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Original Article

Quantitative assessment of *ABCB1* polymorphisms and non-traumatic osteonecrosis of the femur head risk

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Abstract: Several studies have investigated the correlation between genetic polymorphisms in the *ABCB1* gene and Non-traumatic osteonecrosis of the femur head (ONFH) risk. However, the potential association is poorly determined due to the small sample size of a single study. Therefore, we carried out a pooled analysis to try to get a definite evaluation of the association. The relevant studies were retrieved in electronic databases PubMed, Google Scholar, and CNKI. The significance of the relationship was determined via the Z test, and $P<0.05$ was thought statistically significant. Our results suggested that the *ABCB1* rs1045642 was significantly related with decreased risk of ONFH under the allelic model (OR=0.69, 95% CI: 0.59-0.82, $P=0.000$), homozygous model (OR=0.44, 95% CI: 0.31-0.64, $P=0.000$), heterozygous model (OR=0.72, 95% CI: 0.56-0.92, $P=0.010$), dominant model (OR=0.65, 95% CI: 0.51-0.82, $P=0.000$), and recessive model (OR=0.46, 95% CI: 0.32-0.67, $P=0.000$). Similar result was observed for rs2032582 with GC-reduced ONFH under the allelic model (OR=0.74, 95% CI: 0.56-0.99, $P=0.040$), and homozygous model (OR=0.46, 95% CI: 0.25-0.83, $P=0.010$). However, no significant association was found between rs1128503 and ONFH susceptibility under the five genetic models analysis. These results indicated that rs1045642 and rs2032582 are protective factors of ONFH, though these findings must be confirmed in a well-designed case-control study with a larger sample size.

Keywords: ONFH, *ABCB1*, single nucleotide polymorphisms (SNPs), meta-analysis

Introduction

Non-traumatic osteonecrosis of the femur head presents an aseptic and ischemic bone destruction, and mainly affects the femur head. The disruption of blood flow to the subchondral bone leads to osteocyte death (including apotosis and autophagy) and gradual collapse of the sharp and function of the femur head, which seriously influences the quality of life for ONFH patients [1]. Accumulated studies have indicated that intensive glucocorticoid (GC) administration has a predominant effect on the pathogenesis of Non-traumatic ONFH, especially for patients with organ transplantation, autoimmune diseases, and neoplastic diseases, such as childhood acute lymphoblastic leukemia. Besides, alcohol abuse and storage disease are also risk factors for ONFH [2].

Although ONFH has clearly been correlated with these conditions, some patients with high doses of GC or alcohol abuse or storage disease have a low incidence of this disease. The presence of individual difference may be attributed to the varying genetic background, such as single nucleotide polymorphisms (SNPs) in the *VEGF*, *MTHFR*, *APOB* and *ABCB1* gene which have been reported involved in the pathogenesis of Non-traumatic ONFH [3-6].

ATP binding cassette subfamily B member 1 (*ABCB1*), located on 7q21.12, also known as *MDR1*, encodes the membrane transport protein P-glycoprotein which influences the intracellular pharmacokinetics and pharmacodynamics of multiple drugs, including drug absorption, distribution, and excretion [7]. The *ABCB1* polymorphisms rs2032582, rs1045642, and rs1128503 are the most studied SNPs in the association with ONFH risk, but the results in these clinical case-control studies were inconclusive. In 2003, Asano. T et al. found that rs2032582 and rs1045642 were

Table 1. The main characteristics of the included studies in the meta-analysis

Study	Year	Country	Genotyping method	CC ases	Controls	ONFH type	*ABCB1* polymorphisms
X.Y.Yang	2007	China	PCR-RFLP assay	21	106	GC-induced	rs1045642, rs2032582
Kuribayashi	2008	Japan	PCR	30	121	GC-induced	rs1045642, rs2032582
Wei He	2009	China	PCR	31	17	GC-induced	rs1045642, rs2032582
Asano. T	2003	Japan	PCR	30	106	GC-induced	rs1045642, rs2032582
Yanqiong Zhang	2014	China	PCR	94	106	GC-induced	rs1045642, rs2032582
Yun Xue	2014	China	PCR	105	217	GC-induced	rs1045642, rs2032582, rs1128503
Mary V. Relling	2004	US	PCR	25	39	GC-induced	rs1045642, rs2032582
Zongyu Zhang	2015	China	PCR-RFLP assay	113	116	NG	rs1045642, rs1128503
Guoyong Qiao	2016	China	PCR-RFLP assay	100	120	NG	rs1045642, rs1128503

GC, glucocorticoid; NG, not given.

