

mRNA Expression of the CUB and Sushi Multiple Domains 1 (CSMD1) and Its Serum Protein Level as Predictors for Psychosis in the Familial High-Risk Children and Young Adults

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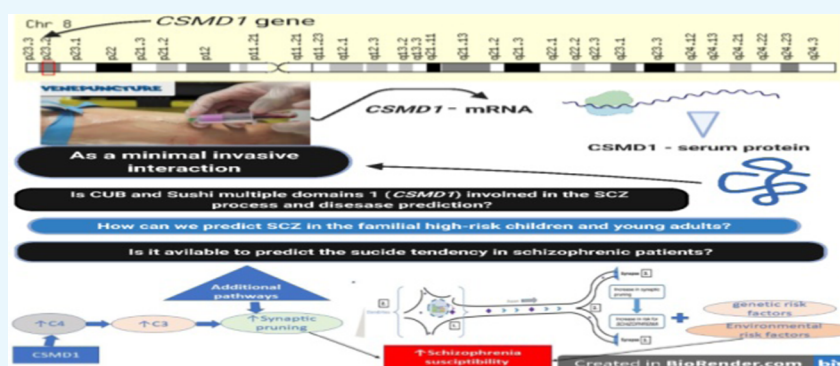
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ABSTRACT: *Background:* Schizophrenia (SCZ) is still a challenging, refractory, and severe disorder. It is not a fully understood disease with genetic and epigenetic susceptibility and about 80% substantial heritability. The CUB and Sushi multiple domains 1 (CSMD1) gene is implicated in neurogenesis, memory, immunity, neuropsychology, and monoamine metabolism. Thus, it is one of the powerful genes involved in the pathogenesis of SCZ. *Purpose:* To evaluate the possible role of the CSMD1 gene's mRNA expression and its serum protein as markers for the early diagnosis of the first-episode SCZ in familial high-risk (FHR) Egyptian children and young adults. *Subjects and methods:* This case–control study included 80 first-episode drug-naïve SCZ patients from FHR Egyptian children and young adults and 80 healthy participants, as controls, from the FHR-susceptible children and young adults but did not develop SCZ. In this study, the CSMD1 gene's mRNA expression and CSMD1 serum levels were measured in the peripheral blood, and these levels were correlated with the lipid profile of the study populations. *Results:* The CSMD1 gene's mRNA expression and its' protein levels were significantly decreased in the SCZ patients compared to controls. The receiver operating characteristic (ROC) curve analysis succeeded in distinguishing SCZ patients from those not having SCZ using cutoff points of ≤ 0.711 and ≤ 4.83 ng/mL for the CSMD1 gene's mRNA expression and serum protein level, respectively. At these levels, the diagnostic sensitivities were 93.75 and 91.25%; specificity was 92.5%; positive predictive value (PPV) were 92.6 and 92.4%; and negative predictive values (NPVs) were 93.7 and 91.4%, respectively. Also, the ROC curve analysis succeeded in discriminating those with suicidal tendencies. *Conclusion:* CSMD1 gene's mRNA expression might be a reliable and early diagnostic predictor of first-episode SCZ in the FHR Egyptian children and young adults.

INTRODUCTION

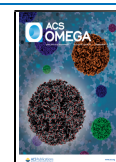
Discovering the full range of Schizophrenia (SCZ) genes and learning the dysregulation that underlies SCZ are difficult.^{1,2} Estimates of the global SCZ prevalence as a psychiatric disorder among noninstitutionalized individuals are 0.33–0.75%.³ SCZ is among the world's top 15 leading causes of disability,⁴ with an increased risk of premature mortality.⁵ Schizophrenia characteristics include negative symptoms, such as a flat affect or poverty of speech; positive symptoms, including hallucinations or delusions; disorganized speech; and impairments in cognition, including attention, memory, learning, and executive functions. Impairments of social and

occupational functioning are generally associated with SCZ.⁶ In adolescence and young adulthood, SCZ and associated psychosis usually arise, while premorbid deficits can also present in childhood.⁷

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When the disease manifests before age 18, it is defined as early-onset schizophrenia (EOS), a subcategory of SCZ associated with more familial vulnerability and poor outcomes.⁸ For early prevention and intervention paradigms, effective prospective detection of individuals who continue to experience persistent psychosis will significantly advance. Unfortunately, the clinical examination is a poor predictor of subsequent psychosis transition even among the ultra-high-risk groups. However, a wide variety of risk factors for SCZ have been identified, including familial susceptibility. At this time, no individual biomarker or combination of biomarkers can allow accurate prospective prediction for individuals at familial high-risk (FHR) of emerging psychosis due to the complex and multilevel nature of the psychotic illness.⁹

Therefore, there is an essential need to identify biomarkers and clinical, physical, or neuroimaging signs that can predict the possibility of subsequent psychosis development in high-risk individuals.

Peripheral blood measurements of biomarkers are non-invasive and widely used methods compared to the difficult accessibility of brain tissue from SCZ patients. Liew et al.¹⁰ showed that ~81.9% of the genes expressed in the brain were also found in the peripheral blood.^{11,12} Most recently, the suggestion of the presence of an SCZ signature by the blood exosomal metabolites concentrations showed a 25 blood-exosome metabolite panel in pathways related to glycerophospholipid metabolism and the biosynthesis of phenylalanine, tyrosine, and tryptophan.¹³

Besides genetics, the epigenetic markers as microRNAs (miRNAs) have generated significant interest in SCZ pathogenesis because of their actions implicated in neural differentiation, synaptic plasticity, and cognitive functions.¹⁴ Interestingly, several SCZ genome-wide association studies (GWAS) loci showed several miRNA-137 target genes that harbor SCZ-associated variants; one of them is the CUB and Sushi multiple domains 1 (*CSMD1*) gene.¹⁵

Elevated expression of complement components genes as complement component 4 (C4) and their protein concentrations in SCZ was suggested in many reports. Even high baseline levels of C4 were proposed to predict worse clinical outcomes of SCZ.^{16–18} This supports the hypothesis that SCZ may be a neuroimmune disorder.¹⁹

