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CLINICAL RESEARCH
Epidemiology and prevention

Mammographic features are associated with cardiometabolic disease risk and mortality

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Aims In recent years, microcalcifications identified in routine mammograms were found to be associated with cardiometabolic disease in women. Here, we aimed to systematically evaluate the association of microcalcifications and other mammographic features with cardiometabolic disease risk and mortality in a large screening cohort and to understand a potential genetic contribution.

Methods and results This study included 57 867 women from a prospective mammographic screening cohort in Sweden (KARMA) and 49 583 sisters. Cardiometabolic disease diagnoses and mortality and medication were extracted by linkage to Swedish population registries with virtually no missing data. In the cardiometabolic phenome-wide association study, we found that a higher number of microcalcifications were associated with increased risk for multiple cardiometabolic diseases, particularly in women with pre-existing cardiometabolic diseases. In contrast, dense breasts were associated with a lower incidence of cardiometabolic diseases. Importantly, we observed similar associations in sisters of KARMA women, indicating a potential genetic overlap between mammographic features and cardiometabolic traits. Finally, we observed that the presence of microcalcifications was associated with increased cardiometabolic mortality in women with pre-existing cardiometabolic diseases (hazard ratio and 95% confidence interval: 1.79 [1.24–2.58], $P = 0.002$) while we did not find such effects in women without cardiometabolic diseases.

Conclusions We found that mammographic features are associated with cardiometabolic risk and mortality. Our results strengthen the notion that a combination of mammographic features and other breast cancer risk factors could be a novel and affordable tool to assess cardiometabolic health in women attending mammographic screening.

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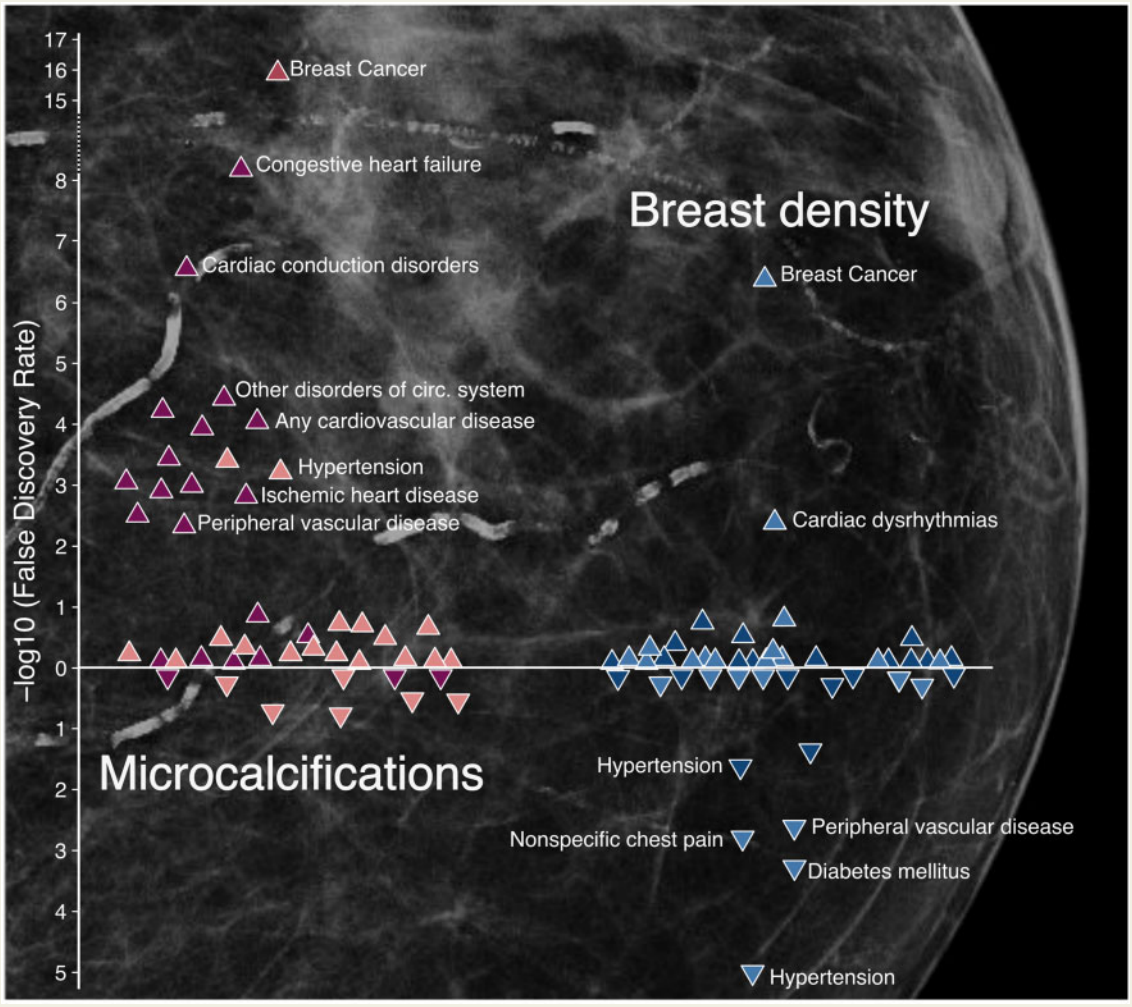
Graphical Abstract



Contrasting association of microcalcifications and breast density with cardiometabolic diseases. Leveraging mammographic features could be useful to predict cardiometabolic health in women attending mammographic screening programmes (mammogram adapted from User: Jmarchn/CC-BY-SA-3.0).

