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Assessment of Serum Copeptin in Relatively Well Controlled and Uncontrolled Children and Adolescents with Type 1 Diabetes Mellitus.

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ABSTRACT

Copeptin is co-released with arginine vasopressin from hypothalamus. The role of copeptin in uncontrolled type 1 diabetes mellitus warrants more investigation. To estimate serum Copeptin in relatively well controlled and uncontrolled children and adolescents with type 1 diabetes mellitus and its relation to clinical, anthropometric parameters and laboratory variables. Also to test if, RS 58542926 variant could affect the serum copeptin level. The study compromised 36 well Controlled, 35 uncontrolled Typ1 diabetic cases and 48 healthy children's controls. Copeptin levels and Genotyping of RS 58542926 were determined. Cases exhibited significantly higher values of the Diastolic BP, FBG, HbA1C, cholesterol and serum copeptin than controls (P=.024, P<.001, P <.001, P= .012 and P < .01 respectively). Statistically significant positive correlation between serum copeptin and gender, diastolic BP, HbA1C and cholesterol in diabetic patients was detected (r =.433**, .252*, .255*, .217* respectively) . RS 58542926 genotype and HbA1C were the most independent predictors of copeptin (P <.001&.003) respectively. Serum copeptin was elevated in uncomplicated diabetic children. It was not an independent predictor of the uncontrolled cases of Type1Diabetes Mellitus . RS 58542926 variant and HbA1C were the most independent predictors of serum copeptin.

Keywords: Copeptin, HbA1C, RS 58542926, Type 1 diabetes mellitus.

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INTRODUCTION

Copeptin, is the C-terminal part of pro vasopressin is known to be co-released with arginine vasopressin (AVP) from hypothalamus (neurohypophysis). As a surrogate marker of the AVP system, copeptin has gradually replaced AVP in several clinical studies largely due to its methodological advantages. Over 90% of AVP is bound to platelets, this hindered pre-analytical processing steps. In several studies the concentration of plasma copeptin has been identified as a surrogate marker for vasopressin **[1, 2]**.

The role of copeptin in uncontrolled Type1Diabetes Mellius(T1DM) warrants more investigation. Zerbe et al. demonstrated that vasopressin is markedly elevated in uncontrolled DM. It was increased mainly, due to associated decreased intravascular volume. However, Schmit et al, reported that several plasma amino acids are increased in diabetic patients and sharing in raising vasopressin level **[3, 4]**.

T1DM is a lifelong problem that can be progressive from childhood into adulthood. Recent epidemiologic data indicate that the prevalence of T1DM is rising globally **[5, 6]** . T1DM in childhood is one of the most complex chronic diseases. Patients are exposed to micro- and macrovascular complications as hypertension, renal failure, coronary heart disease and congestive heart failure **[7, 8]**, but the causes of this interaction are incompletely understood.

Little is known about the role of copeptin in children and adolescents patients with T1DM. Bjornstad and Maahs, reported that copeptin is a promising biomarker for diabetic complications. It will serve as a therapeutic target with respect to the prevention of diabetes-related long-term complications **[9]**. Copeptin, is a stable and sensitive marker for AVP release. It is a39-amino acid glycopeptide that is co-secreted with AVP from the neurohypophysis **[10]**.

The reported results indicate several links between the AVP system and components of the metabolic syndrome **[11-13]**. Furthermore, a study in a hypertensive population reported a relation between copeptin and waist circumference (WC), systolic blood pressure (BP), DM and triglycerides (TG) **[14]**.Even mild to moderate stress conditions contribute to release of copeptin, so these lead to a problem in research on copeptin in various diseases **[15]**.

The TM6SF2 T-allele, encoding the E167K amino acidic substitution, which is expressed in pancreatic β-cells, the liver, adipose tissue and intestine, has unclear biological function.

Musso et al. showed that the single nucleotide polymorphism (SNP) Rs58542926 variant affects glucose homeostasis in nonalcoholic fatty liver disease (NAFLD) **[16]**.The TM6SF2 C>T polymorphism has been linked to a lower LDL-cholesterol level and to an increased risk of DM **[17]**.