The complement system components are groups of plasma proteins that play a role in the immune system function. The C3 is cleaved by C3 convertase into C3a and C3b active particles amplifying the immune cascade. This amplification leads to immune functions through opsonization, chemotaxis, and cell lysis.²⁰ Specific circulating plasma proteins—known as complement control proteins (CCPs)—regulate complement system effects. One of these proteins is *CSMD1*, or membrane-associated complement receptor type 1.²¹ The fragments 1 and 2 of *CSMD1* bind C4 and C3 and facilitate their degradation by Factor I. The *CSMD1* protein comprises CUB domains separated by Sushi domains, followed by an additional 15 tandem segments of the Sushi domains.²²

The *CSMD1* gene is located on chromosome 8p23.2. It encodes a critical cell adhesion molecule that is highly expressed in extracellular and plasma membrane-associated proteins in the central nervous system (CNS) and to a lesser extent in testes; indeed, it is almost undetectable in most other tissues, i.e., it can be said that *CSMD1* has brain specificity. Unlike other complement inhibitors, CFH, C4BP, and DAF are highly enriched in peripheral tissues versus the CNS.^{22–25}

The *CSMD1* protein is most pronounced in the cortex, the hippocampus, and present in a comparatively low quantity in the cerebellum. It is also present in the neuropills and at synapses and is expressed predominantly by neurons.²⁵

The *CSMD1* is involved in brain circuits' development, regeneration, axon and angiogenesis guidance, signaling, connection, neurotransmission, and plasticity. It is showed that a more significant fraction of GABAergic neurons than glutamatergic neurons express *CSMD1*. The *CSMD1* gene knockout induces behaviors reminiscent of blunted emotional responses, anxiety, and depression, suggesting an influence of the *CSMD1* gene on neuropsychopathology and endophenotypes of the negative symptom spectra in SCZ patients.^{25–27}

The *CSMD1* protein is a complement activation and inflammation inhibitor in the brain. There is growing attention to complement activation in neuropsychiatric brain-related disorders because it is crucial for synaptic plasticity.¹⁶ The inappropriate hypofunction of complement inhibitors as *CSMD1* had a role in the pruning hypothesis of SCZ. The most significant risk factor for the onset of SCZ is temporal proximity to the developmental period of late adolescence or early adulthood. Similarly, complement-dependent synaptic pruning processes have been demonstrated to be developmentally regulated.²⁵

A human GWAS on the cerebrospinal fluid (CSF) levels of monoamine metabolites showed that the *CSMD1* gene was regulating the ratio between dopamine and serotonin metabolites in the CSF.²⁷ One of the largest GWAS in SCZ identified five loci; one of them is the *CSMD1* gene locus, which has known functions in the nerve growth cone.^{28–33}

Around 4.9% of SCZ patients die due to suicide, which is higher than the general population, with the highest incidence in the early stages of the disease.³⁴ So, it is essential to predict the suicide tendency in SCZ patients; this will save many souls.

Multiple studies showed the relation of the *CSMD1* gene to schizophrenic patients. To our knowledge, there is no data in the literature highlighting the importance of this gene for the prediction and early diagnosis of first-episode SCZ in drug-naïve FHR Egyptian children and young adults. Due to the brain specificity of *CSMD1*, it can be used in the investigation of SCZ risk. Therefore, we aimed to evaluate the *CSMD1* gene's mRNA expression as a marker for the early diagnosis of the first episode of SCZ in drug-naïve FHR Egyptian children and young adults. It has been presumed that gene expression is a crucial stage at which the genotypes critically influence the SCZ phenotypes. The serum *CSMD1* protein level was also assessed to determine if any other factors affect the *CSMD1* gene's serum protein level to be an easy and early predictive test for psychosis from the peripheral blood.

■ RESULTS AND DISCUSSION

Results. *Demographics and Clinical Data of the Study Population (Table 1).* There was no significant difference between SCZ patients and controls regarding age and sex. Cognitive dysfunction, positive symptoms, and negative symptoms were substantial for the diagnosis of SCZ in SCZ patients. Suicide tendency was observed in 48.8% of SCZ cases (Table 1).

CSMD1 Gene's mRNA Expression Level, Serum Levels of CSMD1 Protein, and Lipid Profile (Table 2). There was a significant decrease in high-density lipoprotein cholesterol (HDL-c) levels, while there was a significant elevation in low-

Table 1. Demographics and Clinical Data of the Study Population^a

	cases (n = 80)	control (n = 80)	test of sig.	P
Sex				
male	35 (43.8%)	43 (53.8%)	$\chi^2 = 1.601$	0.206
female	45 (56.3%)	37 (46.3%)		
Age (years)				
mean \pm SD	17.8 \pm 3.8	17.5 \pm 3.7	U = 3071.0	0.659
median (min–max)	18(12–24)	17(12–24)		
≤ 18 years	43(53.8%)	46(57.5%)	$\chi^2 = 0.228$	0.633
> 18 years	37(46.3%)	34(42.5%)		
mean \pm SD	17.8 \pm 3.8	17.5 \pm 3.7	U = 3071.0	0.659
median (min–max)	18(12–24)	17(12–24)		
Positive symptoms				
no	2 (2.5%)	80 (100%)	$\chi^2 = 152.195^*$	$<0.001^*$
yes	78 (97.5%)	0 (0%)		
Negative symptoms				
no	30 (37.5%)	80 (100%)	$\chi^2 = 72.727^*$	$<0.001^*$
yes	50 (62.5%)	0 (0%)		
Cognitive dysfunction				
no	52 (65%)	80 (100%)	$\chi^2 = 33.939^*$	$<0.001^*$
yes	28 (35%)	0 (0%)		
Suicide tendency				
no	41 (51.3%)	80 (100%)	$\chi^2 = 51.570^*$	$<0.001^*$
yes	39 (48.8%)	0 (0%)		

^a χ^2 : chi-square test U: Mann–Whitney test, * $p \leq 0.05$ is statistically significant.

density lipoprotein cholesterol (LDL-c) levels in SCZ patients compared to controls. The mRNA expression and the serum protein levels of the CSMD1 gene were significantly decreased in SCZ patients compared to controls. Correlating the CSMD1 mRNA level to its serum protein level in the SCZ patients and controls revealed a significant ($p \leq 0.01$) positive correlation ($r = 0.99$ and 0.94 , respectively) (Figure 1 and Table 2).

