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Transsulfuration metabolites and the association with incident atrial fibrillation – An observational cohort study among Norwegian patients with stable angina pectoris

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ABSTRACT

Background/aim: Plasma total homocysteine (tHcy) is elevated in patients with persistent vs. paroxysmal atrial fibrillation (AF), and has been related to increased risk of new-onset AF. Homocysteine is degraded to cystathionine (Cysta) and cysteine (Cys). All three metabolites have been linked to potential proarrhythmic traits such as inflammation and atrial fibrosis. We evaluated the prospective association between these metabolites and new-onset AF among patients with suspected stable angina pectoris.

Methods: Information regarding AF was obtained by linking patient data to national health registries. Risk associations were explored by Cox regression and potential improvements in risk reclassification were calculated by the continuous net reclassification index (NRI > 0).

Results: At baseline, 3535 patients without any prior history of AF were included. During median follow-up of 7.4 years, 392 patients (10.2%) were registered with incident AF. Higher plasma tHcy and tCys were associated with increased risk of incident AF [age and gender adjusted HRs (95% CI) per 1 log transformed SD 1.23 (1.12–1.35) and 1.23 (1.11–1.38)]; multivariate adjustment yielded similar results. Plasma tHcy and tCys also improved reclassification of patients (NRI > 0 (95% CI)) for tHcy 0.118 (0.02–0.22) and tCys 0.107 (0.002–0.21). No association was seen between plasma Cysta and incident AF.

Conclusion: Plasma tHcy and tCys, but not Cysta, were associated with, and improved risk classification of, new-onset AF among patients with stable angina pectoris. Our results motivate further studies to explore the relationship between homocysteine metabolism and cardiac arrhythmias.

1. Introduction

Atrial fibrillation (AF) is the most common clinically significant arrhythmia, and the prevalence and incidence are increasing. AF is associated with increased mortality, morbidity and health care costs, and important risk factors for AF include higher age, male gender, hypertension, smoking and diabetes. Biomarkers, however, have a minor role in the assessment of AF risk [1]. It is therefore of great interest to identify novel biomarkers that can contribute in AF risk prediction of patients with cardiovascular disease (CVD), as well as in exploring potential pathophysiological mechanisms.

Homocysteine (Hcy) is a non-essential amino acid. It is metabolized via the transsulfuration pathway by the pyridoxal 5′-phosphate (PLP) dependent enzymes cystathionine-beta-synthase (CBS) and cystathionase to cystathionine (Cysta) and cysteine (Cys), respectively. Hcy may also be remethylated to methionine [2].

Elevated plasma total homocysteine (tHcy) is associated with CVD and predominantly atherosclerotic CVD [3], however any causal effects are still elusive [4]. Pre-clinical studies suggest that Hcy affects electrical activity in the human atria [5]. Higher plasma tHcy is associated with prevalent AF [6], and notably a recent study link tHcy with incident AF [7], as well as being an independent risk marker for early recurrence

Abbreviations: AF, atrial fibrillation; BMI, body mass index; CBS, cystathionine beta-synthase; CRP, c-reactive protein; CVD, cardiovascular disease; CVDNOR, cardiovascular disease in Norway; Cys, cysteine; Cysta, cystathionine; eGFR, estimated glomerular filtration rate; Hcy, homocysteine; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; NO, nitric oxide; PLP, pyridoxal 5-phosphate; ROS, reactive oxygen species; SAM, s-adenosylmethionine; tHcy, total plasma homocysteine; tCys, total plasma cysteine; WENBIT, Western Norway B-vitamin intervention trial.

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of AF after catheter ablation [8]. Moreover, increased circulating Cysta [9], and total plasma cysteine (tCys) have been linked with CVD [10], but have hitherto not been studied according to AF risk.

We therefore investigated the potential associations between plasma tHcy, Cysta and tCys with incident AF in a large prospective Norwegian cohort of patients evaluated for stable angina pectoris.