Keywords Mammographic screening • Cardiometabolic diseases • Ageing • BMI • Microcalcifications • Breast cancer genetics

Introduction

Cardiovascular disease, diabetes mellitus, chronic renal failure, and other related conditions are considered cardiometabolic diseases, which are the leading cause of morbidity and mortality in women.[1] Despite the huge individual and societal burden of cardiometabolic diseases, there are currently no effective, affordable, and comprehensive screening methods available to detect women with prevalent cardiometabolic diseases.[2–4] Among the current efforts to reduce the mortality from cardiometabolic diseases is the identification and preventive treatment of high-risk individuals.[5,6] However, identification of individuals with a high risk for cardiometabolic disease is challenging,[7] even when accounting for multiple risk factors such as obesity, hypertension, diet, smoking, air pollution and lack of physical activity.[8–10] In addition, the established risk prediction algorithms usually specific for certain age, sex, and ethnic groups and may over- or underestimate the risk in other groups.[11,12]

Similar to cardiometabolic diseases, identification of women at high risk for breast cancer (BC) is important to reduce overall mortality. Multiple risk prediction tools based on reproductive history, hormonal and life-style factors as well as genetic predisposition are available, although they perform even worse compared to established cardiometabolic risk prediction tools.[13] Therefore, mammographic screening programmes were established and have proven

effective at reducing BC mortality world-wide.[14] In addition to detecting early stages of malignant tissue, the mammographic images collected in the screening programme reveal additional features, some of which are considered to be important risk factors for BC.[15,16]

One such feature is microcalcifications (MC) that appear as small bright dots on mammograms and are calcium deposits of <1 mm in diameter. They are likely a consequence of epithelial–mesenchymal transition of epithelial cells, resulting in the formation of stiff extracellular matrix.[17] Depending on the morphology, MC are considered either benign or a sign of malignant BC.[18,19] In a previous study, increased age, family history of BC and a high genetic risk score (GRS) for BC were associated with more MC. In contrast, higher body mass index (BMI), current smoking behaviour as well as moderate to high alcohol consumption resulted in fewer MC.[20] Importantly, multiple reports indicated that mammographic MC are associated with increased prevalence and incidence of cardiovascular and coronary artery disease (summarized in[21]). Similarly, a higher number of MC are frequently observed in women with chronic renal failure,[22,23] thus strongly implicating MC to be involved in multiple cardiometabolic diseases.

Compared to MC, the connection between mammographic density and cardiometabolic diseases is less evident. Milk glands, milk ducts, and supportive tissue are dense tissue[24] and appear white on mammograms. Importantly, the amount of dense tissue is considered a risk factor for BC and can also obscure the detection of early BC stages. The dense area is correlated to most established risk factors for BC, particularly those related to increased oestrogen exposure due to the individual's hormonal and reproductive history. In contrast, the dark (non-dense) areas are indeed fatty tissue, which increase with advanced age, higher BMI, and smoking. Notably, those predictors are also major risk factors for cardiometabolic diseases as well as related conditions and, accordingly, breast density could be a useful indicator for cardiometabolic health.

Hence, in this study, we aimed to further dissect the relationship between mammographic features and cardiometabolic diseases in a large prospective mammographic screening cohort. In addition, we wanted to investigate the occurrence of cardiometabolic diseases in sisters of KARMA women and explore the effect of BC genetics on cardiometabolic diseases to improve our understanding on the shared risk between both conditions.

Methods

Study population

The Karolinska Mammography Project for Risk Prediction of Breast Cancer (KARMA) is a population-based prospective screening cohort of 70 872 women attending the mammography screening programme in Sweden from January 2011 to March 2013.[25] Reasons for exclusion are given in Supplementary material online, *Figure S1*. Briefly, women without mammographic measurements (either microcalcifications or percent mammographic density), women with prevalent BC (i.e. BC diagnosed before recruitment) as well as women with missing BMI information were excluded. Furthermore, all women who underwent breast reduction or enhancement or other breast surgeries were excluded, resulting in an analytical dataset of 57 867 women. All participants signed an informed consent form and Stockholm ethical review board approved the study (2010/958-31/1).

Measurement of mammographic features

Raw mammograms from mediolateral oblique and cranio-caudal views of left and right breasts were collected. We used a computer aided detection system (M-Vu CAD®; iCAD, Nashua, NH, USA) an FDA-approved software, class 3 device (PMA number P010038) to identify suspicious microcalcification clusters as described previously.[26] In this study, we calculated the total number of such clusters in both the breasts for each woman and considered this the main outcome/exposure.[27,28] Hereafter, we refer to those clusters as MC throughout the manuscript.

Percent mammographic density was computed from the dense area (cm^2) divided by the total area (cm^2) of the left and right breast, respectively using the STRATUS method.[29] The total percent mammographic density of a women was computed from the average percent mammographic density of both breasts. We considered women with an average breast density above the mean percent density in the study population (22.46%, standard deviation 19.56) to have dense breast tissue and used this variable as the outcome/exposure in this study.

Breast cancer genetic risk predictors

Family history of BC was ascertained using the linkage to Swedish Multigenerational Register and Swedish Cancer Registry.[26,30] We considered women to have a positive family history of BC in case any first degree relative (parents, siblings or children) were diagnosed with BC up until 31 December 2018.

The GRS for BC was computed as the effect size (log odds ratio) weighted sum of 313 BC risk increasing alleles, previously identified by the BC association consortium.[31] Briefly, we computed two scores, one indicative of increased risk for oestrogen receptor (ER) positive BC and one predisposing for ER negative BC. Thus, each of the 313 variants was multiplied (weighted) by the log odds ratio of the association of the variant with either ER positive or negative BC, respectively, as reported in.[31] Then, we computed mean of the (ER positive or ER negative) weighted allele count of all 313 variants for each individual and scaled the scores to each have a standard deviation of 1 and a mean of 0. The scores were shown to be well calibrated and predictive of high BC risk in the tails of the distribution, even in non-European populations,[32] with area under the curve (AUC) values ranging from 0.60 to 0.64. Women in KARMA were genotyped through the Breast Cancer Association Consortium (BCAC) on a custom Ilumina iSelect genotyping array as part of the Collaborative Oncological Gene-environment Study or on the OncoArray.[33–35] Quality control and imputation of missing and ungenotyped variants was performed by the BCAC. Briefly, missing genotypes were imputed to the 1000 Genomes Phase 3 reference haplotypes using Shapelt[36] and IMPUTE[37] and variants with an imputation quality (R^2) greater 0.3 and a minor allele frequency >0.01 were retained.

Covariates

Participants of the KARMA cohort completed a detailed web-based questionnaire. Established risk factors were categorized as: smoking status (never, former, current, missing), age at first birth (no birth, <20, 20–25, 25–30, 30–35, and >35 years, missing), oral contraceptive use (no, yes, missing), hormone therapy use (never, former, current, missing), and education attainment (less than nine years, high school degree, university degree). The total daily physical activity (in MET-hour/day) represents the amount of physical activity that a participant carries out per day and is computed from the sum of physical activity related to sleeping, work, transportation, leisure time, and sports. We categorized physical activity (low, medium, and high) by splitting the linear variable into tertiles.

