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SHORT COMMUNICATION

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Pharmacogenetic testing of *CYP2D6*, *CYP2C19* and *CYP2C9* in Denmark: Agreement between publicly funded genotyping tests and the subsequent phenotype classification

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1 | INTRODUCTION

The cytochrome P450 (CYP) enzyme family catalyses the metabolism of approximately 80% of all medications.[1,2] Many genes encoding the CYP enzymes exhibit high levels of polymorphism that affect their expression and activity. The most frequent and clinically important variations exist within the genes *CYP2D6*, *CYP2C19* and *CYP2C9*.[1] *CYP2D6*, the most extensively studied drug-metabolizing enzyme, has over 100 allelic variants and is responsible for metabolizing about 25% of all marketed medications.[1,3] *CYP2C19* and *CYP2C9* are collectively responsible for metabolizing about 20% of medications. It is estimated that 74%–97% of Caucasian individuals possess at least one genetic variation in the CYP gene, potentially affecting metabolism for about one quarter of all prescribed medications.[4] Testing for this genetic variation can help identify individuals for whom medication safety and efficacy can be improved through dose adjustment.[1]

Pharmacogenetic (PGx) testing assesses genetic variation by identifying a patient's genotype and assigning a predicted phenotype. The predicted phenotype of an inherited allele can be classified as loss of function, decreased function, normal function or increased function.[3] Numerous PGx tests have been developed for both public and private use. The evidence for whether these tests actually improve treatment outcomes is controversial, but a large implementation study recently showed that pre-emptive PGx testing may reduce the incidence of adverse reactions.[5] PGx tests are normally based on genotyping panels with predefined alleles, but variations in these panels between PGx providers and in the approach for genotype-to-phenotype translation can result in conflicting recommendations.[6,7] Current guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommend classifying phenotypes into four, five and three categories for *CYP2D6*, *CYP2C19* and *CYP2C9*, respectively.[8]

Despite these recommendations, a recent study by Bousman and Dunlop found substantial variation in reported genotype, phenotype and resulting medication recommendations between four commercial PGx tests. Based on this, the authors called for standardization of PGx tests and development of medication guidelines specifying which alleles should be included in the PGx test.[7] In response, recommendation guidelines for selection of relevant *CYP2D6* alleles for PGx testing were published in 2021.[9]

In Denmark, approximately 800 PGx tests of *CYP2D6*, *CYP2C19* and *CYP2C9* are conducted annually across three public laboratories. Evaluation of the agreement in determined genotype and the subsequent phenotype assignment is essential for the credibility of PGx testing to ensure that clinicians will utilize this approach. The objectives of this pilot study were to (1) assess how well the three laboratories agreed on genotypes, (2) assess how well the three laboratories agreed on genotype-to-phenotype translation and (3) identify potential causes of observed discrepancies in genotypes and genotype-to-phenotype translation between the three laboratories.

2 | METHODS

2.1 | Design and study cohort

This is a pilot study based on data from a previously described clinical trial titled Optimization of Nutrition and Medication for Acutely Admitted Older Medical Patients (OptiNAM).[10,11] The OptiNAM trial included older (age ≥65 years) Caucasian patients presenting to the Emergency Department at Copenhagen University Hospital Hvidovre, Denmark. All patients provided informed written consent, and all analyses were approved by the Regional Ethical Review Board (H-18023853) and the Danish Data Protection Agency (H-18023853). The study was conducted in accordance with the Declaration of Helsinki and is registered at ClinicalTrials.gov (NCT03741283). Additionally, the current study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology guidelines for both experimental and clinical investigation.[12] Additional details about the OptiNAM trial including inclusion and exclusion criteria are described elsewhere.[10]

In total, 126 patients from the OptiNAM study received a commercially available PGx test (Personal Medicine Profile, GeneTelligence, Denmark [hereafter: 'PMP-test']). Blood samples (EDTA plasma and buffy coat) were collected from all patients on the day of hospitalization and stored at −80°C in a local biobank. Demographic information such as gender, age, height and weight were collected from the hospital's electronic patient record system.

