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Targeted multi-layer analysis of PANoptosis-associated genes in the etiology of chronic kidney disease

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Abstract

Background Previous studies have examined the cellular and molecular interactions between chronic kidney disease (CKD) and PANoptosis, yet the genetic underpinnings remain unclear.

Materials Data at the summary level regarding the methylation, gene expression, and protein levels associated with PANoptosis were obtained from quantitative trait locus (QTL) studies. Genome-wide association study (GWAS) summary statistics for CKD were derived from a GWAS study, supplemented by a replication dataset from the FinnGen database. Genetic variants proximal to or within genes involved in PANoptosis, which showed robust associations with CKD, were utilized as instrumental variables. These variants were the subjected to SMR analysis to explore their causal relationship. The associations among QTLs were systematically analyzed. Additionally, a colocalization analysis was conducted to ascertain whether the signals identified corresponded to a shared genetic basis.

Results SMR and colocalization analysis revealed 28 methylation sites and 5 genes associated with CKD.Notably, cg01304814 (*PRKAR2A*) and cg09177106, cg15114474 (*CCND1*) were inversely associated with CKD risk. Integrating mQTL and eQTL data, we identified four genes (*CCND1*, *GUCY2D*, *HGF*, *MADD*) causally associated with CKD, with a positive correlation between HGF gene expression and protein levels.

Conclusion Our results provide evidence for the PANoptosis-related genes in the pathogenesis of CKD. Notably, *PRKAR2A*, *HGF*, *CCND1* and MADD, emerged as potential mediators in the pathogenesis of CKD.

Trial registration Not applicable.

Keywords Chronic kidney disease, PANoptosis, Gene, Mendelian randomization

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Background

Chronic kidney disease (CKD) is characterized by the progressive decline in kidney function over time and affects approximately 10% of the global population [1, 2]. Individuals with CKD are at elevated risk for numerous adverse outcomes, such as the need for kidney replacement therapy, cardiovascular events, and mortality [3]. Despite its severity and prevalence, there is currently no effective cure for CKD in clinical practice, and new strategies are needed to extend kidney and patient survival without resorting to dialysis or transplantation [4]. Identifying and understanding the biomarkers involved in the biological pathways of CKD is crucial for identifying potential therapeutic targets.

In recent years, PANoptosis has garnered increasing attention in CKD due to its integration of multiple cell death pathways, including apoptosis, necroptosis, pyroptosis, and ferroptosis, which may profoundly influence disease onset and progression [5]. This new form of cell death is regulated by the PANoptosome protein complex and triggers a cascade of pathological processes through the integration of various cell death signals [6]. The complex interplay between different cell death pathways in CKD suggests that PANoptosis may play a significant role in its onset and progression. For example, studies have indicated that PANoptosis contributes to kidney cell death and functional loss by promoting cell cycle arrest, exacerbating chronic inflammatory responses, and inducing cellular senescence [7]. However, existing studies predominantly rely on molecular manipulation or small-scale cohort designs, limiting statistical power to establish causality between PANoptosis levels and CKD progression. This highlights the urgent need for large-population studies to provide robust statistical evidence and for in-depth mechanistic investigations to elucidate the causal pathways.

MR studies can inform on potential causal relationships between modifiable risk factors and diseases that would require extensive sample sizes and long-term follow-up for sufficient endpoints [8]. A key advantage of this method is its capacity to reduce the influence of confounding factors and reverse causality. Building on this, summary-data-based Mendelian randomization (SMR) represents an extension of the MR method. Through the integration of summary data derived from multiple independent cohorts, SMR substantially enhances statistical power, rendering it especially appropriate for the analysis of large-scale genomic datasets. Additionally, multi-layer integration analysis combines data from genomics, transcriptomics, epigenomics, and proteomics to comprehensively elucidate the molecular mechanisms underlying diseases.

In our study, we applied a summary-data-based Mendelian randomization (SMR) to investigate the potential links between the epigenetic, transcriptional, and proteomic features of genes associated with PANoptosis and their impact on the risk of developing CKD. This approach seeks to offer a comprehensive, multi-layer molecular perspective, highlighting critical interactions and regulatory mechanisms pivotal to the onset of CKD.