Alcohol consumption was ascertained from questionnaire and categorized into low or none (<100 g of alcohol per week), moderate (between 100 and 250 g) and high (>250 g).[38] Hypertension was categorized into five groups according to the 2017 American College of Cardiology/American Heart Association Guideline for the Prevention, Detection, Evaluation and Management of High Blood Pressure in Adults.[39] Briefly, systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured at baseline and we categorized women as follows: both SBP/DBP <120/<80 mmHg was considered normal, 120–129/<80 mmHg was considered elevated, 130–139/80–89 mmHg was considered Stage I, 140–180/90–120 mmHg was considered Stage II, and SBP >180 or DBP >120 mm Hg was coded as hypertensive crisis. We coded BMI and age at mammogram as a continuous variable in our analyses. Women reporting no natural menstruation over the past 12 months before study entry or no menstruation due to oophorectomy were considered postmenopausal. Similarly, women with menses over the past year but no longer menstruating during the 3 months prior to study entry were considered peri-menopausal while women with menstruation in the prior 3 months were considered pre-menopausal. Lipid lowering medication was extracted by linkage to the Swedish Prescribed Drug Register, which contains all prescribed drugs dispensed at pharmacies[40] since 2005. Within this registry, we identified all women using lipid-lowering medications (ATC code C10) between 2005 and study entry/qualifying mammogram.

Cardiometabolic disease ascertainment

We extracted the ICD10 codes of disease diagnoses for all women in KARMA from the In- and Outpatient Registry as well as the Cancer Registry up until 31 December 2018. Since the outpatient registry data (as well as consistent ICD10 coding) was available starting 2001, we restricted the analyses to diagnoses between 01 January 2001 and 31 December 2018 or date of a BC diagnosis, if applicable. Sisters of KARMA participants were identified using the Swedish Multi-generational Register (MGR). Briefly, by linkage to the MGR were able to identify 91.9% of the mothers of KARMA women, which then allowed us to identify a total of 49 583 sisters of KARMA women. Cardiometabolic disease diagnoses for sisters between 01 January 2001 and 31 December 2018 were then retrieved from the same registries as above. The Swedish registries provides almost complete coverage of diseases occurring in both KARMA women and their sisters and the follow-up period is consistent for all women (median: 6.70 years, inter-quartile boundaries: 6.20–7.19 years) due to linkage to the registries occurring for all Karma women and their sisters at the same time.

The extracted ICD10 codes were mapped to phecodes with the phenome-wide association study (*PheWAS*) package[41] as implemented in R.[42] This approach combines and maps different ICD codes from electronic health records to clinically relevant outcomes, which were manually defined by clinical experts. An interactive view of the relevant mapping can be queried at https://phewascatalog.org/phecodes_icd10. For instance, ischaemic heart disease status (phecode 411) is derived from ICD codes I20–I25 (angina pectoris, acute and subsequent myocardial infarction, complications from myocardial infarction, other acute ischaemic heart diseases, and chronic ischaemic heart disease), I34 (mitral valve prolapse), and I52.0 (cardiac septal defect). The hierarchical approach also defines related diseases which are built-in exclusion criteria to prevent contamination of control individuals with cases that have related diseases.[43] In the case of ischaemic heart disease, individuals with myocardial degeneration (I51.5), other ill-defined or unspecified heart diseases (I51.8 and I51.9) would not be used as controls in the analyses. We considered all top level phecodes in our analyses relevant to cardiovascular diseases (i.e. in the *circulatory system* group) and investigated the association of mammographic features with all those phecodes. Furthermore, we included phecodes related to diabetes (phecode 249 and 250) and renal failure (585). Of note, the outcome hypertension (phecode 401) indicates that hypertension was diagnosed in a clinic (inpatient) or outpatient practice, while the hypertension status used as a covariate in our analyses was computed from the blood pressure measurements at baseline exam. We also investigated as BC (phecode 174) to serve as a point of reference for the observed effect sizes.

Since individuals with an existing cardiometabolic trait will also likely be diagnosed again with the same condition after the mammogram, we report the associations with incident cardiometabolic diseases separately for individuals with any pre-existing cardiometabolic diseases as well as individuals without the respective pre-existing condition.

Death from cardiometabolic diseases was ascertained from the Swedish Cause of Death Registry[44] for all KARMA women. Women were followed for death from cardiometabolic disease (ICD codes I00–I99 for cardiovascular diseases, N17–N19 for renal failure, and E10–E16 for diabetes) and censored in case they developed BC, died from other causes or survived until 31 December 2018. Similarly, we also computed cardiovascular mortality alone by restricting the ICD codes to those between I00 and I99.

Statistical analyses

Although the PheWAS could also be investigated by Cox regression, which has slightly more power to detect associations,[45,46] we chose to use logistic regression. This approach does not make any assumptions about whether to censor an individual at the first occurrence of a cardiometabolic diagnosis and thus also is agnostic to the trajectory of cardiometabolic diagnoses in the women over the study period. The results of the PheWAS analyses were plotted with the *phenotypePlot* function implemented in the *PheWAS* package. We controlled the false discovery rate (FDR) to be <0.05 in the PheWAS and thus considered all associations with a *Q*-value of <0.05 to be statistically significant. Statistically significant associations identified in the PheWAS (FDR <0.05) in at least one analysis were further visualized with a correlation plot in for all investigated BC risk factors using the *corrplot* function from the *corrplot* package, implemented in R. In the correlation plot, we deemed associations of genetic BC risk factors with cardiometabolic diseases with an uncorrected *P*-value of <0.05 as statistically significant. Since those analyses are not used to identify novel disease associations but rather to provide additional insights into findings identified in the discovery, we do not adjust for multiple testing in these plots.

Microcalcifications PheWAS

Each incident cardiometabolic disease occurring after the qualifying mammogram was considered as the binary outcome and we estimated the effect of the number of MC on cardiometabolic disease risk using logistic regression, adjusted for the mammographic percent density, age at mammogram, BMI, smoking status and follow-up time (i.e. time between recruitment and either 31 December 2018, the date of a BC diagnosis or date of death if applicable). To uncover potential genetic effects, we investigated the association of MC in KARMA women on the risk of cardiometabolic diseases in their sisters occurring between 01 December 2001 and 31 December 2018 with logistic regression, adjusted for the same covariates and also accounting for the number of sisters.

