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Judul Jurnal : The Role of MMP-9 rs 3918242 genetic variant in association between MMP-9 serum level with risk factors of acute ischemic stroke

Penulis Artikel Ilmiah : 3 orang

Status Pengusul : Meyvita Silviana Dodik Tugasworo Amin Husni

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Vol 10, No 1 (2021): (Available online : 1 April 2021)

Table of Contents

Relationship of gallbladder histopathology towards types of stones in cholelithiasis patients at
Universitas Sumatera Utara Hospital, Medan, Indonesia

Adi Muradi Muhar Denny Rifsal Siregar Doddy Prabisma

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Vitamin D Receptor (VDR) Apa1 gene polymorphism increasing the risk of breast cancer women in Bali,
Indonesia

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Maternal and perinatal outcomes with covid-19: lesson learned from the tertiary hospital
Putri Sekar Wiyati Rabiah Adawiyah Julian Dewantiningrum Besari Adi Pramono
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Finite element analysis of artificial hip joint implant made from stainless steel 316L
Rilo Berdin Taqriban Rifky Ismail J Jamari A Priharyoto Bayuseno
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Sleeve gastrectomy and pancreas omentoplasty improved \hat{I}^2 cell insulin expression and interleukin-1 \hat{I}^2 serum level in non-obese diabetes mellitus rat
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A review of chest X-ray in pulmonary hypertension: correlation of right descending pulmonary artery diameter with mean pulmonary artery pressure
Tara Nareswari Christina Hari Nawangsih Priharsanti Hermina Sukmaningtyas Farah Hendara Ningrum Bambang Satoto Antonius Gunawan Santoso
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The difference in the prevalence of periodontitis degrees between ischemic stroke with diabetes mellitus and prediabetes mellitus
Nanik Sulistyowati Retnaningsih Retnaningsih Widiastuti Rendy Sumali Amin Husni Endang Kustiowati Jimmy Eko Budi Hartono Dwi Pudjanarko
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The association between tension-type headache (TTH) and the severity of insomnia
Runi Asmarani Herlina Suryawati Amin Husni Aris Catur Bintoro Hexanto Muhartomo Dani Rahmawati Suryadi Suryadi
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Factors related to the level of prisoner's participation in implementing the voluntary counseling and testing program in penitentiary Semarang, Indonesia
Untung Sujianto Laura Ayudina Nasyiatul Aisyiyah
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by Dodik Tugasworo

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The Role of MMP-9 rs 3918242 genetic variant in association between MMP-9 serum level with risk factors of acute ischemic stroke



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ABSTRACT

Background: Increased serum MMP-9 level is known to be biomarker of atherosclerotic events and risk of cerebrovascular disease. Risk factors for acute ischemic stroke are known to be related to serum MMP-9 level. The genetic variant of MMP-9 rs3918242 is known to have a role in serum MMP-9 level. The aim of the study is to determine the role of MMP-9 rs 3918242 genetic variant in association between MMP-9 serum level with risk factors of acute ischemic stroke.

Methods: This was a cross-sectional study with an observational analytic design. 62 subjects of acute ischemic stroke treated at Dr. Kariadi Hospital Semarang from October 2018 to February 2019 were involved in this study. Statistical analysis was assessed by the bivariate test and followed by a logistic regression test.

Results: From 62 subjects in this study, 45 (72.6%) had high serum MMP-9 level. The association between serum MMP-9 level with risk factors of acute ischemic stroke was statistically significant in hypertension, diabetes mellitus, dyslipidemia, obesity, and hyperuricemia. There is a positive correlation of serum MMP-9 level with a number of acute ischemic stroke factors. In multivariate analysis, the most influential variables were obesity and hyperuricemia. CC Genetic Variant has a role in the association between serum MMP-9 level with risk factors of acute ischemic stroke, which are hypertension, diabetes mellitus, dyslipidemia, obesity, and hyperuricemia. Meanwhile, CT genetic variants only play a role in the association of serum MMP-9 levels with obesity and hyperuricemia as the risk factors for acute ischemic stroke.

