p = 0.690, respectively). Using the structural equation model, a moderate additive genetic dependence (A: 38%, 95% CI 15–58%) and a greater unique environmental influence (E: 62%, 95% CI 42–85%) was found. Common environmental influence was not identified (C: 0%).

Conclusion: The results of our classical CT-based twin study revealed moderate genetic and greater environmental influences on the phenotypic appearance of hepatic steatosis, commonly referred to as NAFLD. Favorable changes of modifiable environmental factors are of great importance in preventing or treating NAFLD.

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Introduction

Hepatic lipid accumulation in the absence of regular alcohol intake is commonly referred to as non-alcoholic fatty liver disease (NAFLD). In recent years, NAFLD has become increasingly more prevalent, affecting about 25% of adults worldwide, carrying severe vascular and hepatic outcomes [1–3]. It has been demonstrated previously that NAFLD might increase cardiovascular risk. In the Framingham Heart Study fatty liver was associated with several cardiovascular risk factors even after adjustment for other fat compartments [4]. Clinical studies have documented independent associations between NAFLD and increased incidence of cardiovascular events [5, 6]. Furthermore, NAFLD may progress to NASH (nonalcoholic steatohepatitis) and ultimately to cirrhosis, hepatocellular carcinoma, and liver failure [7]. NAFLD is often associated with obesity, type 2 diabetes, hyperlipidemia, hypertension, insulin resistance and, therefore, some authors have suggested that NAFLD should be considered as a component of the metabolic syndrome [8, 9].

In the clinical setting, various imaging modalities including ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) have been used to investigate and quantify hepatic steatosis [10–16]. In daily clinical practice, ultrasonography is routinely used but it has high intra- and interobserver variability. Nevertheless, improvements in methodology resulted in more reliable results [17, 18]. Notably, European guidelines for the management of NAFLD recommend using ultrasonography as a first choice for imaging in adults who are at risk for NAFLD [19]. Recently, CT-imaging for the measurement of hepatic steatosis has become increasingly popular in different research and clinical projects [20, 21]. Importantly, hepatic steatosis is quantifiable on non-contrast-enhanced CT-images and presents with decreased attenuation values of the parenchyma due to the inverse relationship between hepatic fat content and hepatic attenuation [10]. Although MRI proved to be more accurate measurement for evaluating hepatic lipid content, the availability of this method is limited, and its cost is relatively high. Therefore, MRI is limited and reserved mainly for research and clinical trials [22]. Undoubtedly, the exact diagnosis of NAFLD needs liver biopsy and should be based on histopathological investigations. Nevertheless, this invasive method is very rarely justified in practice [23]. Clinicians should base their diagnosis on laboratory findings, biomarkers and results of imaging procedures. Comparative studies with histopathological investigations validated the reliability and usefulness of ultrasonography, unenhanced CT or MRI [13, 21, 22].

Despite the growing knowledge about the pathogenesis of NAFLD, the entire process is still not completely understood. Data about the contribution of genetic and environmental influences on the hepatic steatosis are limited [24, 25]. In an earlier study, our working group found only negligible role for heritability of NAFLD within healthy, middle-aged twin pairs, however, lipid accumulation was visualized by using ultrasonography with its inherent limitations [26]. Currently, we intended to assess the effect of genetic and environmental influences on hepatic lipid accumulation within a cohort of adult twin pairs, undergoing CT-examination. Therefore, our objective was to evaluate the relative contribution of genetics versus environmental factors to hepatic steatosis, using CT-images available in participants of the BUDAPEST-GLOBAL clinical twin study.

Patients and methods

Study population

This study was a prospective, single-center, classical twin study involving monozygotic [MZ] and dizygotic [DZ] same-gender twins of self-reported Caucasian ethnicity. The investigation was conducted under the name of BUDAPEST-GLOBAL (Burden of atherosclerotic plaques study in twins – Genetic Loci and the Burden of Atherosclerotic Lesions) study; participants had been co-enrolled with the large, international, multicenter Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) clinical study (www.ClinicalTrials.gov: NCT01738828) [27]. Detailed study description and enrollment criteria were published previously [28]. The total study population consisted of 202 adult twin subjects (101 twin pairs) of whom 122 were MZ and 80 were same-gender DZ twin subjects. Participants were recruited from the Hungarian Twin Registry [29]. The study was approved by the National Scientific and Ethics Committee (institutional review board number: ETT TUKEB 58401/2012/EKU [828/PI/12], Amendment-1: 12292/2013/EKU [165/2013] and was carried out according to the principles stated in The Declaration of Helsinki. All subjects provided written informed consent.