2.2 | Selection of patients for genotype and phenotype comparison

To achieve a comprehensive dataset, data from 18 individual patients from the OptiNAM study were selected to represent the four, five and three phenotype categories

for *CYP2D6*, *CYP2C19* and *CYP2C9*, respectively, according to the CPIC classification.[13] Patient samples were methodically selected based on their patient identification (ID) numbers and predicted phenotypes for *CYP2D6*, *CYP2C19* and *CYP2C9*, as determined by the PMP-test. Initially, for *CYP2D6*, we chose the sample with the lowest ID number for each metabolizer category, starting with poor metabolizers (PM), followed by intermediate (IM), normal (NM) and ultrarapid (UM) metabolizers. This selection process was replicated for *CYP2C19* and then *CYP2C9*. Our approach ensured inclusion of all phenotypes for each gene according to CPIC classification, resulting in 12 individual patient samples. The six lowest unselected ID numbers were also included, totalling 18 individual patient samples.

2.3 | Genotyping analysis

Genomic DNA was extracted from buffy coat samples using the paramagnetic particle-based platform consisting of the Maxwell® 16 DNA Purification Kit and the Maxwell® 16 Instrument (Promega Corporation, Madison, WI, United States). Genotyping was performed at three public laboratories in Denmark: University College Absalon, Roskilde, Denmark ('Absalon'); Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Roskilde, Denmark ('Sct. Hans'); and The Danish Epilepsy Center, Filadelfia, Dianalund, Denmark ('Filadelfia'). All three laboratories offered to genotype and predict the phenotype of *CYP2D6* and *CYP2C19*, but only Absalon and Filadelfia offered to genotype and predict the phenotype of *CYP2C9*. Genotyping at Absalon and Sct. Hans was done using the commercial platform from Luminex Corporation (Luminex Corporation, Austin, TX, United States). The laboratory kits used by Absalon and Sct. Hans for *CYP2D6* permitted the determination of the alleles *1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *15, *17, *29, *35, *41 and duplications. Using the *CYP2C19* laboratory kits, we determined the alleles *1, *2, *3, *4, *5, *6, *7, *8, *9, *10 and *17. All tests performed at Filadelfia were designed in-house using 5′nuclease-based PCR. The genotyping panel for *CYP2D6* included the alleles *1, *2, *3, *4, *5, *6, *9, *10, *17, *41 and duplications, and the panel for *CYP2C19* included the alleles *1, *2, *3, *4 and *17. Laboratory kits used by Absalon and Filadelfia for *CYP2C9* included the alleles *1, *2 and *3. For all three laboratories, the *1 allele (the most common variant in Caucasians) was identified by the absence of all other alleles. A detailed overview of the included alleles across all laboratories with information on allele function and frequencies can be found in Table S1, and descriptions of all assays and instruments can be found in Table S2.

2.4 | Translation to phenotype

Genotype-to-phenotype translation was inconsistent across the laboratories. Absalon utilized the PharmGKB phenotype classifications published by CPIC.[13] Filadelfia used an internal classification resembling CPIC for predicting phenotypes of *CYP2D6* and *CYP2C9* but did not use the Rapid Metabolizer (RM) classification for *CYP2C19*. Consequently, the genotype-to-phenotype translation for *CYP2C19* was limited to PM, IM, NM and UM. Similarly, Sct. Hans used its own classification system for predicting phenotypes of *CYP2D6* and *CYP2C19* as PM, NM or UM, and the UM phenotype was recognized only in the presence of duplicate alleles (Table 1).