2. Material and methods

2.1. Study population

The study population has been described in detail elsewhere [11]. In short, 4166 patients underwent coronary angiography at two Norwegian university hospitals due to suspected stable angina pectoris in the period 2000–2004. Approximately 2/3 of the patients were included in the Western Norway B-Vitamin Intervention Trial (WENBIT), a randomized controlled trial investigating B-vitamin treatment as secondary CVD prevention [12]. In the present study we excluded patients without valid measurements of plasma tHcy, Cysta or tCys leaving 3856 patients for the final baseline analyses. For the prospective analyses, we also excluded 321 patients with AF at baseline, leaving a total of 3535 patients for the current analyses.

All patients provided written informed consent and the study was carried out according to the Declaration of Helsinki.

2.2. Baseline data

The collection of anamnestic, clinical and anthropometric data has been described in detail elsewhere [11]. Self-reported anamnestic data were provided by the patient, and checked against medical records when available. Health care personnel assessed anthropometric data specific to the study.

2.3. Laboratory analyses

Venous blood samples were drawn 1–2 days prior to or immediately after angiography. The majority of patients were non-fasting (n = 2659). Routine laboratory analyses were performed on fresh blood samples at each recruiting hospital, whereas study-specific analyses were performed at the laboratory of Bevital AS (www.bevital.no) on samples stored at −80 °C and later thawed before analyses by personnel blinded to the study outcomes. Plasma tHcy, Cysta and tCys concentrations were determined by gas chromatography/tandem mass spectrometry [13].

2.4. Endpoint data

The endpoint of interest was receiving a diagnosis of AF (according to the International Classification of Diseases 10th edition I48) during hospitalization or death due to AF throughout 31 December 2009. Endpoint data regarding receiving a diagnosis of AF during hospitalization were retrieved from the Cardiovascular Disease in Norway project (CVDNOR; https://cvdnor.b.uib.no), which provides data on CVD diagnoses at discharge from Norwegian hospitals in the period 1994–2009 [14]. Data regarding death due to AF were retrieved from the Norwegian Cause of Death Registry (https://www.fhi.no/en/hn/health-registries/cause-of-death-registry/cause-of-death-registry-/). Endpoint data were linked to each patient using the 11 digit personal identification number, unique to each Norwegian resident.

2.5. Statistical analyses

Continuous and categorical data are presented as median (25–75 percentiles) and counts (%), respectively, and differences according to new-onset AF were tested by Mann-Whitney-U and Chi square test. We calculated the Pearson correlation coefficient between tHcy, Cysta and tCys. We also calculated the Pearson correlation coefficient between continuous variables and tHcy, Cysta and tCys and geometric mean of the aforementioned metabolites according to categorical variables. We used Cox regression to obtain hazard ratios (HRs) [95% confidence intervals (CIs)] for incident AF according to quartiles and per one standard deviation (SD) increase in log-transformed plasma concentrations of the biomarker of interest. The biomarkers were tested individually when adjusted for age and gender (Model 1) as well as in two multivariate models: Model 2 was further adjusted for known risk factors for AF, including diabetes, hypertension, body mass index (BMI) and smoking. Model 3 extended Model 2 by further including estimated glomerular filtration rate (eGFR), anamnestic heart failure, left ventricular ejection fraction (LVEF), left ventricular end-diastolic pressure (LVEDP), plasma pyridoxal 5'-phsphate (PLP) and C-reactive protein (CRP). The possibility for unmeasured confounding was investigated by calculating E-values to model 3 according to recent recommendations [15].

We explored any non-linear relationships between the biomarkers and future AF risk by constructing generalized additive model (GAM) plots.

We investigated reclassification of patients by adding the biomarker of interest to a multivariate logistic model including age, gender, hypertension, diabetes, BMI and smoking and calculating the continuous net reclassification improvement (NRI > 0) and the Receiver Operating Curve Area Under the Curve (ROC-AUC).

All tests were two-sided and the significance level set to 0.05. We used the software IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., and R for Windows (R Core Team 2016, Vienna, Austria, utilizing the packages #survival, #Hmisc, #PredictABEL, #ROCR, #ggplot2 and #ICC).