Mammographic percent density PheWAS

Logistic regression was used to estimate the association of percent density with incident cardiometabolic disease risk, adjusted for the number of MC, age at mammogram, BMI, smoking status, and follow-up time. Finally, we also investigated the effect of breast density measured in the KARMA women on cardiometabolic risk in their sisters with logistic regression.

Those analyses were also adjusted for the above-mentioned covariates as well as the number of sisters.

Breast cancer genetics and cardiometabolic diseases

We assessed the impact of family history of BC in close relatives on all cardiometabolic diseases (i.e. any cardiometabolic disease occurring between 01 January 2001 and 31 December 2018) with logistic regression, adjusted for age at baseline, number of sisters, number of daughters, BMI, and smoking status.

Logistic regression analyses were used to investigate the association between BC GRS and the risk of all cardiometabolic diseases (i.e. any cardiometabolic disease occurring between 01 January 2001 and 31 December 2018), adjusted for age at baseline, BMI, smoking status, and genotyping platform. We also adjusted for the first two principal components computed from the genotypes to account for potential population differences (i.e. population stratification[47]) as is standard practice in genetic association studies.

Sensitivity analyses

In a sensitivity analyses, we also present the fully adjusted association results for cardiometabolic diseases, which were significantly associated with mammographic features in the PheWAS (FDR <0.05). In those analyses, we adjusted for mammographic percent density, age at mammogram, BMI, smoking status, follow-up time, lipid-lowering medication, hypertension, physical activity, alcohol consumption, education, age at first birth, menopausal status, HRT use, and oral contraception use.

The iCAD system that was used for the automated assessment of MC from mammograms in the breast captures both vascular health and pathological changes due to breast hyperplasia; however, the method used cannot distinguish between these. To address this issue, we have also estimated the association of mammographic features with incident cardiometabolic diseases only in women, which did not develop BC during follow-up. This approach should effectively reduce the impact of malignant microcalcifications associated mainly with BC.

Survival analysis

Death from cardiometabolic diseases was assessed with Cox proportional hazard models as implemented in the *survival* package.[48] The baseline model was adjusted for age at mammogram while the extended model was additionally adjusted for smoking and BMI. The full model was adjusted for age, smoking status, BMI, lipid-lowering medication, hypertension, physical activity, alcohol consumption, education, age at first birth, menopausal status, HRT use, and oral contraception use. Since a BC diagnosis and subsequent treatment may be a significant competing risk for cardiometabolic mortality, we also performed competing risk analyses with the *crr* function from the *cmprsk* package[20] in R. Finally, the age-adjusted mortality rate and confidence intervals per 1000 person-years was computed with *ageadjust.direct* function implemented in the library *epitools* in R.[49]

Results

Study population and determinants of mammographic features

The current study included 57 867 women from the KARMA project (Supplementary material online, *Figure S1* and *Table 1*), who attended mammographic screening in Sweden between 2011 and 2013. In agreement with prior reports, we found that multiple life-style and reproductive covariates were associated with the number of

Table 1 Summary characteristics of KARMA participants at baseline

Variable	KARMA women			
	Without MC	With MC	Non-dense breast	Dense breast
Number of individuals	47 757	10 110	33 560	24 307
Age (SD) (years)	54.85 (9.68)	59.33 (9.85)	58.76 (9.33)	51.32 (8.90)
Body mass index (SD) (kg/m^2)	25.30 (4.24)	24.93 (4.17)	26.85 (4.33)	23.02 (2.86)
Systolic blood pressure (SD) (mmHg)	124.66 (17.55)	128.14 (18.33)	129.10 (17.93)	119.94 (16.01)
Diastolic blood pressure (SD) (mmHg)	75.32 (10.48)	76.21 (10.59)	77.13 (10.43)	73.19 (10.17)
Lipid lowering medication (%)	9.94	14.83	15.28	4.60
Alcohol per week (SD) (g)	49.92 (60.00)	51.12 (62.45)	50.37 (62.47)	49.80 (57.51)
Physical activity per day (SD) (MET)	42.50 (6.32)	42.04 (6.00)	41.90 (6.22)	43.15 (6.26)
Number of sisters (SD)	0.86 (0.99)	0.83 (0.99)	0.86 (1.02)	0.85 (0.96)
University degree (%)	53.36	50.09	46.95	60.85
Ever smoked (%)	53.13	52.58	56.64	48.06
Age at first birth (SD) (years)	27.39 (5.26)	26.32 (5.17)	26.24 (5.12)	28.57 (5.16)
Post-menopausal (%)	52.24	68.85	70.27	34.26
Ever taken HRT (%)	23.73	29.89	29.71	18.04
Ever taken oral contraception (%)	86.60	81.30	83.80	88.26
Family history of breast cancer (%)	10.85	12.04	10.80	11.41
ER positive BC GRS (SD)	-0.02 (1.00)	0.04 (0.99)	-0.04 (1.00)	0.04 (0.99)
ER negative BC GRS (SD)	-0.02 (1.00)	0.06 (1.01)	-0.04 (1.00)	0.05 (1.00)

BC, breast cancer; BMI, body mass index; GRS, genetic risk score with weights for oestrogen receptor positive or negative breast cancer; HRT, hormone replacement therapy; MC, microcalcifications; OCP, oral contraceptives; SD, standard deviation.

microcalcifications (MC) or percent mammographic density (i.e. dense breasts, Supplementary material online, *Figure S2*). In line with prior reports, both an increased number of MC as well as increased breast density were associated with increased risk for BC (*P*-value $<10^{-6}$, *Figure 1* and Supplementary material online, *Table S1*).

PheWAS of mammographic features

To dissect the relationship between mammographic features and cardiometabolic traits, we computed a cardiometabolic PheWAS with the mammographic features as exposure, adjusted for the age at mammogram, BMI, smoking and mammographic density or presence of macrocalcifications.