The aim of this study was to assess serum copeptin levels in relatively well controlled and uncontrolled children and adolescents with T1DM. Additionally, we aimed to investigate the relation between serum copeptin levels and clinical, anthropometric parameters and laboratory variables in this group of patients. Also, to test if, Rs58542926 variant could affect the serum copeptin level.

MATERIALS AND METHODS

Seventy-one children and adolescents (26 males,45 females); previously diagnosed with T1DM were recruited from the Pediatric Diabetic Clinic in Centre of Excellence in National Research Centre. The study protocol was approved by the Human Ethics Committee of National Research Center, and written informed consent was obtained from all children and their parents. A control group included 48 children (9 males, 39 females); they were age-matched healthy subjects.

Exclusion criteria: Obesity, cases suffering of diabetic ketoacidosis , history suggestive of chronic diabetic complications, patients with a significant history of cardiac failure, seizures or a history of significant stroke disease.

- All children were subjected to history taking and clinical examination to fulfill needed data: Insulin therapy, regarding dose in units/kg and type. History suggestive of acute metabolic complications, or chronic diabetic complications was included.
- Blood pressure was measured according to American Heart Association guidelines; three times for patients and controls after 5-min rest in sitting position with the use of mercury sphygmomanometer. The mean value of 2nd and 3rd measurement was calculated. SBP was defined as the onset of the Korotkoff sound (K1), and DBP was defined as the fifth Korotkoff sound (K5).
- Anthropometric indices: Body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. Body mass index was calculated as weight divided by height squared (kg/m2). Waist circumference (WC) was measured at the level midway between the lowest rib margin and the iliac crest. Hip circumference (HIP C) was measured at the widest level over the greater trochanters in a standing position by the same examiner; then waist to hip ratio (WHR) and Waist to height ratio (WHtR) were calculated **[18]**.

Abdominal Ultrasonography

In addition to the routine abdominal ultrasound examination based on the clinical indication, ultrasonography (US) distinctively quantifies visceral fat and subcutaneous fat. We measured the maximum preperitoneal visceral fat thickness (VFT) and the minimum subcutaneous fat thickness (SFT) by US. The visceral fat thickness (VFT) was measured by 3.5 - 5 MHz convex-array probe. VFT is the distance between the internal surface of the abdominal surface of abdominal muscle and the anterior wall of the aorta 1 cm above the umbilicus. The thickness of subcutaneous fat was measured by placement of a 3.75-MHz probe perpendicular to the skin on the epigastrium. Longitudinal scans are obtained along the middle line (linea Alba). The thickness of the subcutaneous fat is defined as the distance between the anterior surface of linea Alba and the fat-skin barrier **[19]**.Ultrasound apparatus model is SA –R3 (No S06YM3 HDC00012F) SAMSUNG MEDISON Company –South Korea .

Laboratory measurements

Ten millimeters of venous blood were withdrawn under complete aseptic precautions from fasting subjects (12 - 14 hrs).five ml of blood were anticoagulated with edta for CBC and DNA purification , rest of sample was left to clot at room temperature for 15 min then centrifuged, sera were collected and aliquated for evaluation of the following parameters:

- 1) lipid profile (serum cholesterol ,triglycerides, High density lipoprotein HDL, Low density lipoprotein LDL) and fasting blood glucose was determined via Olympus AU 400 supplied from Olympus Life and Material Science (Europe GmbH, Wendenstraße, Hamburg, Germany).
- 2) Glycosylated Hb (HbA1c) was measured using ion exchange HPLC (high purified liquid chromatography) Kit supplied by Crystal Chem, USA.
- 4) Copeptin levels were estimated using Elabsciensce Eliza kit (USA) Cat: E-EL-H0851 Lot: CF3TWLX2EZ
- 5) Genomic DNA was extracted from 3 ml whole blood by a commercially available DNA extraction kit (ThermoFischer , Germany) according to manufacturer's protocol.DNA yield was measured by Nanodropper . The purified genomic DNA showed a 260/280 ratio between 1.7 to 1.9.