Methods

Study design

The SMR analysis utilized data from a large-scale dataset as the discovery cohort from the GWAS Catalog, with a replication dataset from the FinnGen database. We identified instrumental variables associated with PANoptosis genes across methylation, gene expression, and protein abundance levels. SMR analyses were then performed to investigate the relationships between these IVs and CKD across these levels and the regulatory relationship between mQTLs and eQTLs. To strengthen the causal inference, colocalization analysis was implemented. The study design, strictly following the STROBE guidelines, is illustrated in Fig. 1. See supplementary materials for details.

Results

PANoptosis-Related gene methylation and CKD

The causal impact of methylation sites in PANoptosis-related genes on CKD was illustrated in Figure S1A, with the complete analysis results provided in Table S2. A total of 101 methylation sites (located in 60 genes) were identified. The findings from the colocalization analysis demonstrated that among the 28 methylation sites situated within 17 genes, there were specific sites that fall within the colocalization region and were correlated with CKD, as indicated by a posterior probability of heterogeneity (PPH4) value exceeding 0.5. The colocalization outcomes for the cg01304814 site, which was positioned in the *PRKAR2A* gene, were depicted in Figure S1B. In the results obtained from the SMR analysis, a total of 15 methylation sites corresponding to 11 genes (*CCND1*, *EXOG*, *GPX1*, *IER3*, *KPNA1*, *MADD*, *PIK3CA*, *PSEN2*, *PSMC3*, *PSMD6*, *UACA*) were validated in the FinnGen cohort (Table S3).

PANoptosis-Related gene expression and CKD

A total of 23 PANoptosis-related genes including *CCND1*, *HGF*, *MADD*, *PRKAR2A* were found to be associated with CKD. The causal relationships between gene expression and CKD were illustrated in Figure S2A, with comprehensive analysis results detailed in Table S4. The colocalization analysis results showed that within the colocalization region window corresponding to SNPs of five genes, there were sites associated with CKD (PPH4 > 0.5). The colocalization findings for the SNP site associated with *PRKAR2A* and CKD were shown