The present analysis included 91 twin pairs (182 twin subjects; 120 women, 62 men); 10 twin pairs from the original cohort were excluded due to inadequate image quality. For the assessment of zygosity a self-reported questionnaire was used [30] and, based on this, 57 MZ and 34 DZ same-gender twin pairs were investigated. Main clinical and laboratory findings of twin pairs are summarized in Table 1.

Computed tomography (CT) scanning protocol

Each subject underwent a non-contrast enhanced CT-scan of the heart with a longer caudal coverage for visualizing the upper part of the abdomen (256-slice CT-scanner; Philips Brilliance iCT, Best, The Netherlands). Details of the study protocol were reported in our design paper [28]. Of note, the native CT-image acquisition resulted in a small (<1 mSv) radiation dose.

Hepatic parenchymal attenuation was measured to quantify hepatic lipid content (Fig. 1). We selected three circular regions of interest (ROI) with an area of at least 300 mm^2 on three cross-sectional images at different hepatic levels (one in the right hepatic lobe above the portal vein, one in the right hepatic lobe below the portal vein, and one in the left lobe). Special attention was taken to avoid hepatic larger vascular

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Table 1. Demographic characteristics, medical history and clinical-laboratory data of twin subjects

Variables	Twin subjects (total, n = 182)	Monozygotic (MZ) twin subjects (n = 114)	Dizygotic (DZ) twin subjects (n = 68)	p Value (MZ vs. DZ twins)
Demographic characteristics, medical history and clinical data				
Female n (%)	120 (65.9%)	70 (61.4%)	50 (73.5%)	0.095
Age (years)	56.0 ± 9.6	54.6 ± 9.7	58.5 ± 8.9	0.006
BMI (kg/m^2)	27.5 ± 5.0	27.6 ± 4.8	27.4 ± 5.3	0.848
Waist circumference (cm)	96.1 ± 13.2	96.0 ± 13.6	96.6 ± 12.8	0.758
Hypertension n (%)	75 (41.2%)	46 (40.4%)	29 (42.6%)	0.760
Diabetes mellitus n (%)	16 (8.8%)	11 (9.6%)	5 (7.4%)	0.600
Current smoker n (%)	31 (17.0%)	19 (16.6%)	12 (17.6%)	0.864
Laboratory parameters				
Fasting glucose (mmol/L)	5.31 ± 1.27	5.34 ± 1.47	5.26 ± 0.85	0.629
HbA1c (%)	5.5 ± 0.9	5.6 ± 1.0	5.2 ± 0.8	0.008
Total cholesterol (mmol/L)	5.54 ± 1.08	5.65 ± 1.13	5.38 ± 0.99	0.095
LDL-cholesterol (mmol/L)	3.47 ± 0.99	3.55 ± 1.06	3.35 ± 0.85	0.180
HDL-cholesterol (mmol/L)	1.60 ± 0.38	1.59 ± 0.40	1.64 ± 0.34	0.357
Triglycerides (mmol/L)	1.57 ± 1.06	1.67 ± 1.23	1.39 ± 0.65	0.043
Serum creatinine (μmol/L)	75.9 ± 8.8	79.6 ± 8.8	70.7 ± 8.8	0.161
ALT (U/l)	20.2 ± 11.6	21.4 ± 12.7	18.1 ± 9.0	0.043
AST (U/l)	22.2 ± 10.8	23.0 ± 12.1	20.7 ± 7.9	0.129
GGT (U/l)	34.8 ± 39.1	37.4 ± 28.9	30.6 ± 39.4	0.260
Insulin (μU/mL)	7.9 ± 7.5	8.4 ± 8.2	7.1 ± 6.0	0.250
C-peptide (ng/mL)	2.3 ± 1.4	2.2 ± 1.3	2.3 ± 1.5	0.635
hsCRP (ng/mL)	2.9 ± 4.5	2.7 ± 2.9	3.1 ± 6.4	0.658

Continuous variables are presented as mean ± SD, while categorical as n (%). P values represent two-sided p values for independent t-tests done between the monozygotic (MZ) and dizygotic (DZ) twin groups. BMI: body mass index, hsCRP: high sensitive C-reactive protein, HbA1c: hemoglobinA1c, HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transpeptidase

structures [21]. For further analysis we used the mean values of CT-attenuation measured in three ROIs of the liver. Hepatic attenuation was measured by two radiologists (ZD, ÁLJ both with 5 years of experience with CT) in consensus. CT-attenuation was expressed in Hounsfield unit (HU). Readers were blinded for the zygosity of subjects investigated.