• Rs 58542926 polymorphism was determined by a predesigned Taqman SNP genotyping assay (Applied Biosystems). Oligonucleotides used for allelic discrimination assays for Rs 58542926 as following:
Context sequences for Rs 58542926 (VIC/FAM) GTGAGGAAGAAGGCAGGCCTGATCT[C/T] GGAGCTGTATTTGCCTTCCATGGTG

The reaction was performed in 25 ul final volume with real time polymerase chain reaction. For genotyping quality control, duplicate samples and negative controls were included to ensure accuracy.

Statistical analysis

Data analysis was carried out using the standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 17.0 (SPSS Inc., USA). All numeric variables were expressed as mean ± standard deviation (SD). The intergroup comparisons were performed by using an independent-sample t test and a one-way analysis of variance and Chi-Square tests for categorical variables. Pearson's and Spearman's correlation tests (r=correlation coefficient) were used for correlating normal and nonparametric variables, respectively. For all tests, a P-value of less than 0.05 was considered significant. Linear multiple regression was run to predict serum copeptin. A receiver operating characteristic curve was configured to test the validity of serum copeptin vs. HbA1C and FBG in detecting the uncontrolled T1DM cases.

RESULTS

Seventy-one patients and fourty eight healthy volunteer controls were studied, with mean age of (13.25±3.84, 12.18 ±4.32 years respectively) with no statistically significant difference. As regard gender: cases (F =45, M =26) and controls (F=39, M=9), with was no statistically significant difference between the 2groups (p > 0.05). Clinical and laboratory characteristics of the studied subjects are represented in Tables 1. Compared with the controls, cases were exhibited significantly higher values of the Diastolic BP, FBG, HbA1C, cholesterol and serum copeptin (P=.024, P<.001, P <.001, P= .012 and P < .01 respectively). However, there was statistically significantly higher level of HDL in the controls (P< .001). As regard BMI, systolic BP, WHR and WHtR there were no statistically significant difference between the 2groups (P>.05). Comparison of serum copeptin in male and females showed that males had statistically significantly higher level of copeptin than females (P< .001). We sub- grouped patients into: controlled patients with HgA1c level less than 8 % (n = 36) with those uncontrolled with a level ≥ 8 % (n = 35). Considering copeptin distribution between subgroups there was statistically significant difference between the three groups (P = 0.026) by ANOVA (Table 2,Table 3, Figure 1).

No correlation existed between serum copeptin, age, duration of disease, BMI ,WC, WHtR, VFT, FBS and Systolic BP in diabetic patients. Interestingly, the observed positive correlation between serum copeptin and gender, diastolic BP, HbA1C and cholesterol in diabetic patients was statistically significant (r =.433**, .252*, .255*, .217* respectively). Details are included in Table 4 and Figure 2.

We investigated associations of serum copeptin, and of allelic variations in TM6SF2 RS 58542926 C>T genotype. TM6SF2 T-allele carriers had significantly higher mean level of serum copeptin (P=.010). We observed no case or control was TT carriers. Details are shown in Table 5.

DISCUSSION

Little is known about the role of copeptin in children and adolescents with type 1 diabetes mellitus. In the present study, copeptin was evaluated in the total group and both sub-groups (patients with controlled, uncontrolled type 1 DM and healthy controls). Results showed a significant difference between controls and both the Uncontrolled & Controlled cases. . Zerbe et al reported that increased plasma vasopressin level in people with type 1 or with type 2 diabetes mellitus **[3]**. The study showed that copeptin is markedly elevated in uncontrolled diabetes and, therefore, plays no role in the extreme dehydration associated with this condition **[3]**.However, Schiel et al showed that the diabetes patients revealed no significant difference with respect to copeptin when compared with normal controls .Moreover; he reported that the level of copeptin may also be related to stress, behavioral and lifestyle factors **[20]**. Knowing, that stress conditions contribute to release of copeptin, these lead to a problem in research on copeptin in various diseases. In addition, in healthy subjects, a 28h water deprivation could lead to a twofold increase in blood level of copeptin **[21]**.Szinnai etal reported that Copeptin showed same changes during water states as shown for AVP **[22]**.