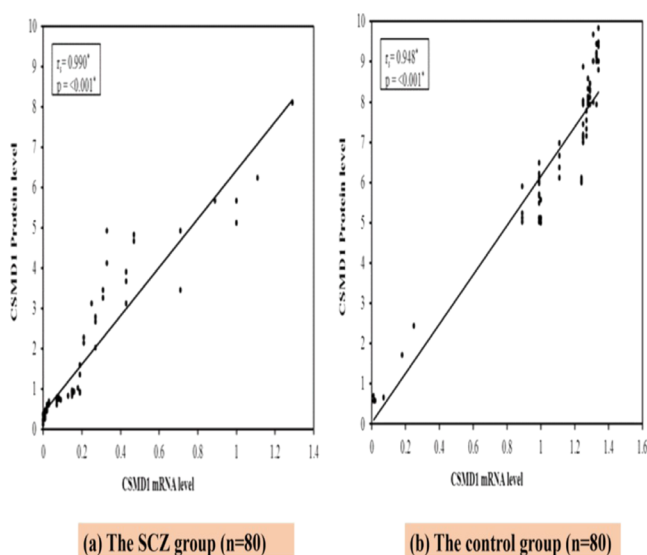


Figure 1. Correlation between the CSMD1 gene's mRNA and serum protein levels (a) in the SCZ patients and (b) in the control group.

Correlating the CSMD1 Gene's mRNA Expression Level and Its Serum Protein Level to Demographics and Clinical Data. In SCZ patients (Table 3), there was a significant correlation between the positive symptoms and increased suicidal tendency, while there was a nonsignificant correlation regarding age, sex, negative symptoms, and cognitive dysfunction. In controls (supplementary data, Table S1), the CSMD1 gene's mRNA expression level and protein level show a significant correlation with sex; these levels were increased in females than males.

Correlation between the CSMD1 Gene's mRNA and Its Serum Protein Levels with Age and Lipid Profile. In SCZ patients (Table 4), there was a significant positive correlation between the CSMD1 gene's mRNA expression level and its serum protein level with HDL ($p = 0.024$) and a significant negative correlation with LDL-c ($p = 0.011$). In controls (supplementary material, Table S2), the CSMD1 gene's mRNA expression level and serum protein level showed a significant positive correlation with HDL, LDL, and cholesterol levels and a significant negative correlation with TAG levels.

Correlation between Suicide Tendency and the Lipid Profile Parameters. In SCZ patients (Table 5), LDL-c, cholesterol, and TAG levels were significantly lower in SCZ patients with suicide tendencies than without. Also, there was a significant correlation of higher cholesterol levels to a younger age (childhood), negative symptoms, and absence of cognitive dysfunction. In controls (supplementary data, Tables S3–S6), LDL and cholesterol were significantly higher levels in females while the TAG levels were significantly higher in males.

Univariate and Multivariate Regression Analysis for the Demographics, Clinical Data, CSMD1 Gene's mRNA Level, CSMD1 Serum Protein Level, and Lipid Profile Affecting SCZ (Table 6). The univariate logistic regression for SCZ risk showed that LDL increased the risk by 1.026-fold, followed by cholesterol by 1.013-fold, then triglycerides (TG) by 1.009-fold. The CSMD1 gene's mRNA level and its serum protein level increased the SCZ risk by 0.007- and 0.426-fold, respectively. The multivariate logistic regression for SCZ risk showed that decreased CSMD1 gene's mRNA level is the only risk factor for SCZ. At the same time, it was a protective factor when its level increases. Adjusted odd's ratio (Table 7) by sex, age, HDL, LDL, cholesterol, and TAG revealed that the CSMD1 gene's mRNA level and its serum protein level maintained their importance as SCZ risk factors by 0.003- and 0.373-fold, respectively (Tables 6 and 7).

The ROC curve analysis was applied to assess the diagnostic utility of the CSMD1 gene's mRNA expression and its serum protein level to predict the SCZ patients of high-risk familial susceptibility (Figure 2 and Table 8). It revealed that the best cutoff point for the CSMD1 gene's mRNA expression is ≤ 0.711 ; at this level, the CSMD1 gene's mRNA level had a diagnostic sensitivity of 93.75% and a specificity of 92.50%, with a positive predictive value (PPV) of 92.6% and a negative predictive value (NPV) of 93.7%. The best cutoff point for the CSMD1 serum protein level is ≤ 4.83 ng/mL; at this level, the CSMD1 serum concentration had a diagnostic sensitivity of 91.25% and a specificity of 92.5%, with a PPV of 92.4% and an NPV of 91.4%.

The ROC curve analysis was applied to assess the diagnostic utility of the CSMD1 gene's mRNA expression and its serum protein level to predict suicide susceptibility (Figure 3 and Table 9). It succeeds in distinguishing those having a suicidal tendency at cutoff points of >0.091 and >0.722 ng/mL,

Table 2. CSMD1 Gene's mRNA Expression Level, Serum Levels of CSMD1 Protein, and Lipid Profile^a

	cases (<i>n</i> = 80)	control (<i>n</i> = 80)	test of sig.	<i>P</i>
CSMD1 mRNA level				
mean ± SD	0.19 ± 0.28	1.11 ± 0.32	<i>U</i> = 384.50*	<0.001*
median (min–max)	0.09(0–1.29)	1.25(0.01–1.34)		
CSMD1 serum protein level (ng/ml)				
mean ± SD	1.6 ± 1.8	6.8 ± 2.1	<i>U</i> = 337.0*	<0.001*
median (min–max)	0.7(0.1–8.1)	7.1(0.6–9.8)		
HDL (mg/dL)				
mean ± SD	46 ± 7.9	60.4 ± 8.2	<i>U</i> = 728.0*	<0.001*
median (min–max)	43.8 (32.2–69.1)	62.6 (42.3–73.9)		
LDL-c (mg/dL)				
mean ± SD	141.7 ± 53.4	101.8 ± 17.2	<i>U</i> = 2296.0*	0.002*
median (min–max)	156.2(70–226.3)	106.4(65.6–133.9)		
Cholesterol (mg/dL)				
mean ± SD	211.6 ± 66.7	181.1 ± 13.2	<i>U</i> = 3040.0	0.585
median (min–max)	251.2(137.3–298.9)	182.1(154.9–198.4)		
TAG (mg/dL)				
mean ± SD	124.1 ± 71.1	100.2 ± 24.2	<i>U</i> = 2956.0	0.405
median (min–max)	163.2(37.5–225.2)	92.2(62.5–151.9)		

^a*U*: Mann–Whitney test * *p* ≤ 0.05 is statistically significant; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TAG: triacylglycerol.