Conclusion: CC genetic variants with high serum MMP-9 levels are associated with more acute ischemic stroke risk factors than CT genetic variants with high MMP-9 serum levels. Therefore, CC genetic variants have the potential to pose more risk of acute ischemic stroke.

Keywords: CIMT, Genetic variant, MMP-9, Acute ischemic stroke.

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INTRODUCTION

Ischemic stroke occurs due to obstruction of blood flow caused by endothelial interactions with blood constituents that can cause thrombosis and blockage of blood flow. The number of ischemic strokes is about 87% of all strokes.¹ The well-known risk factors contribute to ischemic stroke are hypertension, diabetes, dyslipidemia, and obesity, but there are still many other risks.^{1,2} Evidence from twin and familial aggregation studies suggested that genetic risk factors predisposed to ischemic stroke.³ The genes involved in the inflammatory response are under investigation to look for genetic variants that predispose to ischemic stroke. Buraczynska et al. stated that the

risk factors for stroke that could not be modified were also closely related to the MMP-9 gene polymorphism.³

Stroke has been shown to be a cause of long-term disability and death. Ischemic stroke involves hypoperfusion around the infarct area where the cells are still viable, but the cascade leading to the apoptotic process has begun.⁴ Acute cerebral blood flow obstruction results in many pathological conditions, including disturbances in homeostasis, neuronal excitotoxicity, intracellular calcium build-up, depolarization per infarction, free radical accumulation, lipid peroxidation, and protein synthesis disorders that lead to irreversible neuronal damage. The dead neurons will trigger the release of an immune response, which triggers

the activation and infiltration of pro-inflammatory cells. Activation of these cells will produce cytotoxic agents, including Matrix Metalloproteinases (MMPs).⁵⁻⁹

The study which associates serum MMP-9 level with risk factors of ischemic stroke by looking at the genetic background of MMP-9 is promising.^{8,10} Thus, this study aims to determine the role of MMP-9 rs 3918242 genetic variant in association between MMP-9 serum level with risk factors of acute ischemic stroke.

METHODS

This was an observational analytic study, involving 62 subjects determined by consecutive sampling. Subjects were

patients with acute ischemic stroke who were admitted to Neurology department ward in Kariadi Hospital, Semarang, during October 2018 - January 2019. The inclusion criteria were acute ischemic stroke with an onset less than 48 hours as evidenced by non-contrast head CT scan and agreed to participate in the study. Exclusion criteria were patients with transformation hemorrhagic stroke, severe systemic disease (CKD, CHF, chronic liver disease, malignancy), history of drug use that affected MMP-9 level (tetracycline, minocycline, doxycycline, NSAIDs, statins), and peripheral arterial disease.

The subjects were examined in terms of the association between MMP-9 variant genetic and serum levels with risk factors of atherosclerosis. Risk factors include hypertension, diabetes mellitus, dyslipidemia, body mass index, hyperuricemia, and smoking habits. Hypertension was classified by JNC VIII, normal <120/<80, Prehypertension 120-139/80-89, hypertension 140-149/90-99, and hypertension $\geq 160/\geq 100$. It was measured two times or more at onset in ER and in neurology ward. Diabetes Mellitus was classified by Indonesian Consensus of Type 2 diabetic. It was considered normal if HbA1c <5.7, fasting blood sugar <100, blood sugar 2 hours post-meal <140; prediabetic if HbA1c 5.7-6.4, fasting blood sugar 100-125, blood sugar 2 hours post-meal >140; and type 2 diabetes if HbA1c >6.5, fasting blood sugar ≥ 126 , blood sugar 2 hours post-meal ≥ 200 . Blood glucose was taken at less than 48 hours of stroke onset. Dyslipidaemia was classified from The Indonesian Guideline of Dyslipidaemia Treatment 2015. It was considered dyslipidemia if total cholesterol >200, and or triglyceride >150, and or HDL < 40 and or LDL >130. Body mass index was classified from WHO, underweight if <18.5, normal if 18.5-24, overweight ≥ 25.0 -29.9, obese class I ≥ 30.0 -34.9, obese class II > 35.0-39.9, and obese class III ≥ 40 . Hyperuricemia was classified from Indonesian Guideline of Gout Recommendation, high if >6.8 and normal ≤ 6.8 . Smoking habits, yes if the patient was smoker, and no if the patient never ever smoked.