In our study, Copeptin did not correlate with age, similar results were recorded by Schiel etal and Dobša etal [20, 21]. A positive correlation was figured between serum copeptin and gender ; a finding reported by Bhandari etal **[23]**. A strong correlation between copeptin and total cholesterol was detected; this was in agreement with previous report **[20]**. Also, HbA1C showed a significant correlation with serum copeptin while other studies did not report the same result. This can be attributed to inclusion of complicated diabetic cases in the study, but we excluded all complicated cases **[20]**. No correlation existed

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between serum copeptin and BMI, WC, WHtR or VFT. Both Abd El-Fattah et al. and Eltabakh et al. reported a significant positive correlation between copeptin and BMI, WC, WHR but the studied subjects were obese **[24,25]**.

To our knowledge, no other cohort studies have examined the associations of TM6SF2 RS 58542926 variant and copeptin. Previous studies revealed its association with postprandial lipoprotein metabolism and glucose homeostasis in nonalcoholic fatty liver disease (NAFLD) **[16, 26, 27]**.Transmembrane 6 superfamily member 2 (TM6SF2) rs58542926 C>T polymorphism showed that T-allele was associated with higher serum copeptin. Linear multiple regressions, to predict serum copeptin showed that TM6SF2 RS 58542926 variant and HbA1C were the most independent predictors.

Using a receiver operating characteristic curve (ROC curve) to measure the diagnostic accuracy of copeptin as a biomarker of cases with uncontrolled T1DM , it was found that area under the curve (AUC) of serum copeptin was 0.671; which indicates that the overall diagnosis accuracy of copeptin is not significant (p = 0.051). Accuracy is measured by the area under the ROC curve. An area of .60-.70 represents a poor test; no previous study studied this point. The result means that serum copeptin was not as good as FBG OR HbA1C in diagnosing uncontrolled cases of T1DM.Figure 3

Table (1) : Clinical and laboratory characteristics of the studied subjects

	T1DM Cases (No 71)		Controls(No 48)		
	Mean	Std. Deviation	Mean	Std. Deviation	Sig. (2-tailed)
Age (years)	13.25	3.84	12.18	4.32	. 06
BMI, kg/m2	21.1010	5.39543	18.8725	4.20	.126
Systolic BP, mm Hg	106.77	10.805	101.88	5.303	.050
Diastolic BP, mm Hg	69.31	7.174	63.13	7.039	.024
WHR	.8342	.05804	.8356	.06132	.928
WHtR	.4818	.06545	.4850	.12675	.886
FBG level, mg/dL	162.375	40.14496	80.0625	9.80115	<.001
HbA1c, %	8.0324	.82406	5.4077	.71585	<.001
Cholesterol mg/dL	155.416	20.76546	139.875	26.6880	.012
TG, mg/dL	58.4097	18.75008	62.4375	14.8187	.424
HDL mg/dL	38.9722	6.50238	47.0000	19.1485	<.001
Copeptin pg/ml	394 .504	150.43	308.500	94. 804	< .01
Copeptin pg/ml BY Gender	**F** 337.6554	120.79277	**M** 468.2714	155.56396	<.001

(BMI) Body mass index, (WHtR) Waist to height ratio , (WHR)Waist to hip ratio, (FBG)Fasting blood glucose, (TG) Triglycerides, (HDL)High-density lipoprotein ,(LDL) Low-density lipoprotein, (HbA1C) Glycosylated Hb.

Table (2): Multiple comparison of serum Copeptin level between groups

Subgroup	N	Mean	Std.Deviation	Minimum	Maximum	ANOVA SIG
Uncontrolled cases	35	426.60	175.16	188.50	814.40	
Controlled cases	36	363.29	115.98	180.40	507.30	.026
Controls	48	308.50	94.804	160.30	461.40	

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Table (3) : Comparison between clinical and laboratory characteristics of the Uncontrolled & Controlled cases