Notably, in women with pre-existing cardiometabolic disease, more MC were associated with increased relative risk for subsequent cardiometabolic diseases (*Graphical abstract*, *Figures 1* and 2, and Supplementary material online, *Table S1*). In particular, each additional MC was significantly associated with increased risk for diabetes mellitus, hypertension, congestive heart failure, cardiac dysrhythmias and cardiac conduction disorders, ischaemic heart diseases, peripheral vascular diseases, and heart valve disorders (FDR <0.05). Importantly, the absolute risk to develop any cardiometabolic disease within 5 years in women with a pre-existing condition was 57.92% and 51.81% for women with and without MC, respectively (Supplementary material online, *Table S1*).

However, in women without prior cardiometabolic conditions, we found that each additional MC detected in the screening was only associated with the risk for hypertension and congestive heart failure (FDR <0.05). In these women, the 5-year absolute risk to develop any cardiometabolic disease was 21.38% for women with MC and 19.17% for women without MC present at screening.

We found that in women with a pre-existing cardiometabolic disease, breast density was not significantly associated with any incident cardiometabolic diseases (FDR >0.05). In contrast, a higher breast density at baseline in women without cardiometabolic diseases was associated with a lower relative and absolute risk of multiple disease diagnoses such as diabetes mellitus, hypertension, chest pain and peripheral vascular disease, independently of the presence of MC (FDR <0.05, *Figures 1* and 2 and Supplementary material online, *Table S1*).

In addition to those analyses, we performed a sensitivity analysis to investigate the role of potential confounders on our results. To this end, we computed the association of mammographic features with cardiometabolic traits additionally adjusted for lipid-lowering medication, hypertension, physical activity, alcohol consumption, education, age at first birth, menopausal status, HRT use, and oral contraception use (Supplementary material online, *Figure S3* and Supplementary

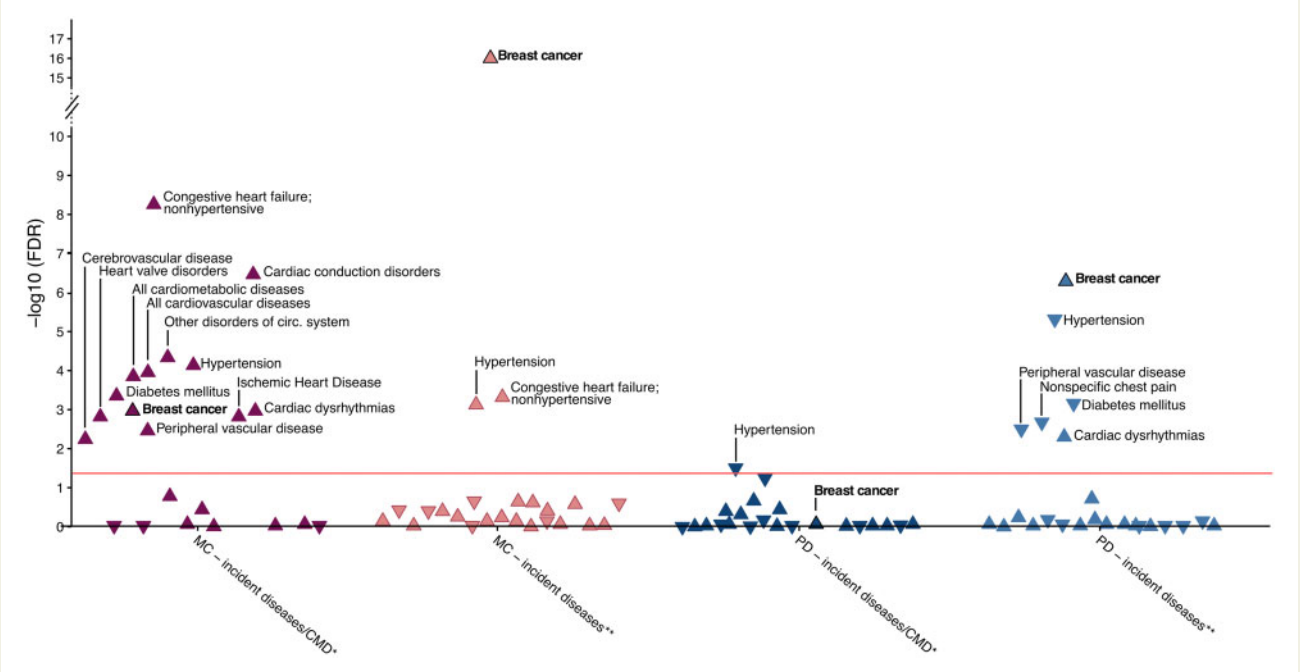


Figure 1 Association of mammographic features with incident cardiometabolic diseases in the KARMA cohort. We conducted phenome-wide association studies for microcalcifications and percent mammographic density (PD). Using logistic regression, we computed the association of an increased number of microcalcifications or dense breasts with cardiometabolic disease risk, adjusted for age, body mass index, smoking status and percent mammographic density or microcalcifications, respectively. Each triangle represents one association. The orientation of the triangles indicates the direction of association, with triangles pointing up indicating increased risk and triangles pointing down reduced risk to develop the respective disease. On the horizontal axis, associations are grouped according to the main exposure (microcalcifications or percent mammographic density) as well as the cohort used in the analyses (with or without pre-existing cardiometabolic disease). The red horizontal bar represents the cut-off for significance at a false discovery rate of <0.05. Breast cancer disease risk is shown to enable the comparison of effect sizes observed for cardiometabolic diseases to those observed for breast cancer. *Incident diseases in women with pre-existing cardiometabolic disease; **incident diseases in patients without pre-existing cardiometabolic disease.