2.5 | Comparison of phenotype classifications between laboratories

Phenotype agreement between laboratories was evaluated based on the official CPIC classifications.[13] However, due to differences in classification systems between laboratories (Table 1), phenotype agreement was also evaluated based on the internal phenotype classifications used by Filadelfia and Sct. Hans. For *CYP2D6*, which includes three phenotypes, predicted IM phenotypes according to CPIC were converted to the NM phenotype according to Sct. Hans' phenotype classification. Additionally, for Filadelfia's phenotype classification of *CYP2C19*, which includes four phenotypes, predicted RM phenotypes were converted to the UM phenotype. Finally, for Sct. Hans' phenotype classification of *CYP2C19*, which includes three phenotypes, predicted IM phenotypes were converted to the NM phenotype. For *CYP2C9*, Absalon and Filadelfia used the same phenotype classification: PM, IM and NM.

2.6 | Outcomes

The primary outcome was agreement of genotype and predicted phenotype classification between the three laboratories.

2.7 | Statistical analyses

Classification agreement was evaluated by percent classification agreement and Conger's Kappa statistic (κ)[14]: 0.21 to 0.40 was interpreted as 'fair' agreement, 0.41 to 0.60 as 'moderate', 0.61 to 0.80 as 'substantial' and 0.81 to 1.00 as 'almost perfect'.[15] Agreement was based on all possible comparisons between laboratories

TABLE 1 Phenotype converting analysis of CYP2D6, CYP2C19 and CYP2C9 between laboratories.

# of phenotype categories	CYP2D6	CYP2C19	CYP2C9
3 phenotypes	PM, NM, UM (Sct. Hans)	PM, NM, UM (Sct. Hans)	PM, IM, NM (Absalon & Filadelfia)
4 phenotypes	PM, IM, NM, UM (Absalon & Filadelfia)	PM, IM, NM, UM (Filadelfia)	
5 phenotypes		PM, IM, NM, RM, UM (Absalon)	

Abbreviations: IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

(i.e., laboratory 1–2, 1–3 and 2–3). For each patient, the possible outcomes were agreement for all comparisons, agreement for one comparison or agreement for zero comparisons. The agreement estimate was calculated as the percent of agreements for all patients out of the total possible number of agreements (three times the number of patients compared). All Kappa estimates are reported with 95% confidence interval (CI). In cases where samples from each laboratory were identical ($\kappa = 1$), the kappa value had a variance of 0 (resulting in a CI with 0 width). Given the relatively small sample, however, we do not believe these samples are sufficiently representative to suggest a CI with 0 width. If a single disagreement was present in these samples, for example, then the width of the CI would expand to 0.26. Therefore, the CI has been omitted in these comparisons. All data calculations and analyses were conducted in R 4.1.0.[16]

3 | RESULTS

Due to the manufacturer's discontinuation of a reagent, genotyping was only completed for 13 of 18 samples for *CYP2D6* at the Absalon laboratory. Therefore, agreement and Kappa estimates for genotyping are based on only 13 samples when the Absalon laboratory is included.

Table 2 shows a detailed overview of patients' genotypes and corresponding phenotypes for *CYP2D6*, *CYP2C19* and *CYP2C9* from all three laboratories. Genotype and phenotype agreement is shown in Table 3.

Overall genotyping agreement between the laboratories was 85% ($\kappa = 0.83$, CI = 0.64:1.00) for *CYP2D6* and 100% ($\kappa = 1.0$) for both *CYP2C19* and *CYP2C9*.

Overall phenotyping agreement between the laboratories was 90% ($\kappa = 0.82$, CI = 0.56:1.00), 52% ($\kappa = 0.32$, CI = 0.14:0.51) and 94% ($\kappa = 0.32$) for *CYP2D6*, *CYP2C19* and *CYP2C9*, respectively. When collapsing the IM into the NM phenotype for CYP2D6 (resulting in three phenotype categories), the agreement increased to 100%. Similarly, when collapsing the IM into the NM phenotype and the RM into the UM phenotype for CYP2C19 (resulting in three phenotyping categories), the agreement increased to 78%.

4 | DISCUSSION

In this study, we found that genotyping assignments for *CYP2D6*, *CYP2C19* and *CYP2C9* were largely consistent across three Danish public-founded laboratories. However, variation in phenotype classification systems between laboratories resulted in notable differences in genotype-to-phenotype translation. There are several considerations when selecting alleles for genotyping panels, including cost, clinical value of additional information and ethnicity of the patient group.[9] The genotyping service is free to patients within the Danish healthcare system, but laboratories are free to decide the "optimal test" based on these considerations.