Table 3. Correlation between the CSMD1 Gene's mRNA and Its Serum Protein Levels with Demographics and Clinical Data in the SCZ Patients^a

	CSMD1 mRNA level			CSMD1 serum protein level	
	no.	median (min–max)	mean ± SD	median (min–max)	mean ± SD
Sex					
male	35	0.1 (0.0–0.9)	0.2 ± 0.2	0.8 (0.2–5.7)	1.7 ± 1.7
female	45	0.1 (0.0–1.3)	0.2 ± 0.3	0.7 (0.1–8.1)	1.4 ± 1.8
<i>U</i> (<i>P</i> -value)		725.0 (0.544)		724.0 (0.538)	
Age					
children (<18 years)	43	0.1 (0–1.1)	0.2 ± 0.3	0.8 (0.1–0.62)	1.6 ± 1.7
adolescents (>18 years)	37	0.1 (0–1.3)	0.2 ± 0.3	0.7 (0.1–8.1)	1.5 ± 1.9
<i>U</i> (<i>P</i> -value)		756.0 (0.703)		747.0 (0.640)	
Positive symptoms					
no	2	0.0 (0.0–0.0)	0.0 ± 0.0	0.2 (0.2–0.2)	0.2 ± 0
yes	78	0.1 (0.0–1.3)	0.2 ± 0.3	0.8 (0.1–8.1)	1.6 ± 1.8
<i>U</i> (<i>P</i> -value)		2.0* (0.003*)		5.0* (0.008*)	
Negative symptoms					
no	30	0.1 (0.0–1.1)	0.2 ± 0.3	0.7 (0.1–6.2)	1.6 ± 1.8
yes	50	0.1 (0.0–1.3)	0.2 ± 0.3	0.8 (0.2–8.1)	1.5 ± 1.8
<i>U</i> (<i>P</i> -value)		687.50 (0.534)		692.0 (0.564)	
Cognitive symptoms					
no	52	0.1 (0.0–1.1)	0.2 ± 0.3	0.7 (0.1–6.2)	1.7 ± 1.8
yes	28	0.1 (0.0–1.3)	0.2 ± 0.3	0.7 (0.2–8.1)	1.3 ± 1.7
<i>U</i> (<i>P</i> -value)		612.0 (0.242)		606 (0.218)	
Suicide tendency					
no	41	0.0 (0.0–1.0)	0.1 ± 0.2	0.6 (0.1–5.7)	1 ± 1.2
yes	39	0.1 (0.0–1.3)	0.3 ± 0.3	0.9 (0.2–8.1)	2.1 ± 2.1
<i>U</i> (<i>p</i>)		557.50* (0.020*)		539.0* (0.012*)	

^a*i*: Mann–Whitney test. * *P* ≤ 0.05 is statistically significant.

respectively. At these levels, the diagnostic sensitivities were 61.54 and 66.67%; specificities were 68.29 and 63.4%; PPVs were 64.9 and 63.4%; and NPVs were 65.1 and 66.7%, respectively.

DISCUSSION

Schizophrenia is a neuropsychiatric condition with a worldwide lifetime prevalence of around 1%. It impairs the mental and social functioning, whose onset typically occurs in late

adolescence to young adulthood.³⁵ The period of late adolescent development is of particular interest to psychiatric study, as the age of onset of major neuropsychiatric disorders, especially SCZ, corresponds to this time frame.

Chronic psychosocial pressures, including childhood adversity, relocation, and urbanity, have been identified as raising the risk of SCZ, in addition to genetic factors, neurodevelopmental risks, and cannabis use. Besides, acute stress plays a part in inducing psychotic symptoms.³⁶

Table 4. Correlation between the CSMD1 Gene’s mRNA and Its Serum Protein Levels with Age and Lipid Profile in SCZ Patients^a

	CSMD1 mRNA level		CSMD1 protein level	
	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>
age (years)	-0.026	0.822	-0.042	0.711
HDL	0.249	0.026*	0.252	0.024*
LDL-c	-0.262	0.019*	-0.283	0.011*
cholesterol	-0.085	0.454	-0.112	0.322
TAG	-0.058	0.608	-0.097	0.391

^a*r_s*: Spearman coefficient. * *P* ≤ 0.05 is statistically significant.

Table 5. Correlation between Suicide Tendency and the Lipid Profile Parameters in SCZ Patients^a

	suicide tendency		<i>U</i>	<i>P</i>
	no (<i>n</i> = 41)	yes (<i>n</i> = 39)		
HDL				
mean ± SD	46.4 ± 9.2	45.7 ± 6.2	754.0	0.661
median (min–max)	44.3 (33.8–69.1)	43.8 (32.2–55.5)		
LDL				
mean ± SD	182 ± 32.3	99.4 ± 35.2	166.0*	<0.001*
median (min–max)	190.7 (84.1–226.3)	88.5 (70–214.1)		
Cholesterol				
mean ± SD	264.5 ± 37.5	155.9 ± 39.5	165.0*	<0.001*
median (min–max)	275.2 (138.8–298.9)	143.4 (137.3–280.1)		
TAG				
mean ± SD	180.9 ± 43	64.4 ± 37.5	112.0*	<0.001*
median (min–max)	193.4 (44.9–225.2)	54.3 (37.5–171.5)		

^a*U*: Mann–Whitney test. * *P* ≤ 0.05 is statistically significant.