	Uncontrolled T1DM (No35)		Controlled T1DM (No 36)		
Item	Mean	Std. Deviation	Mean	Std. Deviation	Sig. (2-tailed)
Age (years)	13.4857	3.68719	12.2917	4.18223	.207
Duration of T1DM (years)	6.029	3.47	5.056	3.0066	.211
BMI	21.51	6.053	20.32	4.68	.363
Systolic BP, mm Hg	108.97	12.420	104.41	8.941	.097
Diastolic BP, mm Hg	71.21	7.153	67.35	6.768	.032
WHTR	.4923	.071	.4706	.05916	.171
Total insulin dose per KG	1.27	.52	1.31	.73	.790
Fasting BG	180.08	47.07	144.00	24.64	>.001
HbA1C	8.63	.65	7.5	.390	>.001
Cholesterol mg/dL	161.028	23.77	150.38	16.16	.030
Triglyceride mg/dL	57.2857	20.92	58.68	15.11	.748
HDL mg/dL	38.6000	7.138	39.27	6.04	.667
LDL mg/dL	62.2857	17.32	62.13	11.125	.966
Copeptin pg/ml	426.60	175.166	363.29	115.98727	.076

(BMI) Body mass index, (WHtR) Waist to height ratio , (FBG)Fasting blood glucose, (HDL)High-density lipoprotein , (LDL)Low-density lipoprotein, (HbA1C)Glycosylated Hb

Table (4) : Correlations between serum Copeptin and biochemical parameters in children with diabetes

		Gender	Age (Ys)	Duration of T1DM (Ys)	BMI	Systolic BP	Diastolic BP	WC	WHtR	VFT	FBS	HbA1C	Chol.	TG
Copeptin pg/ml	r	.433**	.172	-.046	-.101	.178	.252*	.066	-.104	.108	-.034	.255*	.217*	-.185
	P	.000	.152	.706	.406	.162	.034	.586	.392	.350	.780	.032	.047	.121

(*BMI) Body mass index, (BP) Blood Pressure , (WC) Waist Circumference ,(WHtR) Waist to height ratio ,* (VFT) visceral fat thickness, *(FBG)Fasting blood glucose, (HbA1C)Glycosylated Hb* ,(TG) Triglycerides

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Table (5) : Patients and controls grouped according to theTM6SF2RS 58542926 C>T genotype

C>T genotype	Uncontrolled T1DM	Controlled T1DM	Controls	TOTAL
CC Count	29 (90.6%)	28 (96.5%)	41 (86.6%)	70(80.5%)
CT Count	3 (9.4%)	1 (3.5%)	7 (13.4%)	6 (6.9%)
Total Count	32	29	48	109
within RS 58542926	29.4	26.6%	44.0%	100.0%

	RS 58542926	N	Mean	Std. Deviation	Sig. (2-tailed)
Copeptin pg/ml	CC	98	366.09	145.93	.010
	CT	11	490.00	185.20	