Table 2. Meta-analysis of correlations between *ABCB1* polymorphisms and RA risk

SNPs	Comparison model	Test of heterogeneity		Effect model	Test of association	
		I^2	P^a		OR (95% CI)	P^b
rs1045642						
Total	T vs. C	0.0%	0.607	Fixed	0.69 (0.59-0.82)	0.000
	TT vs. CC	24.1%	0.245	Fixed	0.44 (0.31-0.64)	0.000
	TC vs. CC	0.0%	0.792	Fixed	0.72 (0.56-0.92)	0.010
	TT+TC vs. CC	0.0%	0.860	Fixed	0.65 (0.51-0.82)	0.000
	TT vs. TC+CC	0.0%	0.473	Fixed	0.46 (0.32-0.67)	0.000
GC-induced	T vs. C	0.0%	0.467	Fixed	0.73 (0.59-0.91)	0.004
	TT vs. CC	42.6%	0.137	Fixed	0.50 (0.31-0.80)	0.004
	TC vs. CC	0.0%	0.659	Fixed	0.77 (0.57-1.04)	0.090
	TT+TC vs. CC	0.0%	0.812	Fixed	0.70 (0.52-0.94)	0.018
	TT vs. TC+CC	29.6%	0.234	Fixed	0.41 (0.23-0.72)	0.002
rs2032582	T/A vs. G	47.4%	0.107	Fixed	0.74 (0.56-0.99)	0.040
	T/AT/A vs. GG	0.0%	0.448	Fixed	0.46 (0.25-0.83)	0.010
	T/AG vs. GG	46.0%	0.116	Fixed	0.84 (0.54-1.31)	0.439
	T/AT/A+T/AG vs. GG	49.0%	0.098	Fixed	0.75 (0.49-1.14)	0.179
	T/AT/A vs. T/AG+GG	21.8%	0.280	Fixed	0.62 (0.38-1.01)	0.055
rs1128503	T vs. C	0.0%	0.645	Fixed	1.02 (0.83-1.25)	0.865
	TT vs. CC	0.0%	0.548	Fixed	1.07 (0.70-1.64)	0.747
	TC vs. CC	0.0%	0.929	Fixed	0.91 (0.63-1.31)	0.597
	TT+TC vs. CC	0.0%	0.904	Fixed	0.96 (0.68-1.35)	0.803
	TT vs. TC+CC	0.0%	0.385	Fixed	1.09 (0.79-1.50)	0.619

SNPs, single nucleotide polymorphisms. [a]*P* value for heterogeneity based on Q test. [b]*P* value for association calculated by the Z test.

associated with decreased risk of ONFH in a Japanese case with GC therapy after renal transplantation and controls [8]. However, no significant difference of rs2032582 or rs1045642 was observed between ONFH cases and controls without osteonecrosis by Mary V. Relling and colleagues [9]. Several studies demonstrated the homozygous variant type (TT) of rs1045642 was a protective factor of ONFH instead of the heterozygous type (TC) when compared with the homozygous wild type (CC) and rs2032582 was not correlated with osteonecrosis risk in a Chinese population [10-14]. Nevertheless, unlike these results, Wei He and colleagues indicated that rs2032582 was significantly associated with ONFH susceptibility in a Chinese population [15]. Genotype distribution of rs1128503 in osteonecrosis cases and healthy controls did not show any obvious difference in three investigations [10, 13, 14].

Overall, published data on *ABCB1* polymorphisms and ONFH risk have generated inconclusive results. Therefore, to know whether *ABCB1* polymorphisms rs2032582, rs10456-42, and rs1128503 are associated with ONFH susceptibility in a multi-ethnic population, a meta-analysis based on above case-control studies was conducted by us.

Material and methods

Search strategy

Published data were searched without any ethnicity and language restriction in the electronic databases PubMed, Google Scholar, and Chinese National Knowledge Infrastructure (CNKI) based on the following MeSH terms: "*ABCB1* or *MDR1*" and "osteonecrosis or ONFH" and "single nucleotide polymorphisms or SNPs or polymorphisms". The eligible articles had to be issued as a full text and the last search for these studies was up to June 10, 2016.

Inclusion criteria

Eligible studies in our present pooled analysis were selected according to the following criteria: (1) a case-control study that designed to evaluate the relationship between genetic polymorphisms in *ABCB1* and ONFH risk; (2) ONFH was diagnosed based on a combination of X-rays and magnetic resonance imaging and medical history prescribed by Mont MA et al. or Sugano N et al. [1, 16]; (3) provided exact allele and genotype frequency in both cases and controls enough to calculate the pooled odds ratios (ORs) and 95% confidence intervals (CIs); (4) ONFH patients with a history of hip trauma were excluded.

Data extraction

The following data were extracted from the eligible studies independently by two authors in our team: name of the first author, year of publication, ethnicity, ONFH type, total number of cases and controls, genotyping methods, genotype frequency in cases and controls for each *ABCB1* polymorphism.