The only genotyping disagreement between laboratories was for *CYP2D6*. The genotyping panels used by Absalon and Sct. Hans included 16 variants of *CYP2D6*, whereas the panel used by Filadelfia included only 10 variants (see Table S1). Absalon and Sct. Hans used identical assays and instruments for genotyping and identified at least one *35 allele in four out of 13 or 18 patients, respectively. Since both *2 and *35 are classified as NM phenotype, this particular discrepancy does not affect phenotype determination. However, other genotyping discrepancies could result in different predicted phenotypes.[7] This could potentially have clinical implications and indicates the need for cross-validation and genotyping alignment between laboratories.

Due to the different phenotype classification approaches between the three laboratories, we observed higher levels of disagreement in genotype-to-phenotype translation. Absalon follows official CPIC classifications, while Sct. Hans and Filadelfia follow internal phenotyping classification strategies. Sct. Hans' approach was developed to identify phenotype variations with a clinically relevant difference for drug metabolism, and all other variations are classified as the NM phenotype. In

TABLE 2 Diplotypes and corresponding phenotypes for CYP2D6, CYP2C19 and CYP2C9 determined at three laboratories.

	CYP2D6			CYP2C19			CYP2C9	
Patient ID	**ABS**[a]	**Sct. H**	**FIL**	**ABS**	**Sct. H**	**FIL**	**ABS**	**FIL**
1	*35/*35 (NM)	*35/*35 (NM)	*2/*2 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*3 (IM)	*1/*3 (IM)
2	-	*4/*5 (PM)	*4/*5 (PM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*3 (IM)	*1/*3 (IM)
3	*2/*35 (NM)	*2/*35 (NM)	*2/*2 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*2 **(IM)**	*1/*2 **(NM)**
4	-	*4/*35 (NM)	*2/*4 (NM)	*1/*2 **(IM)**	*1/*2 **(NM)**	*1/*2 **(IM)**	*1/*1 (NM)	*1/*1 (NM)
5	-	*1/*41 (NM)	*1/*41 (NM)	*1/*2 **(IM)**	*1/*2 **(NM)**	*1/*2 **(IM)**	*1/*1 (NM)	*1/*1 (NM)
6	*4/*41 **(IM)**	*4/*41 **(NM)**	*4/*41 **(IM)**	*1/*17 **(RM)**	*1/*17 **(NM)**	*1/*17**(UM)**	*1/*1 (NM)	*1/*1 (NM)
7	-	*1/*9 (NM)	*1/*9 (NM)	*1/*17 **(RM)**	*1/*17 **(NM)**	*1/*17**(UM)**	*1/*2 **(IM)**	*1/*2 **(NM)**
8	*2/*5 **(IM)**	*2/*5 **(NM)**	*2/*5 **(NM)**	*1/*2 **(IM)**	*1/*2 **(NM)**	*1/*2 **(IM)**	*1/*1 (NM)	*1/*1 (NM)
9	*1/*41 (NM)	*1/*41 (NM)	*1/*41 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*2/*3 **(PM)**	*2/*3 **(IM)**
10	*1/*2 (NM)	*1/*2 (NM)	*1/*2(NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)
11	*1/*1 (NM)	*1/*1 (NM)	*1/*1(NM)	*1/*17 **(RM)**	*1/*17 **(NM)**	*1/*17**(UM)**	*1/*1 (NM)	*1/*1 (NM)
12	*2/*35 (NM)	*2/*35 (NM)	*2/*2 (NM)	*17/*17 **(UM)**	*17/*17 **(NM)**	*17/*17 **(UM)**	*1/*1 (NM)	*1/*1 (NM)
13	*2/*9 (NM)	*2/*9(NM)	*2/*9 (NM)	*17/*17 **(UM)**	*17/*17 **(NM)**	*17/*17 **(UM)**	*1/*1 (NM)	*1/*1 (NM)
14	*4/*4 (PM)	*4/*4 (PM)	*4/*4 (PM)	*2/*17 **(IM)**	*2/*17 **(NM)**	*2/*17 **(IM)**	*1/*1 (NM)	*1/*1 (NM)
15	*4/*4 (PM)	*4/*4 (PM)	*4/*4 (PM)	*1/*1 (NM)	*1/*1 (NM)	*1/*1 (NM)	*1/*3 (IM)	*1/*3 (IM)
16	*4/*4 (PM)	*4/*4 (PM)	*4/*4 (PM)	*2/*2 (PM)	*2/*2 (PM)	*2/*2 (PM)	*1/*1 (NM)	*1/*1 (NM)
17	-	*1/*2 (NM)	*1/*2 (NM)	*1/*2 **IM**	*1/*2 **NM**	*1/*2 **IM**	*1/*2 **IM**	*1/*2 **NM**
18	*2/*2 × 2 (UM)	*2/*2 × 2 (UM)	*2/*2 × 2 (UM)	*1/*17 **(RM)**	*1/*17 **(NM)**	*1/*17 **(UM)**	*1*1 (NM)	*1/*1 (NM)