3. Results

3.1. Baseline characteristics in the overall population

At baseline 320 (8.3%) patients had previous or current AF. Median (25–75 percentiles) plasma tHcy, Cysta and tCys concentrations were higher among these patients as compared to those without any history of AF (tHcy 10.9 (8.8–13.4) vs. 10.4 (8.7–12.5) μmol/L; P = 0.005, Cysta 0.30 (0.21–0.47) vs. 0.26 (0.19–0.38) μmol/L; P < 0.0001 and tCys 300 (273–329) vs. 289 (266–313) μmol/L; P < 0.0001). Baseline characteristics according to prevalent AF are shown in Supplemental Table 1. According to Supplemental Fig. 1, tHcy, Cysta and tCys were positively correlated with increasing age and CRP and a negatively with eGFR. Supplemental Fig. 2 shows that there is very little difference in geometric mean concentration of all three metabolites in question according to categorical variables.

The Associations Between Plasma Transsulfuration Metabolites and Incident Atrial Fibrillation.

During a median (25–75 percentile) follow-up-time of 7.4 (6.2–8.6) years 392 (10.2%) patients were registered with incident AF. Characteristics of the patients according to new-onset AF are showed in Table 1. Patients with incident AF were generally older and were more often men and having a history of hypertension, diabetes, and heart failure as compared to those who did not develop AF. The former group also more often smoked, and had higher CRP and plasma riboflavin, lower eGFR, as well as more extensive coronary artery disease at angiography.

Plasma concentrations of tHcy, Cysta and tCys were higher among those who developed AF compared to those who did not develop AF. Accordingly, all three metabolites were related to increased risk of AF during follow-up (Supplemental Table 2 and Figs. 1–3). For tHcy and tCys these associations were attenuated in model 2 [HRs (95% CI) per 1 log transformed SD 1.23 (1.12–1.35) and 1.23 (1.11–1.38)]; however, the adjustment rendered Cysta no longer associated with incident AF. Extensive adjusting in Model 3 yielded similar results [HRs (95% CI) per 1 log transformed SD 1.20 (1.08–1.34) and 1.20 (1.06–1.36)], as was the case when including all the three metabolites in the same model, to check for any mutually attenuating effects (Supplemental

Table 1
Patients characteristics according to new-onset atrial fibrillation.

	N	New onset atrial fibrillation		P
		No	Yes	
tHcy, μmol/L	3535	10.2 (8.6–12.4)	11.4 (9.4–14.0)	<0.001
Cysta, μmol/L	3535	0.26 (0.19–0.37)	0.28 (0.21–0.42)	0.001
tCys, μmol/L	3535	288 (265–311)	302 (278–327)	<0.001
Age, years	3535	60 (54–68)	68 (61–74)	<0.001
Male gender, % n	3535	71.0 (2220)	75.5 (392)	0.04
Hypertension, % n	3535	44.3 (1392)	57.9 (227)	<0.001
Diabetes, % n	3535	11.0 (348)	16.1 (63)	0.004
Smoking, % n	3530	32.5 (1019)	26.3 (103)	0.013
BMI, kg/m2	3535	26.0 (24–28)	26.0 (24–29)	0.962
Anamnestic heart failure	3535	5.6 (177)	11.2 (44)	<0.001
Left ventricular ejection fraction, %	3535	66 (60–70)	65 (58–70)	0.041
Previous AMI, % n	3535	39.7 (1247)	43.4 (170)	0.16
LVEDP, mm Hg	3535	16 (12–20)	18 (12–22)	0.073
Extent of CAD at angiography, % n	3535			<0.001
No significant stenosis		26.0 (818)	17.3 (68)	
1-Vessel disease		23.8 (747)	19.4 (76)	
2-Vessel disease		22.2 (698)	23.2 (91)	
3-Vessel disease		28.0 (880)	40.0 (157)	
Plasma methionine, μmol/L	2554	26.7 (22.8–32.1)	27.0 (22.6–35.6)	0.497
C-reactive protein, mg/L	3532	1.7 (0.9–3.5)	2.0 (1.0–3.9)	0.010
Troponin-T, ng/L	3462	4 (3–10)	5 (3–11)	0.220
eGFR, ml/min/1,73m^2	3534	92 (80–100)	85 (70–94)	<0.001
Lipids, mmol/L				
Total cholesterol	3533	5.0 (4.3–5.8)	4.9 (4.2–5.6)	0.201
LDL-cholesterol	3531	3.0 (2.4–3.7)	2.9 (2.4–3.6)	0.397
HDL-cholesterol	3534	1.2 (1.0–1.5)	1.3 (1.0–1.5)	0.243
Alcohol >2 units/week, % n	3535	36.9 (1159)	35.7 (140)	0.077
Physical activity ≥2 days/week, % n	3535	59.3 (1863)	58,4 (229)	0.70
Plasma and serum B-vitamers				
PLP, μmol/L	3516	41.8 (29.9–60.1)	41.7 (29.7–28.5)	0.685
Riboflavin, nmol/L	3516	11.0 (7.3–18.0)	12.6 (8.2–19.1)	0.019
Cobalamin, pmol/L	3153	363 (276–468)	356 (259–465)	0.187
Folate, nmol/L	3534	10.1 (7.42–14.7)	10.0 (7.5–15.4)	0.853