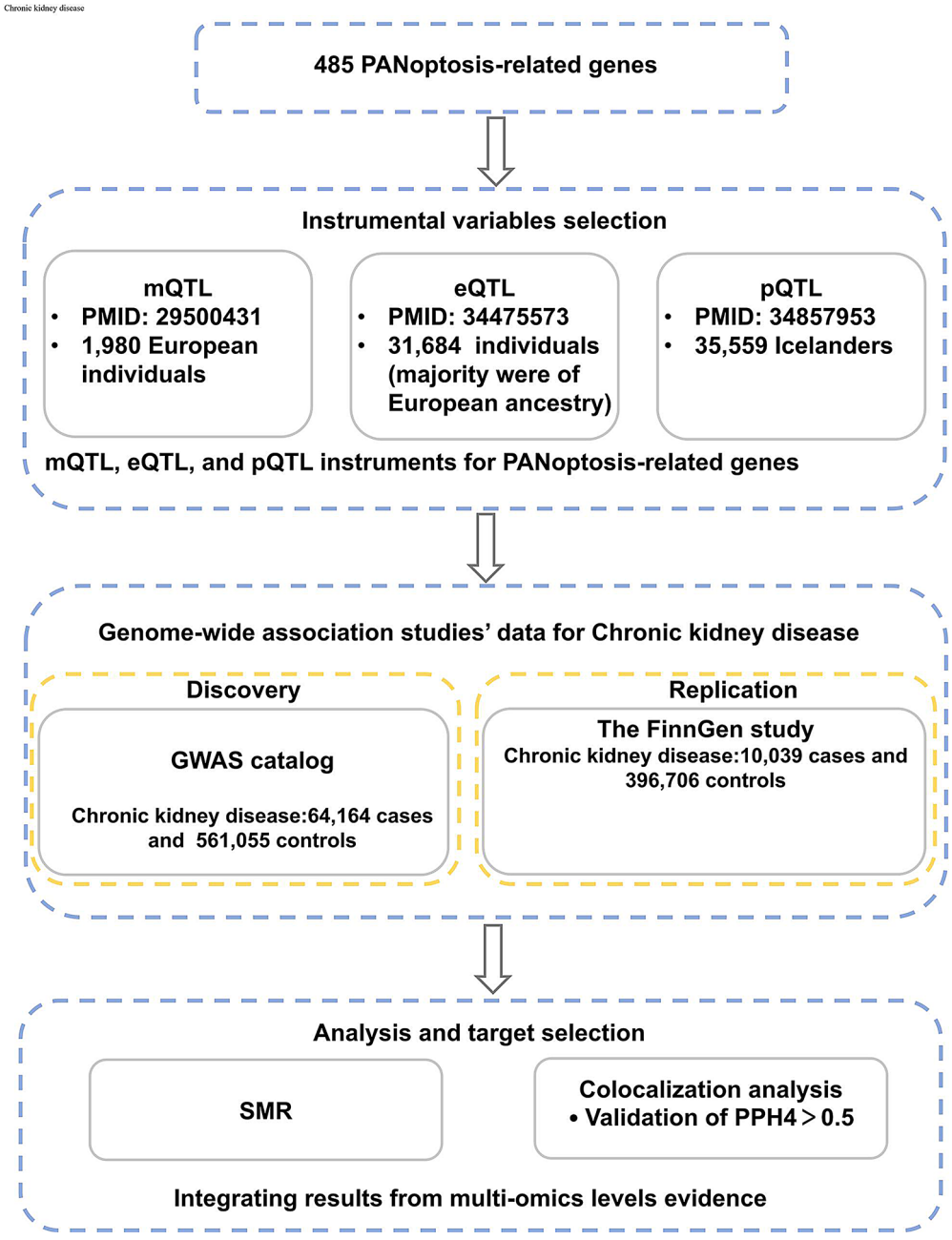

Fig. 1 Study design flowchart

in Figure S2B (OR = 1.269, 95% CI = 1.079–1.493). The validation of genes associated with the risk of CKD in the FinnGen cohort has identified three specific genes: *GUCY2D*, *IFNGR2*, and *MADD* (Tables S5).

PANoptosis-Related protein abundance and CKD

Six proteins were found to be associated with CKD (Figure S3), with the complete analysis results provided in Table S6. Colocalization analysis results showed no strong evidence of colocalization for proteins within the colocalization region window. In the results obtained from the SMR analysis, PSB9 protein, encoded by *PSMB9*, was validated in the FinnGen cohort (Tables S7).

Integrating evidence from Multi-Layer levels

Integrating mQTL and eQTL data, we further explored the regulation of key PANoptosis genes associated with CKD through blood methylation. The results indicated that cg04033857 positively regulates the gene *CCND1* (OR = 1.834, 95% CI = 1.542–2.181), while cg27380288 negatively regulates the gene *GUCY2D* (OR = 0.892, 95% CI = 0.823–0.967), cg18944653 negatively regulates

Table 1 Causal associations between methylation and expression of PANoptosis-related genes

Expo_ID	Outco_Gene	SYMBOL	p_SMR	p_SMR_multi	fdr_SMR	OR_SMR	95% CI_SMR
cg04033857	ENSG00000110092	CCND1	6.85E-12	6.85E-12	2.28E-11	1.834	1.542–2.181
cg27380288	ENSG00000132518	GUCY2D	0.005426	0.005426	0.006383	0.892	0.823–0.967
cg18944653	ENSG00000019991	HGF	1.67E-06	1.67E-06	2.38E-06	0.841	0.783–0.903
cg23844623	ENSG00000110514	MADD	1.20E-08	1.20E-08	2.40E-08	0.787	0.725–0.855

Table 2 Causal associations between expression and protein abundance of PANoptosis-related genes

Expo_ID	Outco_ID	p_SMR	p_SMR_multi	fdr_SMR	OR_SMR	95% CI_SMR
ENSG00000019991	HGF	3.59E-10	3.59E-10	8.44E-09	6.295	3.542–11.188