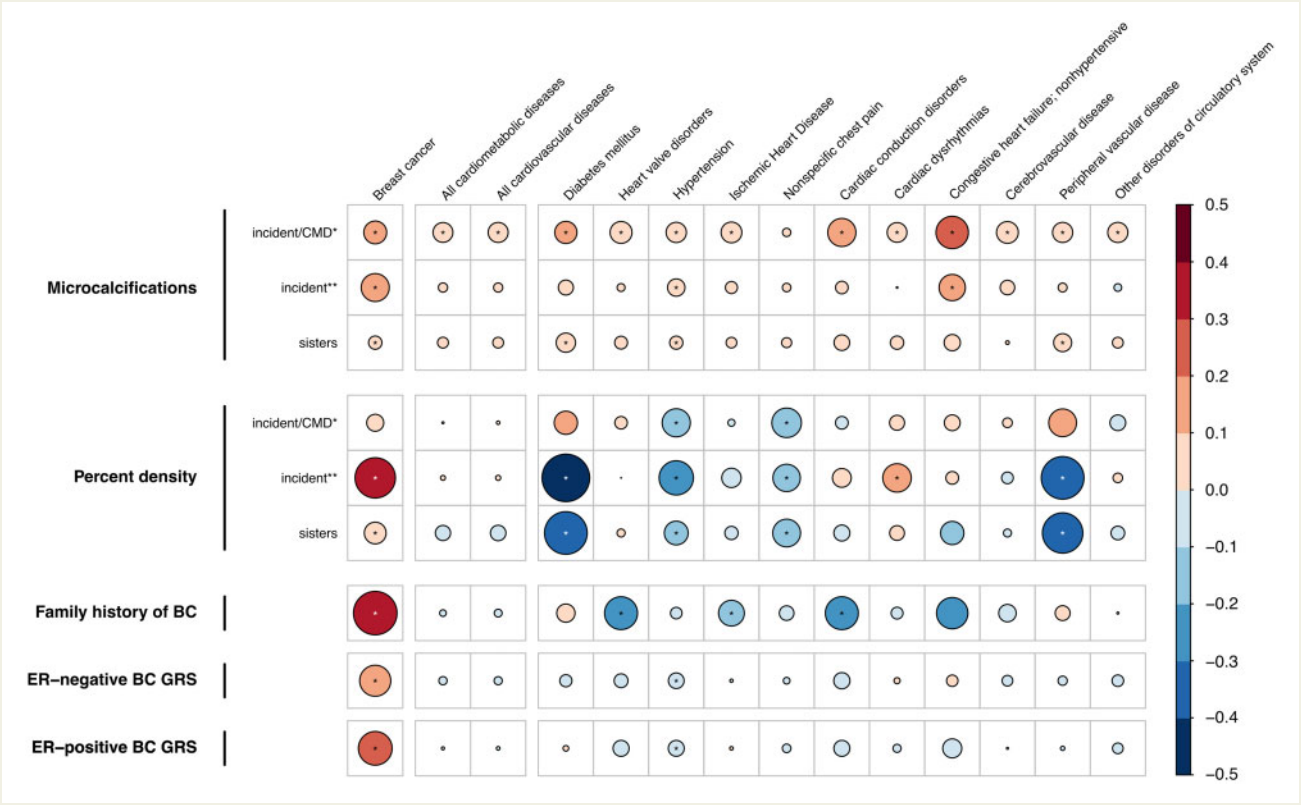


Figure 2 Association of mammographic features and genetic breast cancer risk factors with cardiometabolic diseases. Depicted are the association results of mammographic features and breast cancer risk factors with cardiometabolic diseases, which were statistically significant in the PheWAS (false discovery rate <0.05). The size and colour of the circles indicate the beta of the association (i.e. log odds ratio), while an asterisk indicates statistical significance (*P*-value <0.05). Breast cancer disease risk is shown to enable the comparison of effect sizes observed for cardiometabolic diseases to those observed for breast cancer. GRS = genetic risk score with weights for oestrogen receptor positive or negative breast cancer; *incident diseases in patients with pre-existing cardiometabolic disease; **incident diseases in patients without pre-existing cardiometabolic disease.

material online, *Table S1*). In those analyses, the effect sizes and statistical significance remained largely unchanged, indicating that the observed associations are independent of those reproductive and life-style factors. Further, we have estimated the association of mammographic features with incident cardiometabolic diseases only in women which did not develop BC during follow-up, again adjusted for the same covariates as above. We found that exclusion of women who developed BC during follow-up had little to no effect on the observed associations (Supplementary material online, *Figure S3* and Supplementary material online, *Table S1*).

Genetic dissection of the observed associations

To further investigate a potential genetic contribution to the observed associations, we investigated the association of mammographic features present in KARMA women with cardiometabolic diseases diagnosed in their sisters. We found that an elevated number of MC observed in the KARMA participants was associated with a significantly increased risk for BC, diabetes, hypertension, and peripheral vascular diseases in their sisters (*Figure 2* and Supplementary material online, *Table S1*, $P < 0.05$). In contrast to MC, a high dense breast in KARMA women was associated reduced risk for diabetes mellitus, chest pain, and peripheral vascular disease in their sisters (*Figure 2*), in agreement with the effects observed in the KARMA women.

Next, we aimed to investigate whether BC genetics is also associated with cardiometabolic diseases and thus potentially implicate a shared aetiology between BC and cardiometabolic diseases. To this end, we computed the association of genetic BC risk factors with cardiometabolic disease significantly associated with mammographic features (*Figure 2*). In particular, we evaluated family history of BC as well as the GRS for oestrogen positive and negative BC. Similar to the results for MC and breast density, we found that these factors are significantly associated with incident BC. In addition, we observed a nominally significant ($P < 0.05$) association of family history of BC with reduced risk for heart valve disorders, ischaemic heart disease, and cardiac conduction disorders. Furthermore, we found an association of the oestrogen positive and negative BC GRS with the lower incidence of clinically diagnosed hypertension (*Figure 2* and Supplementary material online, *Table S1*).

