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Original Article

Association of transforming growth factor-β1 polymorphisms with the risk of diabetes mellitus

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Abstract: The association between transforming growth factor-β1 (TGF-β1) polymorphisms with the risk of diabetes mellitus (DM) remains elusive. We aimed to evaluate the relationship between TGF-β1 polymorphisms and DM risk. We searched the association studies according to a predefined criteria using electronic databases. The strength of association between TGF-β1 codon 10/25 polymorphisms and the risk of DM was evaluated by odds ratio (OR) with the corresponding 95% confidence interval (CI). Six case-control studies were identified for the analysis of the association between TGF-β1 codon 10/25 polymorphism and the risk of DM. CC genotype at the codon 10 polymorphism was associated with the risk of type 2 DM (T2DM) (P = 0.026, OR = 1.397, 95% CI = 1.041-1.874). No marked association was observed between codon 25 polymorphism and the risk of DM. No evidence of marked publication bias was observed. CC genotype at the TGF-β1 codon 10 site may be an indicator for the risk of T2DM. However, further larger studies should be performed in the future.

Keywords: Diabetes mellitus, gene polymorphisms, transforming growth factor-β1, meta-analysis

Introduction

Diabetes mellitus (DM), a common, frequently-occurring disease, is accounting for 6% of the world's population [1]. Its incidence is increasing with the aging of the population, economic growth, and changes of lifestyle [2]. The chronic and non-communicable features of DM greatly aggravate people's health. On the other hand, DM is associated with a variety of complications which led to increased morbidity and mortality [3]. Diabetic nephropathy (DN) is one of the most important complications of DM and the main cause of end-stage renal disease (ESRD) in both developed and developing countries, nearly 20-40% of ESRD patients receiving initial therapy were primarily diagnosed as DM [4, 5]. Due to the underlying harms of DM, strenuous efforts have been made to prevent the onset of DM. However, no favorable prevention effects have been obtained. Search for the potential biomarkers for DM susceptibility appears imperative.

Transforming growth factor-β1 (TGF-β1), a pleiotropic cytokine, is a key player in immunoregulation [6]. TGF-β1 plays an important role in the activation of inflammation and the resolution of inflammatory responses in a variety of autoimmune diseases [7]. High glucose induces the increase of TGF-β1 [8]. TGF-β1 also stimulates glucose uptake by enhancing the expression of glucose transporter 1 (GLUT1) in mesangial cells that leads to intracellular metabolic abnormalities in DM [9]. TGF-β1 regulates the production of almost every molecule of the extracellular matrix (ECM) [10]. Glucose intolerance is the hallmark of DM. The central feature of DN is an alteration in the composition of the ECM, including thickening of the glomerular basement membrane (GBM) and expansion of the mesangial matrix [11]. In terms of above-mentioned evidence, TGF-β1 expression may be associated with the risk of DM. Genetic polymorphisms were proven to affect the overall expression and secretion of cytokines [12]. For TGF-β1, the polymorphism at codon 10/25 has been reported to be associated with higher or lower TGF-β1 synthesis [13]. In this sense, TGF-β1 polymorphisms may be associated with the susceptibility of DM.