Abbreviations: tHcy: total homocysteine, Cysta: cystathionine, tCys: total cysteine, BMI: body mass index, LVEF: left ventricular ejection fraction, AMI: acute myocardial infarction, LVEDP: left ventricle end-diastolic pressure, CAD: coronary artery disease, GFR: glomerular filtration rate LDL: low density lipid, HDL: high density lipid, PLP: pyridoxal phosphate.

Table 3). Before adding the three metabolites we checked for collinearity which revealed variance inflation factors between 1.0 and 1.1, thus ruling out collinearity. We calculated the Pearson correlation coefficient between the variables which revealed values between 0.23 and 0.45.

Application of the E-value formula to model 3 revealed E-values for the HR and lower CI of 1.69 and 1.37 for tHcy and 1.69 and 1.31 for tCys.

We found no significant difference in risk estimates between patients receiving B-vitamin treatment in the WENBIT versus patients who did not receive B-vitamin supplements (data not shown).

3.2. Reclassification of patients

When adding plasma tHcy or tCys to a logistic regression model containing age, gender, BMI, hypertension, diabetes and smoking, the NRI > 0 (95% CI) for was 0.118 (0.02–0.22) and 0.107 (0.002–0.21), respectively; however we did not observe any improvement in the ROC-AUC (Supplementary Table 4).

4. Discussion

The current study showed that plasma tHcy, tCys and Cysta were associated with previous AF in a population with suspected stable angina pectoris. Plasma tHcy and tCys were also related to increased risk of incident AF, and either metabolite improved the reclassification of patients beyond traditional risk factors.

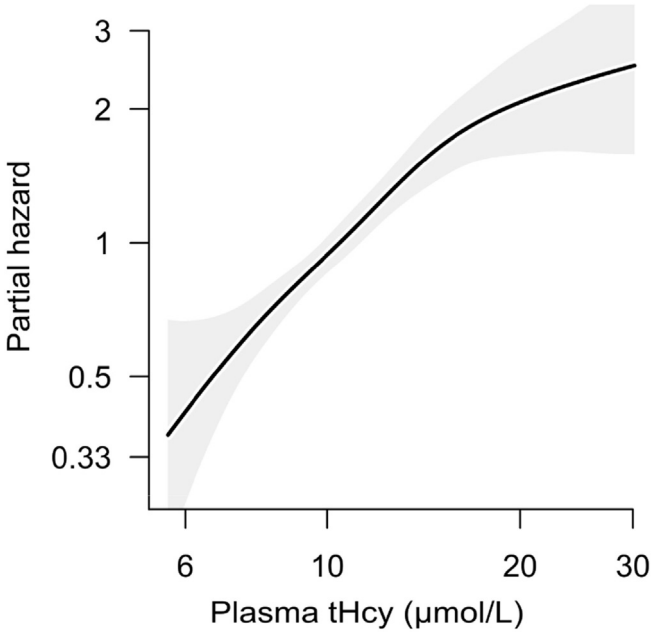

Fig. 1. The unadjusted association between total plasma homocysteine and new-onset atrial fibrillation among patients with suspected stable angina pectoris. The shaded areas surrounding the solid splines depict 95% confidence intervals.