Survival from cardiometabolic diseases

In total, 233 KARMA women died from cardiometabolic disease between recruitment and 31 December 2018. When adjusting for age at baseline, we observed the presence MC was associated with

Table 2 Association of mammographic features with cardiometabolic mortality in KARMA women by pre-existing cardiometabolic disease status

	Variable	Alive	Dead	Baseline model[a]		Full model[b]		Competing risk[b]		Mortality ratec
				HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	
All women	No MC	47 597	160	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		0.55 (0.47–0.65)
	Presence of MC	10 037	73	**1.46 (1.10–1.94)**	**0.009**	**1.46 (1.10–1.94)**	**0.008**	**1.40 (1.06–1.86)**	**0.019**	0.78 (0.61–1.02)
	Non-dense breasts	33 371	189	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		0.67 (0.58–0.79)
	Dense breasts	24 263	44	**0.64 (0.46–0.90)**	**0.010**	0.87 (0.61–1.25)	0.461	0.83 (0.57–1.19)	0.31	0.42 (0.30–0.59)
With CMD	No MC	8382	76	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		1.48 (1.16–1.86)
	Presence of MC	2336	50	**1.77 (1.23–2.55)**	**0.002**	**1.79 (1.24–2.58)**	**0.002**	**1.76 (1.22–2.52)**	**0.003**	2.61 (1.92–3.60)
	Non-dense breasts	7654	105	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		1.88 (1.53–2.31)
	Dense breasts	3064	21	0.73 (0.45–1.18)	0.197	0.92 (0.55–1.54)	0.763	0.90 (0.54–1.51)	0.69	1.39 (0.83–2.19)
No CMD	No MC	39 215	84	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		0.35 (0.28–0.43)
	Presence of MC	7701	23	0.99 (0.62–1.58)	0.966	1.00 (0.63–1.60)	0.991	0.96 (0.61–1.53)	0.87	0.34 (0.21–0.55)
	Non-dense breasts	25 717	84	1.00 (ref.)		1.00 (ref.)		1.00 (ref.)		0.38 (0.30–0.48)
	Dense breasts	21 199	23	0.68 (0.42–1.10)	0.116	0.85 (0.51–1.43)	0.545	0.77 (0.46–1.29)	0.33	0.25 (0.15–0.39)

BMI, body mass index; CI, confidence interval; CMD, cardiometabolic disease; HR, hazard ratio; HRT, hormone replacement therapy; MC, microcalcifications; ref, reference.
[a]The baseline model was adjusted for the age at mammogram.
[b]The model was adjusted for age at mammogram, BMI, smoking status, lipid-lowering medication, hypertension, physical activity, alcohol consumption, education, age at first birth, menopausal status, HRT use, and oral contraception use.
[c]Age-adjusted mortality rate per 1000 person-years.
Significant associations are highlighted in bold (P<0.05).

increased cardiometabolic mortality [hazard ratio (HR) and 95% confidence interval (CI): 1.46 [1.10–1.94], $P = 0.009$, *Table 2*]. This corresponds to an increase in the mortality rate of women with MC compared to women without MC of 0.23 per 1000 person-years. In contrast, we found that dense breasts were associated with a reduced mortality (HR and 95% CI: 0.64 [0.46–0.90], $P = 0.01$, mortality rate reduction of 0.25 per 1000 person-years, *Table 2*). After additional adjustment for BMI and smoking status, we observed similar effect sizes for MC (*Table 2*) but the effect of dense breasts on mortality was attenuated (HR and 95% CI: 0.86 [0.60–1.24], *Table 2*). Importantly, in women with an existing cardiometabolic disease, presence of MC was associated with a markedly increased risk of death (HR and 95% CI: 1.79 [1.24–2.57], $P = 0.002$, mortality rate increase of 1.13 per 1000 person-years, *Table 2*) compared to women with no MC, while we observed no such effect in women without pre-existing cardiometabolic disease. Adjustment for additional lifestyle and reproductive factors did not change the observed associations further. In addition, we conducted a competing risk analyses with cardiometabolic death and BC diagnosis as competing outcomes (*Table 2*). In those analyses, we observed similar effect sizes as above, indicating that a BC diagnosis does not constitute a significant competing event and censoring at BC diagnosis is sufficient. Finally, we also restricted the same analyses outlined above to only deaths from cardiovascular disease, as cardiovascular events are the most common reason for cardiometabolic mortality (Supplementary material online, *Table S2*). In this analysis, we observed similar associations as above, with slightly increased effect sizes observed for the association of MC with cardiovascular mortality (HR and 95% CI: 1.94 [1.33–2.84], $P = 0.001$, mortality rate increase of 1.15 per 1000 person-years, Supplementary material online, *Table S2*) compared to cardiometabolic mortality.

Discussion

In this study, a higher number of microcalcifications resulted in an increased occurrence of cardiometabolic diseases in KARMA participants and their sisters as well as in a higher cardiometabolic mortality in women with pre-existing cardiometabolic diseases (*Graphical abstract*). In contrast, we showed that women with high dense breasts as well as their sisters are less likely to be diagnosed with cardiometabolic diseases. Notably, a family history of BC and a BC-specific GRS were generally associated with lower risk for cardiometabolic diseases.

While it is important to understand diseases that contribute to altered mammographic features, insights into the consequence of mammographic features on cardiometabolic disease risk and mortality can primarily be inferred from the analysis of cardiometabolic disorders occurring after a mammogram. Overall, our results on incident cardiometabolic diseases associated with MC agree with prior cohort studies, which reported effect sizes comparable to ours.[50,51] It is important to note that most previous studies did not account for cardiometabolic disease diagnoses before the mammogram. However, our results indicate MC increase cardiometabolic risk particularly in women with pre-existing cardiometabolic disorders. Those findings, though, are less relevant for cardiometabolic

risk prediction because predicting disease risk in already diseased individuals is unlikely to have a major impact. Nevertheless, ascertaining the presence of MC in mammograms could still be beneficial in these women since we found that the presence of MC was a sign of worse cardiometabolic health and thus associated with increased cardiometabolic mortality, mostly due to cardiovascular complications. Therefore, a detailed medical history of cardiometabolic diseases seems to be vital to precisely assess risk for cardiometabolic mortality in women attending mammographic screenings. In addition, the underlying mechanisms that result in increased cardiometabolic mortality due to MC in women with and not in women without pre-existing cardiometabolic disease need further research. As such, detailed genetic and molecular dissection of the presence of MC in women with and without pre-existing cardiometabolic diseases is warranted, with particular focus on those microcalcifications that are indicative of cardiometabolic death.

In contrast to the results observed in women with pre-existing cardiometabolic diseases, no significant effect of MC on cardiometabolic mortality was observed in healthy women. In addition, despite a comparable number of cardiometabolic disease diagnoses in women with and without prior cardiometabolic diseases, we found fewer and generally weaker statistically significant associations of MC with incident diseases in healthy women. Consequently, accurate assessment of the risk increase due to MC in healthy women is crucial to avoid over-estimating their risk for cardiometabolic diseases. Despite the reduced effect sizes of MC on the risk for cardiometabolic diseases in healthy women, our results still revealed a crucial insight: the effect sizes observed for statistically significant increased cardiometabolic risk due to MC are comparable to the effect sizes observed for BC risk (i.e. about 10% increased risk per MC). This indicates that MC are indeed a strong risk factor for cardiometabolic disease, with each additional microcalcification having comparable effect size to a 5-point increase in BMI.[52,53] The presented results therefore reinforce the notion that MC identified in routine mammographic screening have the potential to improve current risk prediction algorithms[21,54] even in the absence of pre-existing cardiometabolic diseases.