Compared to healthy controls and treated patients, brain imaging investigations have shown that medication-naive patients with SCZ have substantially decreased thalamic and caudate volumes. People with SCZ need medication for a lifetime. Early detection and care of these patients can help in monitoring symptoms before the occurrence of severe problems and boost the long-term outlook.³⁷

Table 6. Univariate and Multivariate Regression Analysis for the Demographics, Clinical Data, CSMD1 Gene’s mRNA Level, CSMD1 Serum Protein Level, and Lipid Profile Affecting SCZ^{ab}

	univariate		multivariate	
	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)
sex (female)	0.207	1.494(0.801–2.786)		
age (years)	0.365	0.987(0.959–1.015)		
positive symptoms	0.996			
negative symptoms	0.997			
cognitive symptoms	0.998			
suicide tendency	0.997			
CSMD1 mRNA level	<0.001*	0.007(0.002–0.024)	0.011*	0.001(0.0–0.177)
CSMD1 protein level	<0.001*	0.426(0.339–0.536)	0.329	1.615 (0.617–4.223)
HDL	<0.001*	0.832(0.790–0.876)	0.263	0.867(0.676–1.113)
LDL-c	<0.001*	1.026(1.016–1.035)	0.815	1.028(0.816–1.295)
cholesterol	<0.001*	1.013(1.006–1.020)	0.936	1.009(0.803–1.269)
TAG	0.006*	1.009(1.002–1.015)	0.479	0.982(0.932–1.033)

^aOR: odd’s ratio, CI: confidence interval, * *P* ≤ 0.05 is statistically significant ^bNB. All variables with *p* < 0.05 were included in the multivariate.

Table 7. Univariate and Multivariate Analysis for the Parameters Affecting SCZ after Adjusting the Odd’s Ratio by Sex, Age, HDL, LDL, Cholesterol, and TAG Levels^{ab}

	crude odd’s ratio		adjust odd’s ratio	
	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)
CSMD1 mRNA level	<0.001*	0.007 (0.002–0.024)	<0.001*	0.003 (0.0–0.035)
CSMD1 protein level	<0.001*	0.426 (0.339–0.536)	<0.001*	0.373 (0.241–0.576)

^aOR: odd’s ratio, CI: confidence interval, * *P* ≤ 0.05 is statistically significant. ^bNB. the odd’s ratio was adjusted by sex, age, HDL, LDL, cholesterol, and TAG.

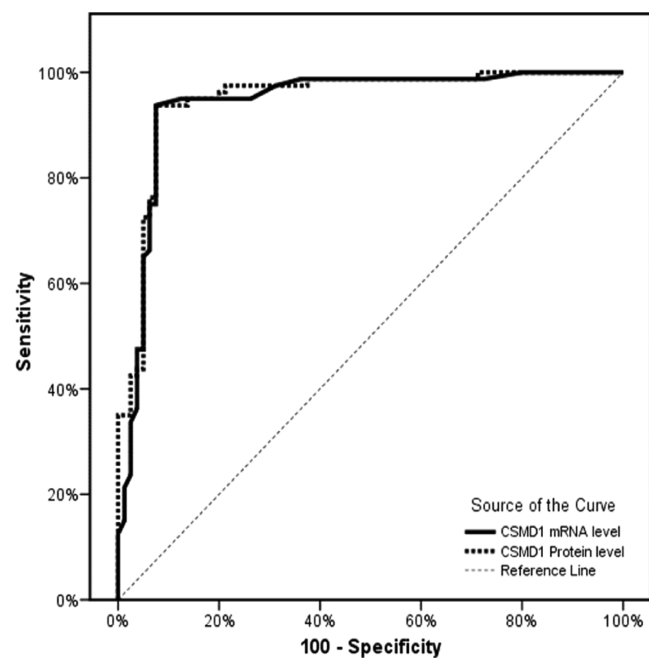


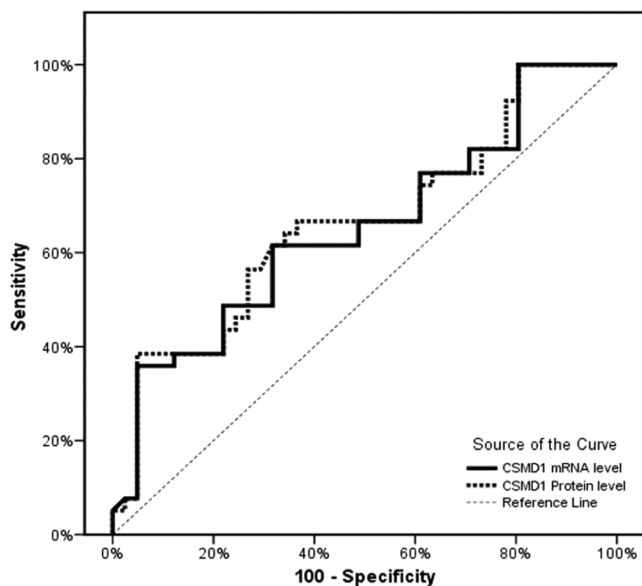
Figure 2. ROC curve for the CSMD1 gene’s mRNA and its serum protein levels to diagnose SCZ patients from the controls.

The origins of the hypothesis of dopamine lie in two lines of testimony: first, clinical studies have indicated that dopami-

Table 8. Agreement (Sensitivity, Specificity) for CSMD1 Gene's mRNA and Its Serum Protein Levels to Diagnose the SCZ Patients from the Controls^{abc}

	AUC	P-value	95% CI	cutoff	sensitivity	specificity	PPV	NPV
CSMD1 mRNA level	0.940*	<0.001*	0.899–0.981	≤0.711	93.75	92.50	92.6	93.7
CSMD1 protein level	0.947	<0.001*	0.911–0.983	≤4.998	93.75	91.25	91.5	93.6

^aAUC: area under a curve, P-value: probability value, CI: confidence interval. ^bNPV: negative predictive value PPV: positive predictive value. ^c* P ≤ 0.05 is statistically significant.

**Figure 3.** ROC curve for CSMD1 mRNA level and CSMD1 serum protein level to predict suicide tendency ($n = 39$).

nergic agonists and stimulants may cause psychosis in healthy people and exacerbate psychosis in SCZ patients, and second, the finding that the dopamine system is affected by antipsychotic drugs.³⁶

Significant associations between the CSMD1 gene and SCZ were observed in many reports, with nominally significant associations recorded for depression and bipolar disorder (BPD). Besides, in healthy subjects, risk variants in the CSMD1 gene affect cognition and brain activation. The CSMD1 protein is one of the complement control proteins. It inhibits C3 in vitro. In the CNS, complement is tightly regulated as it is involved in synaptic pruning and phagocytosis dependent on microglia. In fact, it is proposed that the CSMD1 gene may play a role in aberrant synaptic elimination in many neurodegenerative disorders. Therefore, the impact of CSMD1 protein on the pathogenesis of SCZ and depression-related phenotypes can be mediated by immune and synaptic dysregulation.³⁸

In this work, all of the SCZ patients were drug-naive with first-episode FHR-SCZ. The study population was homogeneous in age; all had no other principal physical diagnosis.