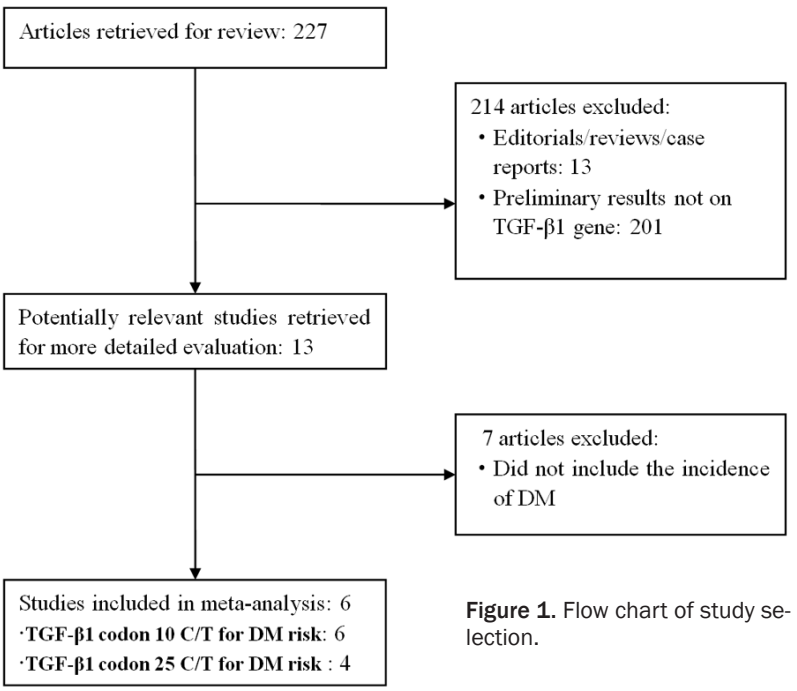

Figure 1. Flow chart of study selection.

Currently, a number of studies have been performed to test the association between TGF-β1 codon 10/25 polymorphism and DM risk. However, the results remain in conflict among the reported studies [14-19]. A previous meta-analysis by Jia et al. [20] showed that TGF-β1 codon 10 polymorphism conferred an elevated risk of nephropathy in T2DM. A meta-analysis of the relationship between TGF-β1 polymorphism and DM risk was rare. With the accumulating evidence, we, therefore, summarized the available publications to perform a meta-analysis with the aim of clarifying the association between TGF-β1 polymorphisms and DM risk.

Materials and methods

Search strategy

According to the recommendations of the PRISMA statement [21] (Supplemental Table 1). The published papers were searched through April 2014 for relevant studies that tested the association between TGF-β1 gene polymorphisms and the risk of DM in humans using PubMed, Embase, Cochrane and China National Knowledge Infrastructure (CNKI) databases. No restriction was imposed on search language. The used search terms were as follows: (1) diabetes mellitus, DM, type 1 diabetes mellitus, type 2 diabetes mellitus, T1DM, T2DM; and (2) gene polymorphisms, transforming growth factor-β1, TGF-β1, codon 10, codon 25, 869T/C and 915G/C. We also scrutinized the reference lists of extracted articles and reviews. If the same data was included in more than one study we chose the study with the most complete analysis.

Inclusion and exclusion criteria

Inclusion criteria: 1. A case-control study. 2. The outcome of interest was DM. 3. A minimum of two comparison groups (DM group vs control group).

Exclusion criteria: 1. Case reports, reviews and editorials. 2. Association of other genes with DM risk. 3. Multiple publications of the same data. 4. Investigation of the role of TGF-β1 gene to diseases.

Data extraction and synthesis

We extracted the following characteristics from each study: First author's last name, publication year, ethnicity of study population, number of cases and controls for TGF-β1 gene codon 10/25 genotype. Frequencies of allele (C, G) were calculated for case and control groups, from the corresponding genotype distribution. Two authors independently performed the data extraction and synthesis with any disagreements resolved by discussion.

Statistical analysis

STATA version 12.0 (Stata Corp, College Station, TX) was used to calculate the available data from each study. The pooled statistic was odds ratio (OR) to measure the association between TGF-β1 gene codon 10/25 polymorphism and DM risk across studies. Heterogeneity of ORs among studies was tested by using the *Q* statistic (significance level at $P < 0.10$). The I^2 statistic, a quantitative measure of inconsistency across studies, was also calculated. The

Table 1. Characteristics of studies evaluating the effects of TGF-β1 polymorphisms on DM risk

First author, year	Ethnicity	DM				Controls				M allele (%)		HWE
		MM	MN	NN	Total	MM	MN	NN	Total	Case	Control	P
TGF-β1 codon 10 C/T[1]												
Tsiavou, 2004[4]	Caucasian	9	11	12	32	7	24	8	39	45.3	48.7	0.351
Buraczynska, 2007[4]	Caucasian	126	224	153	503	76	181	143	400	47.3	41.6	0.387
Javor, 2010[3]	Caucasian	17	56	75	148	27	71	41	139	30.4	44.9	0.931
Jahromi, 2010[3]	Caucasian	78	208	102	388	75	80	74	229	46.9	50.2	$< 10^{-4}$
El-Sherbini, 2013[4]	Caucasian	10	50	39	99	3	27	68	98	34.3	35.1	0.332
Bazzaz, 2014[3]	Caucasian	39	126	83	248	15	57	47	119	41.1	36.5	0.383
TGF-β1 codon 25 G/C[2]												
Tsiavou, 2004[4]	Caucasian	26	6	0	32	35	4	0	39	90.6	94.9	0.945
Javor, 2010[3]	Caucasian	137	11	0	148	117	22	0	139	96.3	92.1	0.599
El-Sherbini, 2013[4]	Caucasian	79	20	0	99	81	9	8	98	89.9	87.2	$< 10^{-4}$
Bazzaz, 2014[3]	Caucasian	201	44	3	248	97	21	1	119	90	90.3	0.905