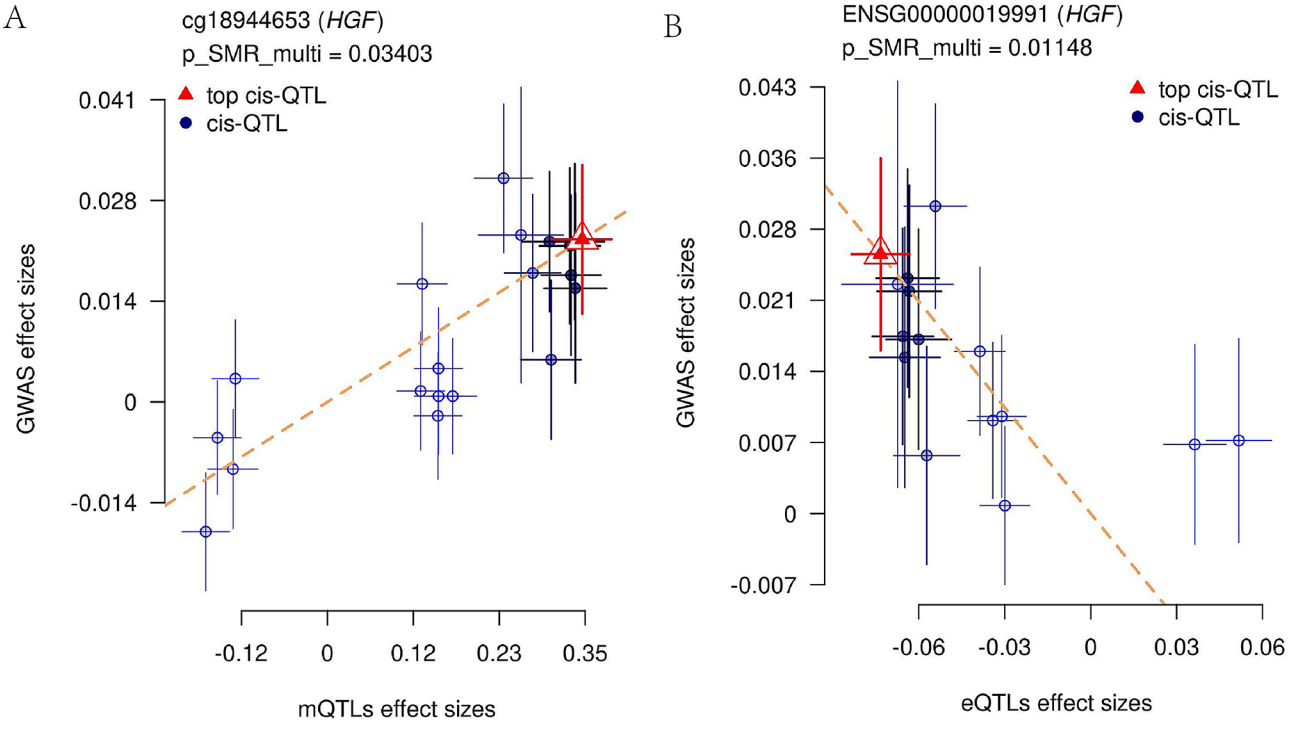

Fig. 2 SMR Effect Plot between HGF-related results of m/eQTLs and GWAS. SMR (**A**) Effect plot for *HGF* at cg18944653 in mQTL and its association with CKD., (**B**). Effect plot for *HGF* in eQTL and its association with CKD

the gene *HGF* (OR = 0.841, 95% CI = 0.783–0.903), and cg23844623 negatively regulates the gene *MADD* (OR = 0.787, 95% CI = 0.725–0.855) (Table 1). Furthermore, the integrated eQTL and pQTL data demonstrated that the regulatory relationship between *HGF* gene expression and its protein abundance was validated (OR = 6.295, 95% CI = 3.542–11.188) (Table 2). Figure S4 display Manhattan plots illustrating the chromosomal localization of methylation sites, gene expression, and protein levels associated with CKD. To graphically display the outcomes of our SMR analysis, we created locus plots that illustrate the associations between *HGF* methylation, gene expression and CKD (Fig. 2A-B).