Table 9. Agreement (Sensitivity, Specificity) for CSMD1 mRNA Level and CSMD1 Protein Level to Predict Suicide Tendency in SCZ Patients ($n = 39$)^{abc}

	AUC	P-value	95% CI	cut off	sensitivity	specificity	PPV	NPV
CSMD1 mRNA level	0.651	0.020*	0.530–0.772	>0.091	61.54	68.29	64.9	65.1
CSMD1 protein level	0.663	0.012*	0.543–0.783	>0.722	66.67	63.41	63.4	66.7

^aAUC: area under a curve, P-value: probability value, CI: confidence interval. ^bNPV: negative predictive value, PPV: positive predictive value. ^c* P ≤ 0.05 is statistically significant.

Since patients were young at an early stage of the disease, they were expected to grow their brains. Therefore, we speculate that these patients' transcription changes are likely to be essential to the disease process.

In our study, the CSMD1 gene's mRNA and its serum protein levels in the FHR-SCZ were significantly decreased compared to FHR controls. These results support the CSMD1 genetic susceptibility to SCZ. Our results agreed with Liu et al.,²⁷ who demonstrated that expression levels of the CSMD1 gene in Han Chinese patients with SCZ were lower in peripheral blood mononuclear cells (PBMCs) than in healthy controls. In the PBMCs of SCZ patients following anti-psychotic therapy, the expression levels of the CSMD1 gene were higher than those in the baseline SCZ patients. These findings ensure that the CSMD1 gene's mRNA expression is involved in the SCZ molecular pathogenesis and can affect the efficacy of antipsychotics against SCZ.³⁹

The CSMD1 has been validated as one of microRNA (MiR)-137 targets. MiR-137 was involved in both regulation of adult neurogenesis and neuron maturation, and hence could be involved in the pathogenesis of SCZ. Several studies have shown that the CSMD1 gene rs10503253's A allele is associated with cognitive dysfunction and reported as a primary and core SCZ deficiency. The CSMD1 gene is, therefore, regarded as an essential susceptibility for SCZ.⁴⁰

In this study, multivariate logistic regression for SCZ risk showed that the CSMD1 gene's mRNA level is the only risk factor for SCZ when its level decreases. At the same time, it was a protective factor when its level increases. This risk is maintained after adjusting the odd's ratio by sex, age, HDL, cholesterol, and TAG levels. Also, both mRNA and protein expression's ROC curves demonstrated high specificity and sensitivity of the CSMD1 gene in early diagnosis of SCZ in FHR individuals and predicting suicide tendencies with good NPV and PPV. Also, its value as an early predictor is confirmed by the significant correlation between the CSMD1 gene's mRNA and protein expression with the positive symptoms and increased suicidal tendencies. These results were in agreement with Liu and Sainz.^{27,39} They reported that the level of CSMD1 was decreased in SCZ patients who did not receive any medications. They also found that the level of CSMD1 was increased after taking antipsychotic medication with marked relief of SCZ symptoms.

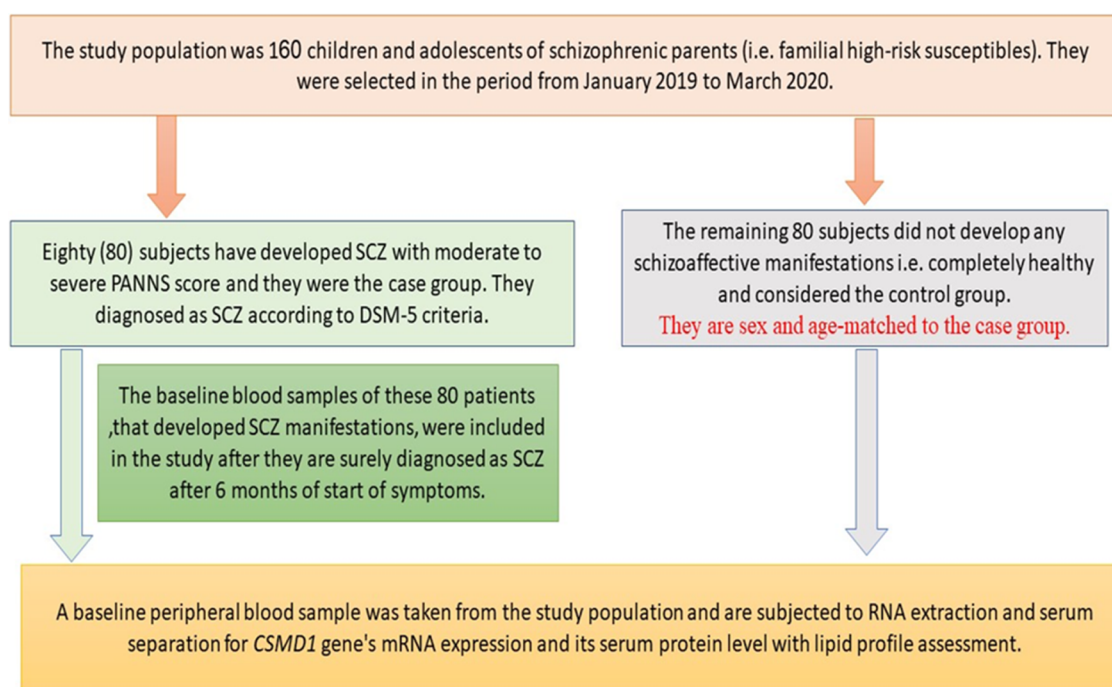


Figure 4. Study design.