[1]C/T = M/N; [2]G/C = M/N; [3]Type 1 diabetes; [4]Type 2 diabetes.

pooled ORs were calculated using either fixed-effects model or, in the presence of heterogeneity, random-effects model.

Mantel-Haenszel or I-V heterogeneity model was used.

Furthermore, 95% confidence intervals (CIs) were also calculated. A chi-square test using a web-based program was used to determine whether genotype distribution of the control groups reported conformed to Hardy-Weinberg equilibrium (HWE) (HWE; $P < 0.05$ was considered significant). Chi-square and exact tests were used. Sensitivity analysis was conducted when studies with controls were not in HWE. Potential publication bias was assessed by Begg's test and Egger's test at the $P < 0.05$ level of significance when the number of enrolled studies was more than two. Begg's test and Egger's test were used. $P < 0.05$ was considered statistically significant, except where otherwise specified.

Results

Study characteristics

We firstly retrieved 236 citations from the PubMed, Embase, Cochrane and China National Knowledge Infrastructure (CNKI) databases. Of these, 221 papers were excluded according to the inclusion and exclusion criteria. Six studies [14-19] were enrolled in our analysis for the association between TGF-β1 gene codon 10/25 polymorphism and DM risk (**Figure 1**).

Study characteristics for TGF-β1 gene codon 10 polymorphism with DM risk

Six studies [14-19] were identified for the analysis of the association between TGF-β1 gene codon 10 polymorphism and DM risk (**Table 1**). All studies were performed in Caucasians. A total of 1418 cases and 1024 controls were included. The average frequency of the C allele was 43.5% in cases and 41.3% in controls.

Study characteristics for TGF-β1 gene codon 25 polymorphism with DM risk

Four studies [14, 16, 18, 19] were enrolled for the analysis of the association between TGF-β1 gene codon 25 polymorphism and DM risk (**Table 1**). All studies were performed in Caucasians. A total of 527 cases and 395 controls were included. The average frequency of the G allele was 91.7% in cases and 90.6% in controls.

Association of TGF-β1 gene codon 10 polymorphism with DM risk

C allele and TT genotype were not associated with the risk of T1DM and T2DM (**Table 2**). No significant association between CC genotype and T1DM risk was observed (**Table 2**). CC genotype conferred a significantly increased risk of T2DM (**Figure 2**; **Table 2**). Sensitivity analysis

Table 2. Meta-analysis of the association of TGF-β1 polymorphisms with the risk of DM

Genetic variant	DM	Studies	Q test	Model selected	OR (95% CI)	*P*-value
			p-value			
TGF-β1 codon 10						
C vs T	T1DM	3	0.066	Random	0.901 (0.698-1.162)	0.421
	T2DM	3	0.033	Random	1.304 (0.858-1.984)	0.214
CC vs (CT+TT)	T1DM	3	0.137	Fixed	0.738 (0.478-1.140)	0.171
	T2DM	3	0.406	Fixed	1.397 (1.041-1.874)	0.026
TT vs (CT+CC)	T1DM	3	0.022	Random	1.043 (0.663-1.640)	0.856
	T2DM	3	0.095	Fixed	0.826 (0.528-1.292)	0.402
Sensitivity analysis						
C vs T	T1DM	2	0.021	Random	0.875 (0.531-1.441)	0.601
CC vs (CT+TT)	T1DM	2	0.107	Fixed	0.862 (0.415-1.791)	0.690
TT vs (CT+CC)	T1DM	2	0.024	Random	1.202 (0.601-2.402)	0.603
TGF-β1 codon 25						
G vs C	T1DM	2	0.767	Fixed	1.019 (0.866-1.199)	0.820
	T2DM	2	0.790	Fixed	1.010 (0.789-1.292)	0.939
GG vs (GC+CC)	T1DM	2	0.674	Fixed	1.044 (0.825-1.320)	0.721
	T2DM	2	0.876	Fixed	0.949 (0.664-1.356)	0.774
CC vs (GC+GG)	T1DM	2	0.738	Fixed	1.802 (0.282-11.520)	0.534
	T2DM	2	0.085	Fixed	0.532 (0.020-14.194)	0.706
Sensitivity analysis						
G vs C	T2DM	1	-	Fixed	0.955 (0.593-1.539)	0.851
GG vs (GC+CC)	T2DM	1	-	Fixed	0.905 (0.454-1.805)	0.778
CC vs (GC+GG)	T2DM	1	-	Fixed	3.646 (0.144-92.551)	0.433