4.1. Transsulfuration metabolites and CVD

Previous studies regarding tHcy and transsulfuration metabolites with CVD have mainly looked into the relationship between the metabolites and atherosclerotic cardiovascular events, including acute myocardial infarction and stroke, in addition to all-cause death [3,16–19]. However data regarding transsulfuration metabolites and AF in particular is scarce. A positive prospective association between plasma tHcy and AF is in line with findings from a recent study from a general population [7]. However, no association between levels of plasma tHcy and new-onset AF was found in the Framingham cohort [20]. Discrepancies

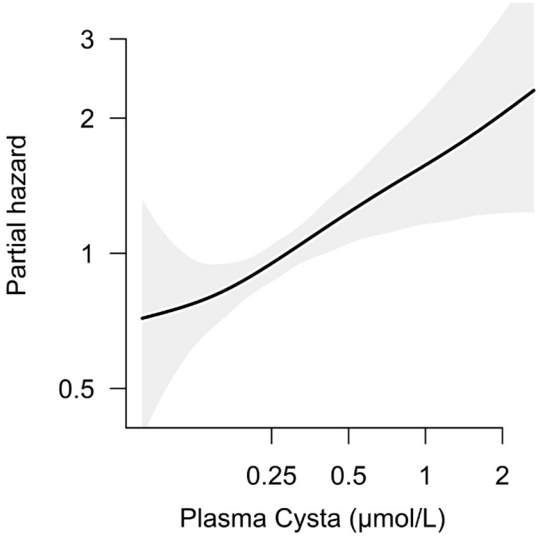

Fig. 2. The unadjusted association between total plasma cystathionine and new-onset atrial fibrillation among patients with suspected stable angina pectoris. The shaded areas surrounding the solid splines depict 95% confidence intervals.

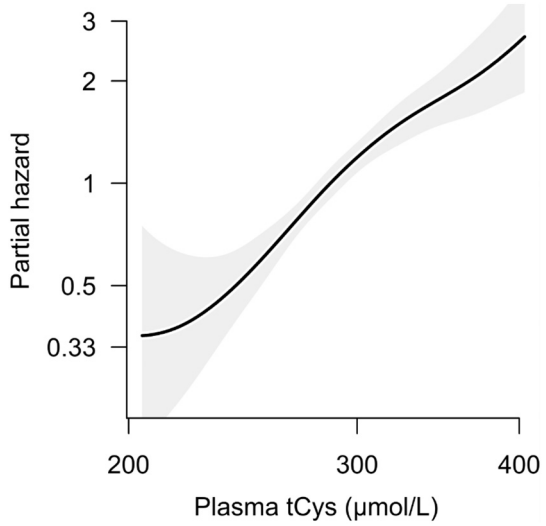

Fig. 3. The unadjusted association between total plasma cysteine and new-onset atrial fibrillation among patients with suspected stable angina pectoris. The shaded areas surrounding the solid splines depict 95% confidence intervals.

between epidemiological studies may relate to different population characteristics, as we studied patient with presumed coronary heart disease.

4.2. Potential mechanisms

4.2.1. Transsulfuration and methylation

Transsulfuration primarily takes place in hepatocytes; however some studies suggest that it also can take place in human blood as endothelial cells have been shown to secrete CBS. Hydrogen sulfide (H_2S) is produced during transsulfuration and has been linked to pulmonary vein and atrial arrhythmogenesis in rats [21,22]. By remethylation, Hcy is a precursor of S-adenosylmethionine (SAM), which is crucial for remethylation reactions. Elevated tHcy has been associates with altered DNA methylation [23], a feature observed in the atrial and pulmonary vein tissue of patients with AF [24], and which also has been linked to increased risk coronary heart disease [25]. In this study those who developed AF were older and more often had hypertension and diabetes, features which have been linked to altered DNA methylation [26–28].

4.2.2. Electrophysiological Changes

AF is caused by electrical instability in cardiomyocytes in the atria [29]. Hcy has been shown to affect sodium and potassium ion channels in human atrial myocytes and may thereby contribute to cardiac excitability and arrhythmogenesis [5,30,31]. tCys has been shown to regulate cardiac ion channels through its redox state [32], this is further discussed below.