Our study showed a significant positive correlation of the *CSMD1* gene's mRNA expression and its protein level with HDL-c and a significant negative correlation with LDL-c in SCZ patients. In patients with suicidal attempts, there was no significant statistical difference between HDL and those with no suicidal history in the SCZ group. At the same time, there was a significant statistical decrease in LDL, cholesterol, and TG in patients experiencing suicide attempts. These results agreed with the series of Ainiyet et al.⁴¹

There is a neurochemical linkage between suicide and decreased serum cholesterol levels. This hypothesis suggests a reduced serotonergic activity due to a decrease in cholesterol in the lipid rafts of synaptic membranes and the incidence of steroid modulation that leads to increased impulses to suicide in those patients.⁴²

Cholesterol and mental disorder have an appealing, complex, and perplexing relationship, but with contradictory evidence. The lack of response to psycho-pharmacotherapy can be predicted even by the blood cholesterol level.⁴³

The SCZ, obsessive-compulsive disorder, panic disorder, generalized anxiety disorder, and posttraumatic stress disorder have been correlated with elevated cholesterol levels. Depression, antisocial personality disorder, borderline personality disorder, and dissociative disorder have been related to low cholesterol levels.⁴⁴

Our results showed higher blood cholesterol levels in SCZ patients than the FHR controls but still higher in the healthy FHR controls than the standard reference levels. Also, our SCZ patients with suicidal thoughts and cognitive dysfunction have had lower cholesterol levels than those without suicidal thoughts. It was previously suggested that decreased cholesterol levels contributed to decreased serotonergic transmission due to changes in serotonin receptors and affinity and function of transporters. Low peripheral and central cholesterol levels have been hypothesized to reduce the lipid viscosity of neuronal cell membranes, causing a reduction in the availability of presynaptic serotonin transporters and

postsynaptic serotonin receptors., increasing suicide risk in SCZ.⁴¹

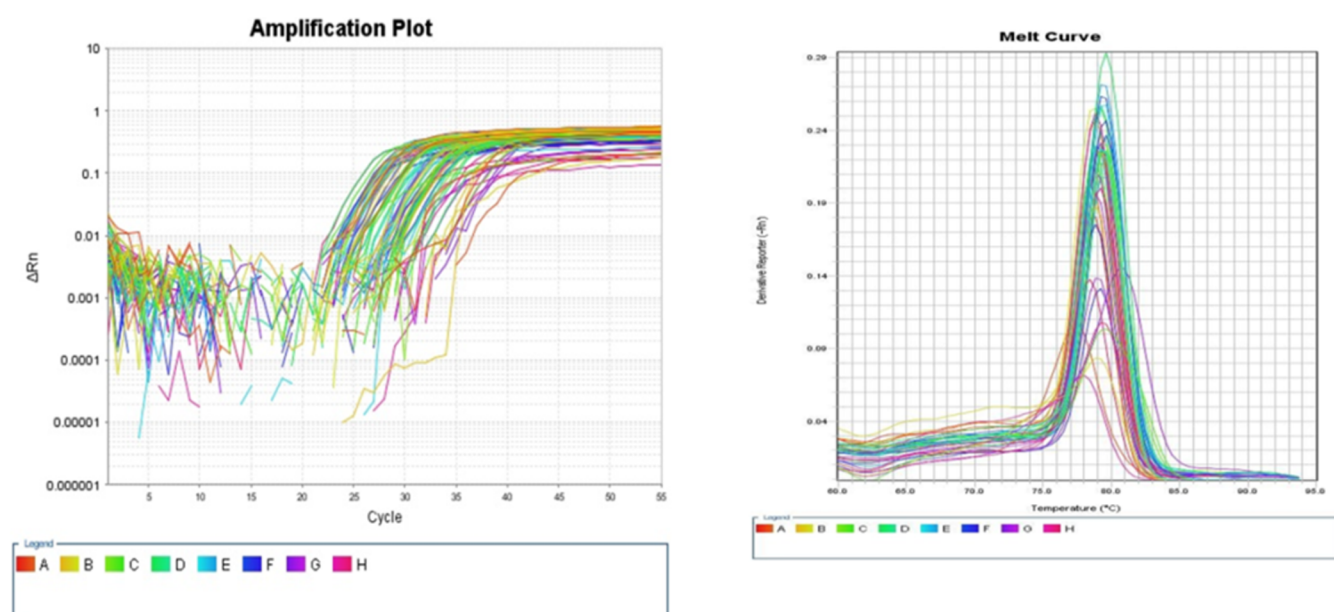
Schizophrenia's pathophysiology involves inflammatory and immune pathways. They are integrated with redox regulation, which affects membrane lipids' composition and serum lipids causing dyslipidemia. Thus, serum and membrane lipids' abnormalities and the neuronal membrane lipid composition change in neuronal cells can affect neurotransmission, symptoms, and behavior in SCZ.⁴⁵

There was a significant statistical difference between SCZ patients and controls regarding cognitive symptoms, negative symptoms, and positive symptoms in the current study. This finding was consistent with the results of Sarkar et al.⁴⁶ and Mitral et al.⁴⁷ Sarkar et al.⁴⁶ reported that negative symptoms of SCZ that persist longer than positive ones are more challenging to treat. The deficit syndrome depends on the severity of SCZ.

This study had some limitations. First, the few drug-naïve patients with an accurate EOS diagnosis made the study sample relatively limited. Second, the mRNA expression and serum protein analyses were performed in the peripheral blood; therefore, due care should be taken when inferring these results into the brain. Peripheral biomarkers have been proposed to mimic identical pathological processes occurring in the brain. Therefore, abnormalities in the blood are potential disease pathology indicators. Third, no extended follow-up was performed for the healthy FHR controls due to the COVID-19 pandemic.

CONCLUSIONS

Our study indicated that the *CSMD1* gene is associated with SCZ pathogenesis. The *CSMD1* gene might be a promising predictor marker for SCZ in Egyptian FHR children and young adults.



(A) The amplification plot.

(B) The melting curve.

Figure 5. *CSMD1* gene's mRNA expression.

MATERIALS AND METHODS

Study Population and Setting. This study was approved by the ethical committee of the Faculty of Medicine, Menoufia University, with approval number 112020BIO. Oral and written informed consent was obtained from the participants or their legal guardians after discussing the study's aim and procedures. This research was conducted in the Medical Biochemistry and Molecular Biology Department in collaboration with the Neuropsychiatry Department and Central Laboratory, Menoufia University and Hospitals, between January 2019 and March 2020.