As much as 5 cc of venous sampling was taken ≤ 48 hours stroke onset to examine

Table 1. Characteristics of the subjects

Variable	F	%	Mean \pm SD	Median (min – max)
Age			59.42 \pm 12.12	59 (17 – 84)
Gender				
Male	31	50.0		
Female	31	50.0		
BMI				
Underweight	4	6.5		
Normal range	15	24.2		
Overweight	27	43.5		
Pre-obese	1	1.6		
Obese class I	15	24.2		
MMP-9			1203.26 \pm 734.70	991.5 (219 – 3064)
Normal	17	27.4		
High	45	72.6		
MMP-9 Gene				
CC	43	69.4		
CT	19	30.6		
Blood Pressure				
H0(Pre-hypertension)	12	21.1		
H1(HT Grade I)	22	38.6		
H2(HT Grade II)	23	40.4		
Blood Glucose				
D0 Non-DM	20	32.3		
D1(Prediabetes)	14	22.6		
D2(DM type 2)	20	32.3		
Dyslipidemia				
E0 (No)	11	17.7		
E1 (Yes)	51	82.3		
Hyperuricemia				
A0 (No)	30	48.4		
A1 (Yes)	32	51.6		

serum MMP-9 level. Serum MMP-9 serum level was examined in Prodia Clinic Laboratory, Semarang used the ELISA (Enzyme Linked Immunosorbent Assay) method with the Quantikine® ELISA Human MMP-9 reagent kit (R&D Systems, Inc., Minneapolis, USA) stain: DMP900, Lot: P176247. The calibration standard range was 0.313-20 ng / mL, with detection limits ≤ 0.156 ng / mL, 100x dilution factor, measurements using Microplate Reader Bio-Rad Model 680 (USA) instruments with Microplate Manager ver 5.2.1 software (Bio-Rad Laboratories Inc., CA, USA), with a normal range in healthy subjects was 169-705 ng / mL.

As much as 3 cc of venous sampling was taken ≤ 48 hours stroke onset to examine MMP-9 rs3918242 genetic in the Biomolecular Laboratory of the

Faculty of Medicine, Gadjah Mada University, Yogyakarta with the PCR-RFLP method. The DNA sequence primers that were examined were forward 5'-GCCTGGCACATAGTAGGCC-3' and reverse 5'-CTTCCTAGCCAGCCGGCATC-3'. PCR was optimized with the KAPA Taq Extra HotStart PCR Kit KR0366-v3.13 and dNTPs. After PCR product was formed, it was continued by examining RFLP using the SphI enzyme.

The data were analyzed with SPSS for Windows version 22. It was carried out in two stages, namely descriptive statistics to determine the basic characteristics of subjects and analytic to determine the correlation between variables. The chi-square test was used to analyze the level of MMP-9 and genetic variant with risk

Table 2. Statistical test results of the role of MMP-9 genetic variant on the association between serum MMP-9 level and a number of risk factors for acute ischemic stroke