Anthropometric data, medical history and laboratory analysis

We recorded basic anthropometric parameters (weight, height, waist circumference) in each subject. Brachial blood pressure was measured prior to the CT-exam. We used questionnaires for evaluating past medical history and current lifestyle (smoking, dietary habits, physical activity). Fasting peripheral blood draw was performed before the CT-examination and we used standard methods in certified laboratory for assessing laboratory parameters.

Statistical analysis

For evaluating genetic and environmental influences on NAFLD, first we assessed co-twin correlations of HU in MZ and DZ pairs separately, and then we evaluated the

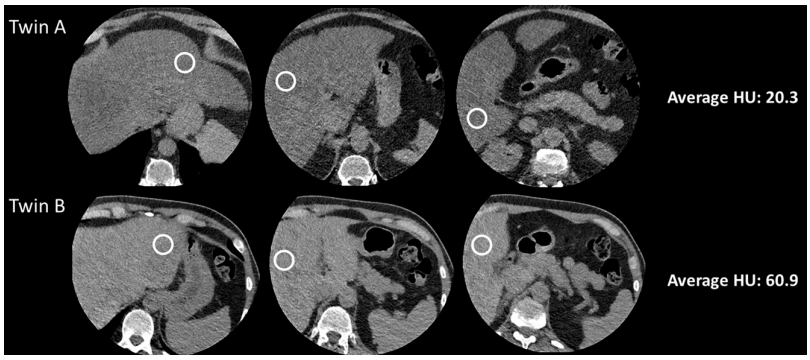

Figure 1. Evaluation of hepatic attenuation in a dizygotic twin pair. Measurements were performed in three regions of interest with at least 300 mm^2 (white circles), and average values of the three measurements were used for analysis. HU: Hounsfield unit

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heritability using structural equation models often called ACE models. In the ACE model, the best fit model substantiates the quantification of genetic or environmental effects on phenotype investigated (in our study: hepatic lipid accumulation assessed by CT-attenuation, expressed in HU). All models were corrected for age, sex, BMI and HbA1c values. Log likelihood-based 95% confidence intervals (CI) were calculated for all estimated parameters. The most parsimonious model best describing our data was found by eliminating, C (AE model), A (CE model) or AC (E model). Deterioration of fit was assessed using −2*log-likelihood values. All calculations were performed using R version 3.6.0. [31]. Twin modelling was performed using OpenMx version 2.12.2. [32]. Using the structural equation model, the effect of genetic and environmental influences on a given phenotype can be partitioned into additive genetic effects (A), common (or shared) environmental (C) and unshared (or unique) environmental (E) factors, which drive the variance in the phenotype for each twin. Additive genetic effect (A) is perfectly ($r = 1.0$) correlated across MZ twins and correlated less ($r = 0.5$) across DZ twins. Environmental components are grouped as common factors (C), which equally effect the siblings, and unique factors (E), which cause differences within families. Both MZ and DZ twins shared 100% of their C factors and none of their E factors. Since measurement error in the phenotype is also uncorrelated across measurements, it appears as part of the unique environmental component.

Continuous variables are expressed as mean ± standard deviation (SD) whereas categorical variables are expressed as numbers and percentages. MZ and DZ twins were compared using Student's t-tests and Chi-square tests. Correlations were calculated using Pearson correlation coefficients. Descriptive statistics and correlations were calculated using IBM SPSS Statistics version 23 (IBM, Armonk, NY, USA). The level of significance was considered as $p < 0.05$.

Results

Our study cohort (91 twin pairs, 182 twin subjects) represented a moderately overweight, middle-aged Caucasian population with a slight female predominance (Table 1). Mean values of laboratory data (including hepatic, renal and metabolic parameters) were within the normal range.

There was no significant difference between MZ and DZ twin subjects regarding hepatic CT-attenuation (57.9 ± 12.6 HU and 59.3 ± 11.7 HU, $p = 0.747$, respectively).

Age-, sex-, BMI- and HbA1c-adjusted co-twin correlations between the siblings showed that MZ twins have stronger correlations of HU values than DZ twins (rMZ = 0.592, $p < 0.001$; rDZ = 0.047, $p = 0.690$, respectively).