Note: Underlining denotes disparities in measured diplotypes, while bolded phenotypes indicate differences in phenotype scores.
Abbreviations: ABS, Absalon; FIL, Filadelfia; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; Sct. H, Sct. Hans; UM, ultrarapid metabolizer.
[a]$n = 13$ for Absalon.

this way, Sct. Hans' approach is focused on implementation and clinical relevance, which is simpler but more conservative than CPIC's approach. Filadelfia's approach nearly complies with CPIC, but the laboratory does not include the RM phenotype for *CYP2C19* and instead identifies it as the UM phenotype.[17] This discrepancy is likely explained by the lack of international consensus on the clinical relevance of the *17 allele when the phenotype conversion analysis was developed; the field has since evolved without reassessment of this approach. This simplified and clinically-oriented approach may also have some disadvantages. For example, identifying *CYP2C19*

TABLE 3 Geno- and phenotype agreement (%) of CYP2D6, CYP2C19 and CYP2C9.

	Agreement %	Conger's Kappa (95% CI)
CYP2D6		
All laboratories[a]		
Genotype	84.6	0.83 (0.64–1.00)
4 phenotypes	89.7	0.82 (0.56–1.00)
3 phenotypes	100.0	1.00[b]
Filadelfia & Sct. Hans		
Genotype	77.8	0.76 (0.54–0.98)
4 phenotypes	94.4	0.88 (0.64–1.00)
3 phenotypes	100.0	1.00[b]
Filadelfia & Absalon[a]		
Genotype	76.9	0.75 (0.47–1.00)
4 phenotypes	92.3	0.87 (0.59–1.00)
3 phenotypes	100.0	1.00[b]
Absalon & Sct. Hans[a]		
Genotype	100.0	1.00[b]
4 phenotypes	84.6	0.73 (0.35–1.00)
3 phenotypes	100.0	1.00[b]
CYP2C19		
All laboratories		
Genotype	100.0	1.00[b]
5 phenotypes	51.9	0.32 (0.14–0.51)
4 phenotypes	59.3	0.41 (0.24–0.57)
3 phenotypes	77.8	0.51 (0.27–0.75)
Filadelfia & Sct. Hans		
Genotype	100.0	1.00[b]
5 phenotypes	38.9	0.24 (−0.15–0.63)
4 phenotypes	38.9	0.41 (−0.11–0.94)
3 phenotypes	61.1	0.30 (−0.16–0.75)
Filadelfia & Absalon		
Genotype	100.0	1.00[b]
5 phenotypes	77.8	0.93 (0.85–1.00)
4 phenotypes	100.0	1.00[b]
3 phenotypes	100.0	1.00[b]
Absalon & Sct. Hans		
Genotype	100.0	1.00[b]
5 phenotypes	38.9	0.33 (−0.15–0.80)
4 phenotypes	38.9	0.41 (−0.11–0.94)
3 phenotypes	61.1	0.30 (−0.16–0.75)
CYP2C9		
Absalon & Filadelfia		
Genotype	100.0	1.00[b]
3 phenotypes	77.7	0.00 (0.00–0.00)

Note: Percentage patients ($n = 18$) with agreement and kappa coefficient (95% CI).