MMP-9 Gene Variable	MMP-9 serum				p
	Normal		High		
	n	%	n	%	
CC genetic variant (n =43)					
Age					
< 65 y.o	10	100	14	42.4	0.001
≥ 65 y.o	0	0	19	57.6	
Gender					
Male	5	50	14	42.4	0.728
Female	5	50	19	57.6	
Blood pressure					
Normal	0	0	0	0	0.001*
Pre HT	6	60	3	9.1	
HT gr 1	3	30	12	36.4	
HT gr 2	1	10	18	54.5	
Blood Pressure					
Non DM	5	50	5	15.2	0.013*
Prediabetes	4	40	8	24.2	
Type 2 DM	1	10	2	60.6	
Dyslipidemia					
Yes	5	50	31	93.9	0.004*
No	5	50	2	6.1	
BMI					
Underweight	3	30	0	0	<0.001*
Normal range	6	60	2	6.1	
Overweight	1	10	20	60.6	
Pre-obese	0	0	1	3	
Obese Class 1	0	0	10	30.3	
Hyperuricemia					
Yes	1	10	23	69.7	0.002*
No	9	90	10	30.3	
Smoking					
Yes	0	0	4	12.1	0.558
No	10	100	29	87.9	
CT genetic variant (n=19)					
Age					
< 65 y.o	6	85.7	11	91.7	1.000 ^y
≥ 65 y.o	1	14.3	1	8.3	
Gender					
Male	5	71.4	7	58.3	0.656
Female	2	28.6	5	41.7	
Blood pressure					
Normal	0	0	1	8.3	0.754
Pre-HT	2	28.6	2	16.7	
HT grade I	2	28.6	5	41.7	
HT grade II	3	42.9	4	33.3	
Blood Glucose					
Non DM	4	57.1	3	25	0.075*
Prediabetes	3	42.9	3	25	
Type 2 DM	0	0	6	50	
Dyslipidemia					
Yes	4	57.1	11	91.7	0.117
No	3	42.9	1	8.3	

MMP-9 Gene Variable	MMP-9 serum				p
	Normal		High		
	n	%	n	%	
BMI					
Underweight	1	14.3	0	0	0.026*
Normal range	5	71.4	2	16.7	
Overweight	1	14.3	5	41.7	
Pre-obese	0	0	0	0	
Obese class 1	0	0	5	41.7	
Hyperuricemia					
Yes	0	0	8	66.7	0.013*
No	7	100	4	33.3	
Smoking					
Yes	0	0	2	16.7	0.509
No	7	100	10	83.3	

*Significant (p<0.05)

factors of stroke. To analyze the most influence variable on serum MMP-9 level, a multivariate test was performed using logistic regression analysis.

RESULTS

Table 1 showed the mean serum MMP-9 level of ischemic stroke patients taken at day 1-2 from onset was higher than normal (1203.26 (SD 734.70) ng/ml) and was found in 45 subjects (72.6%), ranged 219-3.604 ng/ml (median 991.5 ng/ml). Examination of the MMP-9 genetic variant showed that 43 subjects (69.4%) had CC genotype and 19 subjects (30.6%) had CT genotype, while TT genotype was not found in all study subjects.

Based on Table 2, subjects who had high serum MMP-9 level at day 1-2 from the onset in the CC genotype group were the ones with these following risk factors, such as aged > 65 years (57.6%), female (57.6%), high degree of hypertension (90.9%), type 2 DM (60.6%), dyslipidemia (93.9%), and hyperuricemia (69.7%). While, subjects who had high serum MMP-9 level at day 1-2 from the onset in the CT genotype group had these following risk factors, they were aged < 65 years (61.7%), male (58.6%), high degree of hypertension (83.3%), type 2 DM (50%), dyslipidemia (91.7%), and hyperuricemia (66.7%). Based on Table 2, there is a significant MMP-9 genetic variant's role in the association between serum MMP-9 level and a number of risk factors for acute ischemic stroke.

DISCUSSION

In subjects with genetic variation of MMP-9 in CC allele, most of them who had high MMP-9 level also had grade 2 hypertension (54.5%), followed by grade 1 (36.4%), and pre-hypertension (9.1%). It showed that the higher the blood pressure, the more subjects with high MMP-9 levels. On the contrary, the lower the blood pressure value, the greater the number of subjects with normal MMP-9 level. Statistically, the association between the status of the hypertension and MMP-9 level in the CC allele is significant (p < 0.05). This finding is not in line with previous studies in which subjects carrying the T allele either CT or TT were associated with a risk of hypertension with high level of MMP-9 instead of the CC allele, but this was conducted in Chinese population.¹⁰ Another study showed that the risk of hypertension with T allele was significantly found in Chinese Han and Caucasians. While, in the Javanese, the risk of hypertension was more common in the genetic variation of MMP-9 R279Q, although this was insignificant and require further study.¹¹ In a meta-analysis study with 6 residual members, the CC, CT, or TT genotypes were respectively associated with the risk of essential hypertension, although further links are needed.¹²