Discussion

Our research employed SMR and colocalization analysis to investigate the genetic correlations between DNA methylation, gene expression, and protein levels of ferroptosis-associated genes in CKD. We found that cg01304814 (located in *PRKAR2A*) and cg09177106, cg15114474 (located in *CCND1*) are inversely associated with the risk of CKD, while *HGF*, *CCND1*, *MADD*, *GUCY2D* genes were associated with CKD with multilayer evidence.

As a regulatory subunit of Protein Kinase A (PKA), PRKAR2A modulates PKA activity by influencing substrate specificity, localization, and cAMP responsiveness [9]. While direct studies on *PRKAR2A* in CKD are limited, its role can be inferred through its integration into PKA signaling networks, which are dysregulated in renal conditions [10, 11]. In renal tissues, PKA has been

implicated in gluconeogenesis, ion transport, and podocyte function [11]. PRKAR2A is able to anchor PKA to specific subcellular compartments via A-kinase anchoring proteins (AKAPs), enabling spatially restricted cAMP signaling. Furthermore, PKA has been shown to inhibit TGF-β-mediated fibrogenesis in other tissues [12]. Given that interstitial fibrosis is a hallmark of CKD progression, it is plausible that PRKAR2A could mitigate renal scarring and CKD progression by antagonizing fibrotic pathways in the kidney through restricted PKA-cAMP signaling. However, our findings suggested that the expression levels of *PRKAR2A* were positively associated with CKD risk, with contrasted with previous reports. The discrepancy could stem from the fact that our data were derived from blood samples rather than kidney tissues, highlighting the potential impact of tissue-specific differences. Blood-based measurements may reflect systemic responses or indirect effects on renal function, whereas kidney tissue data would more directly capture the local pathological changes within the organ. This distinction underscores the importance of considering tissue-specific contexts when interpreting the role of *PRKAR2A* in CKD. Future studies should explore both blood and kidney tissue data to reconcile these differences and provide a more comprehensive understanding of its involvement in CKD progression.

HGF, which binds to its receptor MET to modulate cellular proliferation, migration, and tissue regeneration, plays a crucial role in the pathogenesis of inflammatory and immune-mediated conditions [13]. The HGF-MET signaling pathway is vital for initiating reparative processes and promoting regeneration in the kidney following injury [14]. Extensive research has been conducted on *HGF* in the context of diabetic kidney disease (DKD). Studies have demonstrated that exogenous administration of HGF can mitigate proteinuria and tubulointerstitial fibrosis by suppressing TGF-β expression and enhancing renal repair mechanisms in murine models [15–17]. Additionally, it has been observed that *HGF* mRNA levels are decreased in individuals with DKD, indicating a potential renoprotective function of *HGF* in this condition [13]. Consistent with prior findings, our SMR study show that *HGF* levels were associated with decreased CKD risk across eQTL and pQTL levels. Therefore, HGF represents a potential therapeutic target for CKD treatment, with modulation of its activity potentially suppressing fibrosis and decreasing CKD risk.

CCND1 is a key regulator of cell cycle progression and is central to oncogenic transformation due to its role in promoting cellular proliferation [18]. Within the glomerulus, *CCND1* expression is predominantly observed in podocytes, as evidenced in the Heymann nephritis rat model [19] and in human focal segmental glomerulosclerosis (FSGS) cases [20]. Bioinformatics analysis identified *CCND1* as a potential marker associated with both cancer and membranous nephropathy [21]. In the context of CKD, studies have shown that higher levels of *CCND1* are associated with higher estimated glomerular filtration rates (eGFR), suggesting a potential protective effect on kidney function [22]. On the other hand, while cell cycle progression can aid in repair after acute kidney injury, ongoing cell cycle activity in chronic kidney disease may exacerbate damage. By interacting with CDK4 or CDK6, CCND1 facilitates the transition from the G1 to S phase of the cell cycle, potentially exerting a significant impact on the pathogenesis of CKD [23]. Moreover, eQTL analyses have linked variants in *CCND1* to eGFR in genome-wide association studies [22]. Therefore, *CCND1* may hold promise as a valuable target for intervention in cases of CKD. Here, our research has elucidated that *CCND1* expression levels exhibited an inverse correlation with CKD risk, suggesting that *CCND1* served as a protective factor agaisnt CKD. While the promotion of cell cycle progression through CCND1 is a double-edged sword, with the potential to both aid in repair and exacerbate damage depending on the context, its association with higher eGFR in CKD patients highlights its potential protective effects. Future research should focus on elucidating the precise mechanisms through which CCND1 exerts its influence on kidney function and identifying specific pathways that can be modulated to harness its protective potential.