OR: odds ratio, CI = confidence interval.

showed similar results compared to those from non-sensitivity analysis.

Association of TGF-β1 gene codon 25 polymorphism with DM risk

TGF-β1 gene codon 25 polymorphism was not associated with the risk of DM (**Table 2**). Sensitivity analysis did not changed the overall results significantly.

Evaluation of publication bias

No significant publication bias was observed (codon 10 C vs T for T1DM/T2DM: Begg *P* = 0.602/0.603, Egger *P* = 0.382/0.325; codon 10 CC vs. (CT+TT) for T1DM/T2DM: Begg *P* = 0.602/0.117, Egger *P* = 0.793/0.286; codon 10 TT vs (CT+CC) for T1DM/T2DM: Begg *P* = 0.117/0.602, Egger *P* = 0.663/0.444).

Discussion

Increasing attention has been focused on the etiology of DM. The confirmation of possible genetic origin of DM would give an insight to early prevention of DM.

A number of investigations reported that gene polymorphism was associated with the susceptibility of DM and could be used as a marker to predict the onset of DM, such as ACE I/D gene polymorphism [22, 23]. In our meta-analysis, we found that CC genotype at the TGF-β1 codon 10 site was a risk factor for the onset of T2DM. Several facts may account for the association of TGF-β1 gene polymorphism with DM risk. First, TGF-β1 plays a role of both pro-inflammation and anti-inflammation in many pathophysiological conditions. TGF-β1 inhibits and reverses the activation of macrophages and down-regulates central effector mechanisms of the innate immunity [24]. The innate immune system modulates the effects of many factors, such as genes, fetal programming, nutrition, and age on the later development of metabolic sequela associated with insulin resistance [25]. On the other hand, TGF-β1 can also positively regulate immune responses. For exam-

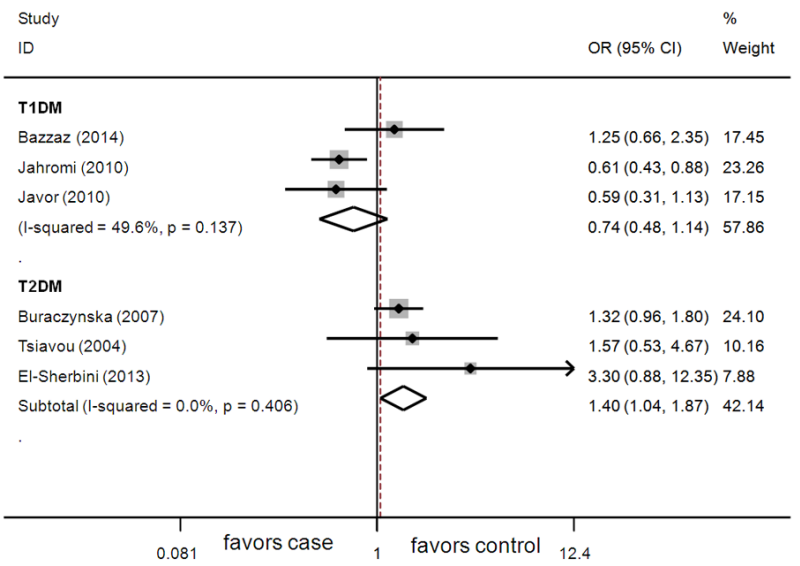

Figure 2. Association between CC genotype and DM risk.