The group of CC allele variant with high MMP-9 level mostly had diabetes mellitus (60.2%), followed by prediabetes (24.2%), and non-diabetes (15.2%). The association between diabetes and high MMP-9 level in

this group was significant (p-value < 0.05). There has been no previous study of CC genotype that proved a significant association between diabetes mellitus status and increased level of MMP-9. Thus, this result needs to be confirmed again because MMP-9 level in this study is not only due to diabetes mellitus, but also can relate to other factors.

Whereas in the CT allele variant, the group who had high MMP-9 the most was diabetes mellitus group (50%), followed by prediabetes (25%), and nondiabetic (25%). None of the subjects with normal MMP-9 levels in the diabetic subjects had the CT allele. Most normal MMP-9 level (57.1%) were owned by subjects who were non-diabetic. The association between diabetes mellitus and increased MMP-9 level in the CT allele group was insignificant (p > 0.05). However, the increase in MMP-9 level in diabetes patients with T allele was evidenced in another study by Singh et al.¹³ Other studies have shown that the T allele in the CT and TT genotypes showed a significant increase MMP-9 level.¹⁴ It can be concluded that diabetes is associated with serum MMP-9 level, but if viewed from its genetic potential, the CC genotype has a significant association to an elevated level. High MMP-9 level was not associated with diabetes mellitus subjects with CT genotype.

In patients with CT genotype, high MMP-9 level was found predominantly in the dyslipidemia group, but it was insignificant (p < 0.05). Our finding is not in line with previous studies reporting

that CT or TT genotype significantly increased MMP-9 level with an odds ratio of 4.54 times. Whereas the CC genotype mostly had significantly high MMP-9 level (93.9%). This result is in accordance with some previous studies, which there were a total of 505 subjects who had a CC genotype at NIHSS score <16 and NIHSS \geq 16 and 381 of them were with dyslipidemia. However, the results were not significant.^{15,16} It can be concluded that dyslipidemia is related to serum MMP-9 level, but if viewed from its genetic potential, the CC genotype has a significant relationship to the increase in MMP-9 level in subjects with dyslipidemia, while the CT genotype is not related to the high MMP-9 level in subjects with dyslipidemia.

Imbalance of food intake with the amount of energy consumed can lead to massive adipose tissue expansion, which generally underlies obesity. Adipose tissue consists of a wide variety of cells which can undergo volumetric expansion during progression to an obese state, for example, an increase in cell size. The massive expansion of the adipose tissue requires consistent remodeling of the stromal matrix. Tissue remodeling itself is a normal physiological process, where there is a balance between degradation and extracellular matrix synthesis. MMPs are proteolytic enzymes that are responsible for the remodeling of the extracellular matrix through the degradation and turnover mechanisms of connective tissue and basement membrane proteins such as collagen, proteoglycans and elastin.¹⁷ Subjects with CC genotype who had high MMP-9 levels were predominantly overweight (60.6%), followed by obese class 1 (30.3%), normal range (6.1%), and pre-obese (3%). Subjects with the CC genotype who had normal MMP-9 levels were mostly in the normal BMI (60%). The association between obesity and MMP-9 level in CC genotype group was significant ($p < 0.05$). However, in general, if only seen from obesity, most patients with CC alleles who were not obese had the highest level of MMP-9 (69.7%), although it was not significant ($p > 0.05$). Our findings are different with previous studies where the genotypes with the T allele, namely CT and TT, had a risk of obesity with a high

MMP-9 level with a 4.54 times higher odds ratio and a risk of developing non-alcoholic fatty liver disease than the CC group.¹⁸ The number of subjects with the T allele in our study was not representative enough to find a significant relationship. Whereas in the group with CT genotype, high MMP-9 level was mostly owned by subjects who were obese and overweight. Statistical analysis showed that there was a significant association between high MMP-9 in CT allele group and obesity ($p < 0.05$). This is in accordance with the study of Andrade et al.¹⁹ which stated that genotypes carrying T alleles tend to be obese with high level of MMP-9, this group was less able to modulate MMP-9 polymorphisms, thus pathogenic processes occurred more frequently, especially in women. It can be concluded that dyslipidemia is associated with serum MMP-9 level and seen from the genetic variation CC and CT genotypes have a significant association with increased MMP-9 levels in obese subjects.