MADD has been implicated in various cellular processes, including cell growth, apoptosis, and immune responses [24]. Our findings revealed that *MADD* expression levels were negatively associated with CKD risk. However, evidence from other diseases provides valuable insights. For example, a study by Medlyn et al. [25] showed that MADD regulates natural killer cell degranulation, which is crucial for immune surveillance and may have implications for the inflammatory processes observed in CKD. Given these findings, it is plausible that alterations in *MADD* expression could contribute to the pathogenesis of CKD. Further efforts are required to clarify the specific pathways by which MADD exerts its protective effects on CKD.

This study is the first to evaluate the associations between PANoptosis-related genes and CKD using SMR and colocalization methods. A key strength of the study is its application of SMR, which enables a simultaneous assessment of the relationships between methylation, expression, and protein abundance of PANoptosis-related genes and CKD across independent European populations. However, it is important to take limitations into account when evaluating our findings. Firstly, the focus on cis-acting regulatory elements, while effectively minimizing pleiotropic effects, may have inadvertently excluded the influence of trans-acting

regulatory factors [26]. This could explain why certain genes well-recognized to be associated with CKD, such as *FOS*, *PTGS2*, *TRAIL* and *DR5* [7, 27], were not identified in our study. Additionally, the stringent colocalization threshold (PPH4 > 0.5) may have filtered out genes with weaker associations. Secondly, the GWAS data are confined to populations of European ancestry, omitting gene expression or eQTL data from diverse ethnic groups, which may limit the applicability of our findings to a more comprehensive racial and ethnic spectrum. However, it is important to note that CKD is genetically heterogeneous across different populations [28]. Future studies should aim to include multi-ethnic cohorts to better understand the genetic factors associated with CKD in a broader context. In the meantime, our findings from European ancestry populations can serve as a foundation for further research, but caution should be exercised when extrapolating these results to other ethnic groups.

Conclusion

Our investigation delved into the causal associations among epigenetic alterations, gene expression, and protein levels in CKD. This study underscores the pivotal role of these genetic elements and their regulatory mechanisms in the advancement of the disease, enriching our comprehension of the fundamental pathophysiological processes and paving the way for novel therapeutic interventions.

Abbreviations

CKD	Chronic kidney disease
AKI	Acute kidney injury
CRP	C-reactive protein
ATRA	All-trans retinoic acid
ESRD	End-stage renal disease
MR	Mendelian Randomization
RCTs	Randomized controlled trials
SMR	Summary-data-based Mendelian randomization
LD	Linkage disequilibrium
GWAS	Genome-wide association studies
SNPs	Single nucleotide polymorphisms
OGD	Oxygen-glucose deprivation
HGF	Hepatocyte growth factor
CCND1	Cyclin D1
FSGS	Focal segmental glomerulosclerosis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-025-00768-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Not applicable.

Author contributions

Tong Li and Yingyue Zhang carried out the studies, participated in collecting data, and drafted the manuscript. The two authors contributed equally to this work. Tong Li, Xiaofei Ma and Xingzhi Wang performed the statistical analysis and participated in its design. Yingyue Zhang, Qi Liu, Manshu Sui and Xingzhi Wang participated in acquisition, analysis, or interpretation of data and draft the manuscript. All authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

All authors declare that they have no any conflict of interests.

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