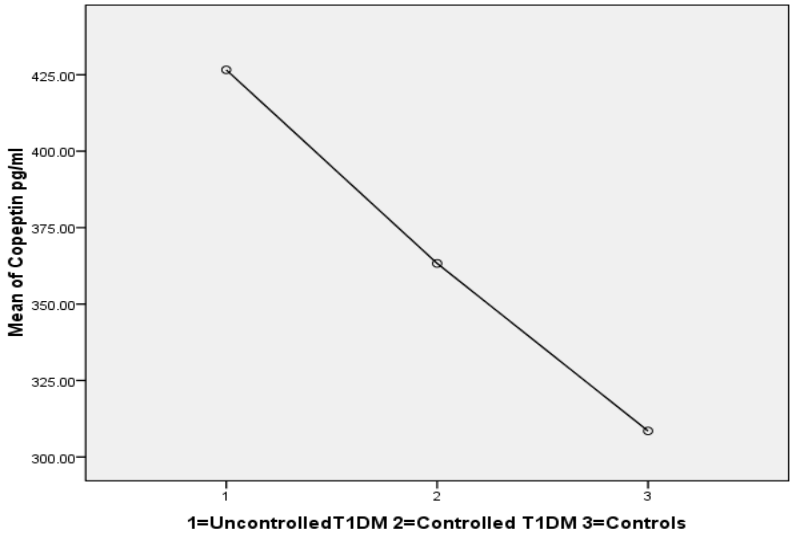

Figure (1) : Mean serum Copeptin levels in various groups

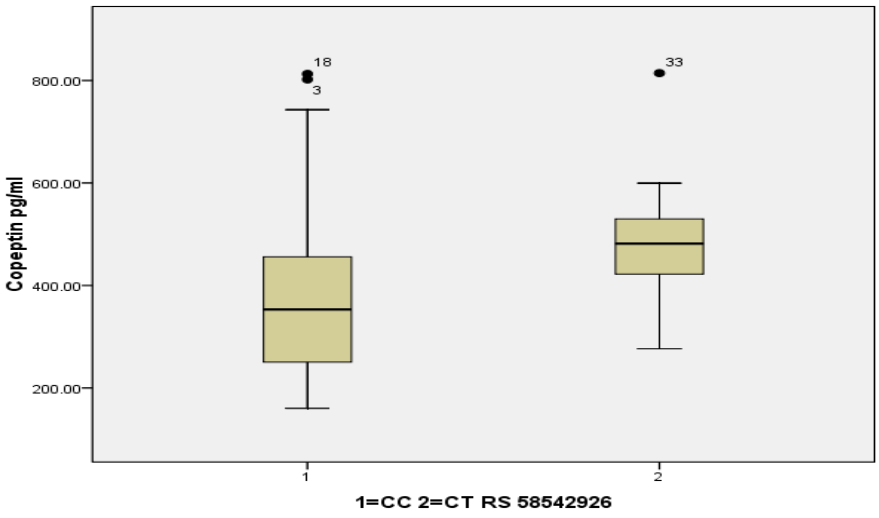

Figure (2) : Boxplot of serum copeptin in theTM6SF2 RS 58542926 C>T genotype

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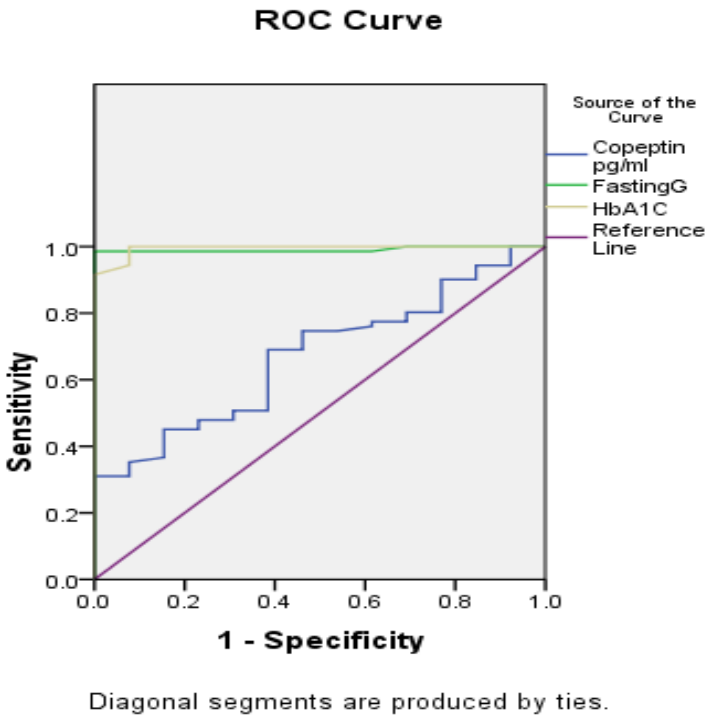

Figure (3) : ROC curve of serum copeptin vs. HbA1C and FBG in predicting Uncontrolled DM

CONCLUSION

Based on the current study results, serum copeptin was elevated in uncomplicated T1DM children. Serum copeptin was not an independent predictor of the uncontrolled cases of T1DM. TM6SF2 RS 58542926 variant and HbA1C were the most independent predictors of serum copeptin. The present study is the first to investigate the associations between TM6SF2 RS 58542926 variant and serum copeptin, a large study is needed to evaluate this association and its causal inference with T1DM abnormalities.

Weaknesses of the study; the study was a cross sectional not a prospective longitudinal study; the small number of controls. More research is needed in order to prove its clinical usefulness of copeptin in T1DM especially in complicated cases and its relation to TM6SF2 RS 58542926 variant.

Conflict of interest statement: The authors have nothing to disclose. No substantial part of this article has been published before or introduced in any conference.

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