The study population was 160 children and adolescents of schizophrenic parents (i.e., FHR-susceptible) with an age range of 12–24 years (Figure 4). Eighty subjects have developed first-episode SCZ (represent the SCZ patients group) with mild to moderate PANNS score. The first episode of SCZ was selected to avoid the influence of the antipsychotic medication on our results. The patient must have had at least two of the following indications for the diagnosis of SCZ: delusions, hallucinations, disorganized speech, disorganized or catatonic actions, and negative symptoms. These symptoms persist for at least 6 months, during which the patient must have had at least 1 month of active symptoms (or less if effectively treated) associated with social or occupational decline. The SCZ diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).⁴⁸ We excluded patients with (1) previously diagnosed and treated chronic SCZ patients; (2) schizoaffective disorder, depressive, and bipolar disorders with psychotic features; (3) severe neurologic diseases that might affect their cognitive functions (i.e., significant head injury, cerebrovascular disease, seizure disorder); (4) chronic unstable medical condition, e.g., poorly controlled diabetes, and hypertension; (5) BMI ≥ 25.1 kg/m²; (6) severe visual or hearing impairment; (7) substance abuse or dependence in the past 3 months, and any substance

addiction have been ruled out; (8) the presence of any other neuroinflammatory or any autoimmune disease; and (9) the patients with metabolic syndrome. The remaining 80 subjects did not develop SCZ manifestations, i.e., wholly healthy, and considered the control group. They are sex- and age-matched to the case group.

All of the study participants were subjected to complete history and clinical examination. Laboratory molecular investigations for the *CSMD1* gene's mRNA expression were performed by the real-time quantitative polymerase chain reaction (qPCR) technique. The concentration of serum *CSMD1* was measured using the enzyme-linked immunosorbent assay (ELISA) method. Lipid profile (including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c)) was measured by spectrophotometric methods using Cobas-C.

Procedures. Samples Collection. Whole peripheral blood (5 mL) was taken under complete aseptic conditions, and 1 mL was evacuated in an ethylenediaminetetraacetic acid (EDTA) Vacutainer tube for RNA extraction. The remaining 4 mL of the blood sample has been put into a plain tube and allowed to clot for 2 h at room temperature. Then, they were centrifuged at 3000 rpm for 15 min to obtain the serum. The RNA extract and the serum were stored in sterilized Eppendorf tubes at -80 °C till analysis.

Measurement of the Serum *CSMD1* Concentration. This is performed using an ELISA kit supplied from MyBiosource, Inc. (Southern California, San Diego) with (catalog number MBS7206464) according to the manual instructions.

Estimation of mRNA Gene Expressions. The total RNA was extracted from blood leukocytes using miRNeasy RNA Mini Kit (Qiagen, Applied Biosystems), depending on the manufacturer's guidance. Then, QuantiTect Reverse Transcription Kit (Qiagen, Applied Biosystems) was used to

synthesize the complementary DNA (cDNA) in compliance with the manual's instructions. Finally, the QuantiTect synergy brands (SYBR) Green master mix PCR Kit (Qiagen, Applied Biosystems) with primers (supplied from Thermo Fisher Scientific, Inc.) was used to perform the second-stage real-time PCR for measuring *CSMD1* gene's mRNA levels. The forward and reverse primers for the human *CSMD1* gene are 5'-GTCTGGGCTCGTGGATATGT-3' and 5'-CAGGTCTCGGAAGGACAGAG-3', respectively. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal reference gene with the following forward and reverse primers for the human *GAPDH* gene: 5'-GCACCGTCAAGGCTGAAGAC-3' and 5'-TGGTGAAGACGCCAGTGGGA-3', respectively. A final volume of each PCR reaction was 20 μ L, containing 10 μ L of SYBR Green 2 \times QuantiTect PCR Master Mix, 3 μ L of cDNA, 1 μ L of forward primer, 1 μ L of reverse primer, and 5 μ L of RNase-free H₂O. The cycling conditions of the PCR reaction mix were as follows: incubation for 3 min at 94 °C, followed by 50 cycles, denaturation for 30 s at 94 °C, annealing for 40 s at 55 °C, and extension for 31 s at 72 °C. Data analysis was done by version 2.0.1 of Applied Biosystems 7500 program. The comparative $\Delta\Delta C_t$ method was used for the relative quantification (RQ) of the gene's expression.⁴⁹ The mRNA expression level of the *CSMD1* gene was normalized to the housekeeping gene's mRNA levels, *GAPDH*. To confirm the precision of the amplification and absence of primer dimers, the melting curve analysis was performed (Figure 5B).

Statistical Analysis. Data were fed to the computer and analyzed using IBM-SPSS software package version 20.0. (IBM Corp, Armonk, NY). The Kolmogorov–Smirnov test was used to verify the normality of the distribution of variables. Comparisons between groups for categorical variables were assessed using the chi-square test. The Mann–Whitney test was used to compare two groups for abnormally distributed quantitative variables. The Spearman coefficient was used to correlate between distorted distributed quantitative variables. The receiver operating characteristic (ROC) curve was used to determine the diagnostic performance of the markers. Area more than 50% gives an acceptable presentation, and area of 100% is the best performance for the test. To detect the most independent/ affecting factor for affecting SCZ used logistic regression analysis. The significance of the obtained results was judged at the 5% level.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c03637>.

Relation between the *CSMD1* gene's mRNA level and *CSMD1* serum protein level with different parameters in the control group (Table S1); relation between the *CSMD1* gene's mRNA level and *CSMD1* serum protein level and different parameters in the control group (Table S2); relation between HDL and LDL with different parameters in the cases and control groups (Tables S3 and S4); and relation between cholesterol and TAG with different parameters in the cases and control groups (Tables S5 and S6) (PDF)

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Author Contributions

N.R.B. (the corresponding author) and E.M.A.E.G. contributed to the conception, design, and acquisition of data; laboratory work; analysis and interpretation of data; and drafting and revising the manuscript. M.S.R. performed laboratory work and revised the manuscript. A.N.R. selected the study participants and revised the manuscript.

Notes

The authors declare no competing financial interest.

The datasets generated and/or analyzed during the current study are not publicly available as the privacy of this pilot study could be compromised but are available from the corresponding author on reasonable request.

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