Importantly, we found that increased breast density was associated with a reduced incidence of cardiometabolic diseases, especially in women without pre-existing cardiometabolic diseases. Conversely, women with a low dense breast are at increased risk for cardiometabolic diseases. Potentially, those findings could be explained by the contrasting influence of BMI on breast density and cardiometabolic diseases, particularly diabetes mellitus and cardiovascular diseases. However, we adjusted our analyses for BMI and found no association of prevalent diabetes mellitus with breast density (data not shown), indicating that our adjustments sufficiently accounted for differences in BMI. Nevertheless, we have not ascertained and accounted for all risk factors for cardiometabolic diseases such as blood lipid levels; thus, residual confounding by those variables could still be responsible for the observed association.

Contrary to the generally reduced risk for cardiometabolic diseases observed for women with dense breasts, we found that women with dense breasts had increased risk for incident cardiac dysrhythmias. This is surprising since women with high dense breasts have fewer cardiometabolic disorders that are known risk factors for dysrhythmias. A potential explanation for the observed association could be prior reports showing that post-menopausal women taking HRT are at increased risk for arrhythmias[55–57] as well as have higher dense breasts.[58] Therefore, increased levels of oestrogen in the body seem to be a significant risk factor for arrhythmias as well as denser breast tissue. Since we adjusted the analyses for current and previous HRT as well as for menopausal status, our evidence suggests that high oestrogen levels independent of those factors are responsible for the increased occurrence of arrhythmias. Thus, even though women with dense breast are at generally reduced risk for cardiometabolic diseases, their concomitant medications and overall hormonal exposure should be considered when including breast density in risk prediction and prevention efforts. Importantly, the associations we describe for dense breasts were adjusted for the number of MC and thus represent independent associations. Therefore, including breast density might be useful to improve cardiometabolic risk prediction based on MC alone, particularly in women without prior cardiometabolic diseases.

To ascertain a potential genetic overlap between cardiometabolic disease risk and mammographic features, we investigated the occurrence of cardiometabolic diseases in sisters of KARMA women. Those analyses are only feasible due to linkage of both KARMA women and their sisters to the same nationwide registries with virtually no missing data, allowing accurate assessment of cardiometabolic health in sisters. We observed generally consistent effect sizes in both KARMA women and their sisters. This implicates that either shared environment or shared genetics is indeed partially responsible for the observed association. Therefore, we extended our PheWAS to include strong genetic BC risk factors such as family history of BC and GRS. Here, genetic BC risk factors were associated with reduced cardiometabolic disease risk. Thus, the known BC genetics is unlikely responsible for the observed associations with microcalcifications. Identification of the actual shared genetic and environmental risk factors has the potential to reveal further insights into the shared molecular basis and thus warrants further studies.

It is important to note that the automated iCAD system used in this study has been developed to identify calcifications that are markers for BC and may not necessarily be able to identify all arterial calcifications. In addition, the approach cannot distinguish between arterial and non-arterial MC but is likely detecting both at the same time. Consequently, additional research is necessary to see whether BC risk-associated MC and breast arterial calcifications are differentially influencing cardiometabolic risk. Furthermore, the observed associations are likely underestimating the true effect sizes and thus a novel automated assessment of breast arterial calcifications could provide even more accurate cardiometabolic health assessment, particularly if the training includes relevant outcome measures such as cardiometabolic disease incidence or mortality. Nevertheless, in our sensitivity analyses, we found that excluding women that developed BC during follow-up did not change the observed associations. This finding strongly suggests that the iCAD system is able to capture not only MC due to breast hyperplasia but also those MC that predispose for cardiometabolic risk and mortality. Therefore, using the iCAD system as an automated assessment of MC promises a rapid and cost-effective estimation of cardiometabolic health in women. Such an application to existing screening programmes is particularly warranted since women are at greater risk of cardiometabolic

mortality than men and generally have poorer prognosis following an acute cardiovascular event. Since the iCAD system is FDA approved and in use at several sites indicating that a rapid clinical implication of our findings is feasible. To this end, women with adverse mammographic features (i.e. more MC or lower dense breasts, automatically assessed via the iCAD system and STRATUS) and/or pre-existing cardiometabolic disease will need to be included in a clinical trial. In these women, the efficacy of cardioprotective measures according to established guidelines[59] or with a specifically tailored regime should be assessed, thus paving the way to reduce cardiometabolic mortality in our ageing population.

Taken together, we found that an increased number of microcalcifications were associated with elevated risk for cardiometabolic diseases and mortality particularly in women with pre-existing cardiometabolic diseases. In contrast, high mammographic density was associated with reduced cardiometabolic disease risk, predominantly in women without pre-existing cardiometabolic diseases. Our results indicate that automated quantification of microcalcifications and breast density could be useful at no additional cost or radiation to improve cardiometabolic risk prediction in women attending mammographic screenings. Crucially, automated assessment of mammographic routine screening images might be suitable to identify women with poor cardiometabolic health at-risk for cardiometabolic death.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Authors' contributions

F.G., P.H., and K.C. conceived and designed the study. F.G. performed data management, all statistical analyses, and interpreted the results with the support of H.Y. F.G. drafted the manuscript. M.E. and S.A. measured mammographic features. H.Y., M.E., P.H., S.A., and K.C. critically reviewed, commented, and approved the manuscript.

Ethical standards

The study was approved by the ethical review board in Stockholm (2010/958-31/1). Informed consent was obtained from all individual participants included in the study. All experiments comply with the current Swedish laws.

Conflict of interest: PH is owner of iCAD stocks and a member of the iCAD Scientific Advisory Board. All other authors declare that they have no conflict of interest.

Data availability

Access to phenotypes, biospecimen and genotypes from the KARMA study can be requested from https://karmastudy.org/contact/data-access/.

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