[a]$n = 13$ for Absalon.

[b]CI not calculated due to variance estimate of 0.

as the NM phenotype instead of the IM phenotype could impact prescribing of medications such as clopidogrel.[18] Similarly, identifying *CYP2C19* as the UM phenotype instead of the RM phenotype could impact initial dosing for proton pump inhibitors or the decision to prescribe citalopram/escitalopram.[19,20] Altogether, our results indicate that alignment of genotype–phenotype translation schemes in Denmark would be clinically relevant, and we recommendcontinuous updating of the phenotype approach is essential.

The lack of national alignment between public laboratories in Denmark poses several challenges. First, disagreement in phenotyping classification may complicate the use of PGx prescribing and dosing guidelines based on internationally accepted phenotyping classification systems. Second, differences in genotype-phenotype translation makes it challenging to use a patient's phenotyping results across a nationwide healthcare system. Third, the lack of alignment in phenotyping classifications may limit the implementation and future clinical use of PGx.[21,22]

This is the first study to evaluate the national agreement between all public providers of PGx tests in Denmark. The primary limitation is the limited sample size, which may compromise accuracy and generalizability of our findings. The retrospective study design also did not allow for assessment of patient-related outcomes and relevance to everyday clinical practice. Another limitation is the focus on exclusively Caucasian patients, who likely have a high degree of genetic homogeneity. Including patients with a range of different ethnicities may have yielded lower assay sensitivity, which would indicate an even greater need for concordance between PGx testing providers. Finally, phenotype prediction from the three laboratories could theoretically be conducted without performing genotyping in this pilot study. However, genotyping across laboratories represents an assessment of the laboratories' performance in clinical reality, which is a strength of our work. Altogether, our findings demonstrate that alignment of both genotyping and phenotyping classifications is pivotal to ensuring consistent implementation of PGx testing within Denmark.

AUTHOR CONTRIBUTIONS

Concept, design and methodology: Morten Baltzer Houlind, Luise Hansen, Henrik Berg Rasmussen, Jens Borggaard Larsen, Kim Dalhoff, Per Damkier, Anne B. Walls, Charlotte Vermehren, Trine Rune Høgh Andersen, Thomas Kallemose, Lona Christrup, Niels Westergaard; collection of data: Morten Baltzer Houlind, Esben Iversen, Henrik Berg Rasmussen, Jens Borggaard Larsen, Steffen Jørgensen, Niels Westergaard; data analysis and interpretation: Morten Baltzer Houlind, Luise Hansen, Henrik Berg Rasmussen, Jens Borggaard Larsen, Kim Dalhoff, Per Damkier, Anne B. Walls, Thomas Kallemose, Lona Christrup, Niels Westergaard; statistical analysis: Morten Baltzer Houlind, Luise Hansen, Thomas Kallemose; funding: Morten Baltzer Houlind, Lona Christrup; writing—original draft preparation: Morten Baltzer Houlind, Luise Hansen; writing—review and editing: Morten Baltzer Houlind, Luise Hansen, Esben Iversen, Henrik Berg Rasmussen, Jens Borggaard Larsen, Steffen Jørgensen, Kim Dalhoff, Per Damkier, Anne B. Walls, Charlotte Vermehren, Trine Rune Høgh Andersen, Thomas Kallemose, Lona Christrup, Niels Westergaard. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available on request due to restrictions. The data presented in this study are not publicly available due to Danish legislation. Request to access the dataset will require an individual inquiry to the Danish Data Protection agency for approval.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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