ple, TGF-β1 supports the differentiation of T-helper 17 (Th17) cells that are activated in many proinflammatory conditions in the presence of Interleukin-6 (IL-6). Of note, IL-6 levels were increased before the onset of T2DM [26]. TGF-β1 could possibly prevent or slow down the autoimmune-mediated destruction of pancreatic Langerhans islets, leading to an absolute lack of insulin production [27]. In this sense, the activation of the innate immune system and the development of a systemic low-grade chronic inflammation are closely involved in the development of T2DM. In terms of above-mentioned evidence, TGF-β1 is closely associated with the susceptibility of DM. Second, the ability of an individual to produce high or low levels of TGF-β1 may be genetically predetermined. For example, the inflammatory and anti-inflammatory activities of TGF-β1 and its signaling pathway is often inactivated by mutation or altered expression of its components [28]. Gene polymorphisms can influence cytokine production or function, they may contribute to genetic predisposition to the disease. Polymorphism at codon 10/25 may be associated with higher or lower TGF-β1 synthesis [13]. SNPs in codon 10/25 of TGF-β1 alter the amino acid sequence (Leu10Pro/Arg25Pro) and also affect TGF-β1 level. TGF-β1 Pro10 (C) allele secrets almost twice as much as Leu10 (T) allele [29]. The C allele was repeatedly associated with increased TGF-β1 production, resulting from a leucine-to proline substitution in the signal amino-acid sequence of the protein, which indicated that certain allele/genotype may affect the risk of DM.

Our findings agreed partially with the above-mentioned evidence. We found that codon 10 CC genotype increased the risk of T2DM, which was consistent with the notion that C allele was linked to an increased production of TGF-β1. However, we observed that codon 10/25 polymorphism was not associated with the susceptibility of T1DM, which might be due to the following facts: first, T1DM develops as a result of progressive T cell-mediated autoimmune destruction of only pancreatic β cells [30]. TGF-β1 is not directly associated with the damage of pancreatic β cells, which indicated that TGF-β1 gene polymorphism may not affect the T1DM risk obviously.

Second, the etiology, metabolic status and disease-specific genetic background vary quite a lot between T1DM and T2DM. Compared with T1DM, T2DM is a complex metabolic disease characterized by insulin resistance and/or pancreatic β-cell dysfunction, and is always associated with metabolic abnormalities, including obesity, hypertension and hyperlipidemia, which were associated with the inflammatory responses [31]. We speculated that TGF-β1 may interacted with these factors through its pro- and anti-inflammatory effects to increase the risk of T2DM. Interestingly, although TGF-β1 gene polymorphism at codon 25 is significantly associated with TGF-β1 production, we observed that TGF-β1 codon 25 polymorphism was not associated with DM, which might be due to the facts that only six studies were included in the investigation, and the studied populations were limited to Caucasians, which might not preclude the possibility that TGF-β1 gene polymorphism exerts effects in DM risk in a population-specific manner. In the past, the association between TGF-β1 gene polymorphism and metabolic diseases were investigated in a number of studies. Scaglione et al. [32] reported that TGF-β1 gene polymorphism was associated with left ventricular geometry and

function in hypertensive subjects. Long et al. [33] reported that TGF-β1 genes were associated with obesity phenotypes. Argano et al. [34] reported that TGF-β1 T29C gene polymorphism was associated with the severity of hypertension. Rosmond et al. [35] reported that Pro10 allele in the TGF-β1 gene pathway contributed to obesity. Fuku et al. [36] reported that total, leg, and appendicular fat-free mass index were significantly lower in male subjects with CT/TT genotypes compared with those with CC genotypes. All these evidences strongly indicated the possibility that TGF-β1 gene polymorphism might be associated with the development of DM.

Several limitations should be considered in our study. First, the heterogeneities might affect the results of our meta-analysis, although a random-effects model had been conducted. Second, most participants were Caucasians, which might result in selection bias. More studies in other regions, such as Africa and Asia should be conducted in the future. Finally, although no evidence of significant publication bias was observed, the relative small number of participants decreases the statistical power. The stages of DM and interactions among different sites of TGF-β1 gene or other genes might affect the results. More in-depth analysis in terms of these factors should be performed in the future.

Taken together, the results of our study suggest that CC genotype at the TGF-β1 codon 10 site may be an indicator for the risk of T2DM. However, more studies are needed in the future.

Acknowledgements

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Disclosure of conflict of interest

None.

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Supplementary Table 1. PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	2
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	2
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	2
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	2
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	4

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	6
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	6
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	6

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