The group with the CC allele and high MMP-9 level mostly had hyperuricemia (69.7%), and the association was significant ($p < 0.09$). This finding also happened in the CT allele group with statistically significant association (66.7%). This is in line with the study of Mehde et al.²⁰ which showed that in the CC and CT genotypes, serum MMP-9 level had a positive correlation with high uric acid level. The higher the uric acid level, the higher the MMP-9 level will be. This positive correlation is appropriate with the presence of T allele, where the relationship between TT > CT > CC.²⁰ It can be concluded that hyperuricemia is associated with MMP-9 level and in terms of genetic potential, CC and CT genotypes have a significant association with increased MMP-9 level in subjects with hyperuricemia.

Previous experimental studies found that the T allele had a higher promoter activity than the C allele due to its higher binding ability to transcription repressors.²¹ Several previous studies reported results regarding the association of genetic variation MMP-9 rs3918242 in ischemic stroke, but the results were inconsistent.²²⁻²⁶ Nie et al. conducted a study on a population in China that

showed that the genetic result of MMP-9 rs3918242 was associated with ischemic stroke.²⁶ Zhang, et al. conducted another study on a population in China whose results reported that the C allele was associated with an increased risk of ischemic stroke.²² Some studies actually reported that there was no association between genetic variation of MMP-9 rs3918242 on stroke.²³⁻²⁵ These result differences could be due to differences in ethnicity, study design, and sample size.²⁷

The genetic variation of MMP-9 rs3918242 has several genotypes, namely CC, CT and CT. The T allele is an allele that can significantly increase the gene promoter in expressing MMP-9 so that MMP-9 CT levels are higher than CC, and TT is higher than CT.^{28,29} In this study, we found the number of CC was 69.4%, CT 30.6%, and none for TT. This result is consistent with many previous studies that reported that consecutively the most considerable population was CC followed by CT and TT.^{21-26,28,29} The small number of sample ($n = 62$) made us could not find the TT variant in this study. Previous studies using a sample of 1274 patients found 2.4% variation in TT and from 1258 healthy controls, they found 1.2% variation in TT.³⁰ In our study, high level of MMP-9 was found in the CC group (73.3%), which is consistent with the study of Zhang et al.²¹ Although ours are insignificant ($p > 0.05$).

This study has some limitations, for instance we could not find a genotype that had a small frequency in Asian races (i.e TT genotype), so it could not be analyzed. We also did not carry out a detailed registration of the subject's risk factors which could also affect the results, including when it was diagnosed, what treatment was previously consumed, how to control risk factors, and history of family's disease.

CONCLUSION

CC genetic variants with high serum MMP-9 level are associated with more acute ischemic stroke risk factors than CT genetic variants with high MMP-9 serum levels. Therefore, CC genetic variants have the potential to pose more risk of acute ischemic stroke.

ETHICAL APPROVAL

The ethical approval for this study was issued by the Health Research Ethics Committee of Medical Faculty Universitas Diponegoro No. 51/EC/FK-RSDK/I/2018

CONFLICTS OF INTEREST

There is no conflict of interest.

FUNDING

All financial resources are borne by the researcher.

AUTHOR CONTRIBUTIONS

The conceptualization, writing preparation, validation, formal analysis, investigation, and data curation of this study was supported by Dodik Tugaworo. While, methodology, writing, and reviewing were supported by Amin Husni.

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The Role of MMP-9 rs 3918242 genetic variant in association between MMP-9 serum level with risk factors of acute ischemic stroke

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PAGE 3

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