Using the structural equation model, the role of environmental influences was greater (E: 62%, 95% CI 42–85%) compared to a moderate, additive genetic dependence (A: 38%, 95% CI 15–58%). Common environmental influence was not identified (C: 0%). Detailed model information is given in Table 2.

Table 2. Detailed model information regarding single trait classical twin model of CT-based liver attenuation

Variable	Full model	Estimated parameters	A	95% CI	C	95% CI	E	95% CI	Model-*2LL*	AIC	BIC	Difference to full model -*2LL*	Difference to full model *p*
Liver attenuation (HU)	ACE	ACE	0.38	[0.05–0.58]	0.00	[0.00–0.24]	0.62	[0.42–0.85]	471.03	119.03	−320.93		
		AE	**0.38**	[0.15–0.58]			**0.62**	[0.42–0.85]	471.03	117.03	−325.43	0.00	1.00
		CE			0.23	[0.03–0.42]	0.77	[0.58–0.97]	475.71	121.71	−320.75	4.68	0.03
		E					1.00	[1.00–1.00]	480.73	124.73	−320.23	9.70	0.008

A: additive genetic factor, C: common environmental factor, E: unique environmental factor, HU: Hounsfield unit

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Discussion

Based on the results of our CT-based classical twin study, the contribution of unique environmental factors appears to have a greater role than genetic determinants in the development of hepatic steatosis.

We were uniquely positioned to address this question by performing a classical twin study which has value even in the current omics era of molecular genetic studies [33]. Our cohort consisted of middle-aged (56.0 ± 9.6 years) adult twin subjects (n = 182), with a female predominance (65.9%) and a higher number of MZ vs. DZ twin subjects (n = 114 vs. n = 68, respectively). These numbers are in line with former investigations as females and MZ twin pairs are more willing to participate in different research projects [34]. The sample size of our current investigation is also comparable to that of former classical twin studies [34].

We used non-enhanced CT-images for evaluating hepatic lipid accumulation, similarly to prior studies [10]. There is no consensus for CT-based criteria for NAFLD; both attenuation absolute values (expressed in HUs) and derived numbers (spleen-to-liver attenuation ratio or difference in attenuation values between liver and spleen) have been used [10, 21]. In our twin study, we preferred to use absolute numbers of HUs instead of derived ratios or differences. We considered more appropriate for assessing hepatic lipid content by measuring absolute attenuation values.

We found that environmental influences outweighed genetic influences in the development of hepatic lipid accumulation. Our current CT-based findings corroborate our previous investigation using ultrasonography [26], although the results of ACE models are numerically slightly different. Nevertheless, we expected a slight numerical difference as we used different methodology in a different twin population.

Our results have profound clinical implications. Since our study suggests that the development of NAFLD is predominantly influenced by environmental factors, the importance of lifestyle changes (including healthy diet, medical nutrition therapy, nutraceutical/dietary supplements, regular physical activity or behavioral intervention) is substantial in the primary prevention of NAFLD and related conditions [35, 36]. In addition, bariatric surgery in obese people may reduce NAFLD [37]. Although several different drugs have shown some potential promising results in early clinical Phase-2 and Phase-3 trials, there are no currently approved drugs for the treatment and prevention of NAFLD and NASH [38, 39].

Our study has several limitations. Hepatic steatosis was estimated based on CT-attenuation, without any histological validation. Nevertheless, unenhanced CT-based evaluation of NAFLD is accepted, and histopathological correlations have been already published by others [21]. Furthermore, while biopsy-based steatosis measurements are severely hindered by "geographic miss" [40] our CT-based approach allows for the quantification of hepatic steatosis in a large, substantial portion of the liver. In our study, zygosity was classified using validated questionnaires; a method widely used in clinical twin studies [30]. Our study was performed in adult twin subjects of Caucasian population; therefore, the generalizability of our findings is limited.

In conclusion, our classical CT-based twin study documented moderate genetic and greater environmental influences on the phenotypic appearance of hepatic steatosis commonly referred to as NAFLD. Therefore, favorable changes of modifiable environmental factors are of great importance in preventing or treating NAFLD.

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Authors' contribution: GJ, PMH conceived the study, GJ, ZDD drafted the manuscript, MK performed the statistical analysis, contributed to interpretation, ALJ, ZDD, AP, IFS, JK, ADT, DLT performed the clinical study, collected data, PMH provided continuous supervision of the study, SV, BM, PMH critically reviewed the manuscript with intellectual contribution to interpretation. GJ wrote the final version of manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read the final version of the manuscript and agreed to submit it to IMAGING for Open Access publication.

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