4.2.3. Inflammation, oxidative stress, atrial cardiomyopathy and fibrosis

AF is associated with inflammation [33]. Elevated tHcy is associated with up-regulation of NF-kB in mice and increased vascular inflammation [34], and also known to induce oxidative stress. This may lead to endothelial dysfunction and reduced NO bioavailability [35], both of which are associated with AF [36–38].

Moreover, tCys is an antioxidant and takes part in redox reactions through its reactive thiol groups [39], besides being a precursor of glutathione, an important intracellular antioxidant [2]. The redox state of Cys is associated with activation of inflammatory gene expression, tissue fibrosis and has also been shown to regulate cardiac ion-channels suggesting a possible link to AF [32,40–42]. Notably, recent studies suggest that AF is the symptom of an underlying atrial cardiomyopathy [43], and also link AF to atrial fibrosis [44,45]. Mild hyperhomocysteinemia is reported to increases collagen formation in rabbit aortic smooth muscle cells [46], as well as increasing metalloprotease-driven deterioration of elastic fibers [47] and Cys is also shown to contribute to tissue fibrosis [48]. Hence, the association between tHcy and tCys with AF could be due to underlying altered atrial morphology and electrophysiological properties related to inflammation and fibrosis, supported by the finding of increased risk of AF recurrence after radio frequency ablation among patients with elevated tHcy and established AF [8]. The lack of an association between Cysta and AF, despite the positive relationships between tHcy and tCys with AF, suggests that tHcy and tCys may be associated with AF through partly independent mechanisms.

In the current study, patients who developed AF had higher serum CRP, a marker of cardiac fibrosis [49]; however adjusting for CRP as well as LVEDP as a proxy for elevated left atrial pressure, did also not alter the risk estimates significantly, suggesting that other mechanisms are involved in the development of AF.

4.3. Strengths and limitations

The strengths of this study include the large number of patients, the long-term follow-up and its prospective design. All blood samples were analyzed by the same core laboratory, reducing the risk of analytical bias. The biomarkers have shown good reproducibility within subjects and stability when frozen to −80 °C [50], which favor their use as biomarkers.

The current study also has limitations. First, although we have made substantial efforts to control for potential confounders, we cannot rule out the possibility for residual or unmeasured confounding, as is always the case for observational studies. However the calculated E-value suggests that such confounding needs to be associated both with tHcy/tCys and incident AF with a strength of RR > 1.69. We are not aware of any potential confounder of this strength, conditional on the variables already included in the fully adjusted model.

Second, the potential for residual confounding is an inherent limitation of observational studies and cannot be ruled out. Second, misclassification of endpoints may also have occurred when obtaining endpoint data from health registries. Patients with higher plasma concentrations of the metabolites of interest also appeared sicker, thus being more prone to later hospitalization. Third, although tHcy and tCys moderately improved reclassification, it is uncertain whether this information can be utilized in a clinical setting to improve risk stratification. Finally, the population in this study was highly selected and consisted primarily of middle-aged, Caucasian men with established or suspected coronary heart disease. The risk-associations should therefore be externally confirmed in cohorts with other characteristics.

5. Conclusion

This observational cohort study suggests a link between metabolites in the transsulfuration pathway and incident AF. Our results motivate further studies in proarrhythmic mechanisms related to one-carbon metabolism, and in particular the status of transsulfuration metabolites.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2020.05.010.

CRediT authorship contribution statement

Mads M. Svenningsson: Writing - original draft, Formal analysis, Methodology. **Gard F.T. Svingen:** Writing - original draft, Formal analysis, Methodology. **Vegard Lysne:** Writing - review & editing, Visualization, Formal analysis. **Per M. Ueland:** Writing - review & editing. **Grethe S. Tell:** Writing - review & editing, Investigation, Validation. **Eva R. Pedersen:** Writing - review & editing, Investigation, Validation. **Indu Dhar:** Writing - review & editing. **Dennis W. Nilsen:** Writing - review & editing, Investigation. **Ottar Nygård:** Writing - review & editing, Investigation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to report.

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Disclaimers

Data from the Norwegian Cause of Death Registry have been used in this publication. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by this registry is intended, nor should be inferred.

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