Statistical analysis

To explore the association between *ABCB1* polymorphisms and ONFH risk, pooled ORs and 95% CIs under the allelic model, homozygous model, heterozygous model, dominant model, and recessive model analysis were examined. Subgroup analysis by ONFH type was also conducted for rs1045642. The significance of the relationship was determined via the Z test, and $P<0.05$ was thought statistically significant. The heterogeneity among included studies were assessed using the *Q*-statistic test and I^2 test [17, 18]. If the $P_H>0.05$ or $I^2<50\%$, which were considered less heterogeneity among included studies, the fixed-effects model was then applied to calculate the pooled ORs; otherwise, the random-effects model was applied. The possible publication bias among included studies in each model analysis was evaluated visually in a Begg's funnel plot and the degree of asymmetry was examined by an Egger's linear regression test ($P<0.05$ for Egger'stest was considered significant). All statistical tests were managed using the STATA statistical software package (version 11.0; Stata Corporation, College Station, TX).

Results

Characteristics of included studies

Electronic literature retrieval following our search strategy resulted in the identification of 23 articles initially. After screening their titles and abstracts, 14 were then excluded based on the predefined inclusion criteria. Where after, full-text reading of the remaining 9 articles were carried out and the repeated data about the association of rs1045642 with ONFH risk were observed (Asano et al., 2003 and Kuribayashi et al., 2008). The 9 included studies in our present analysis collected 9 articles of rs1045642, 6 articles of rs2032582, and 3 articles of rs1128503. The main characteristics of the included studies are listed in **Table 1**. And the three SNPs of *ABCB1* in controls did not deviate from Hardy-Weinberg equilibrium ($P>0.05$).

Quantitative synthesis

A summary of the analysis results of the correlations between the *ABCB1* variants and ONFH risk was listed in **Table 2**. Nine articles reported the role of rs1045642 in ONFH susceptibility, and one of them was eliminated due to the duplicate data. Significant heterogeneity among included studies was observed under

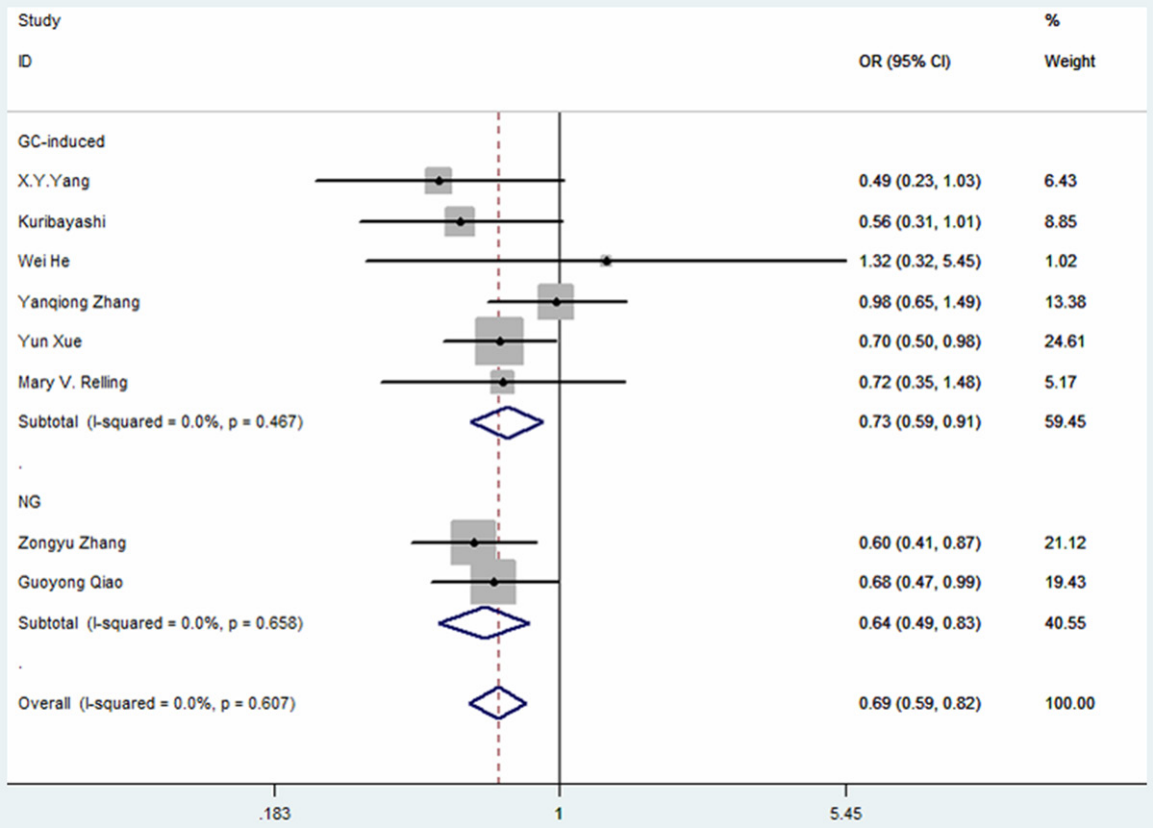

Figure 1. Forest plot for T vs. C of the association between *ABCB1* rs1045642 and ONFH risk.

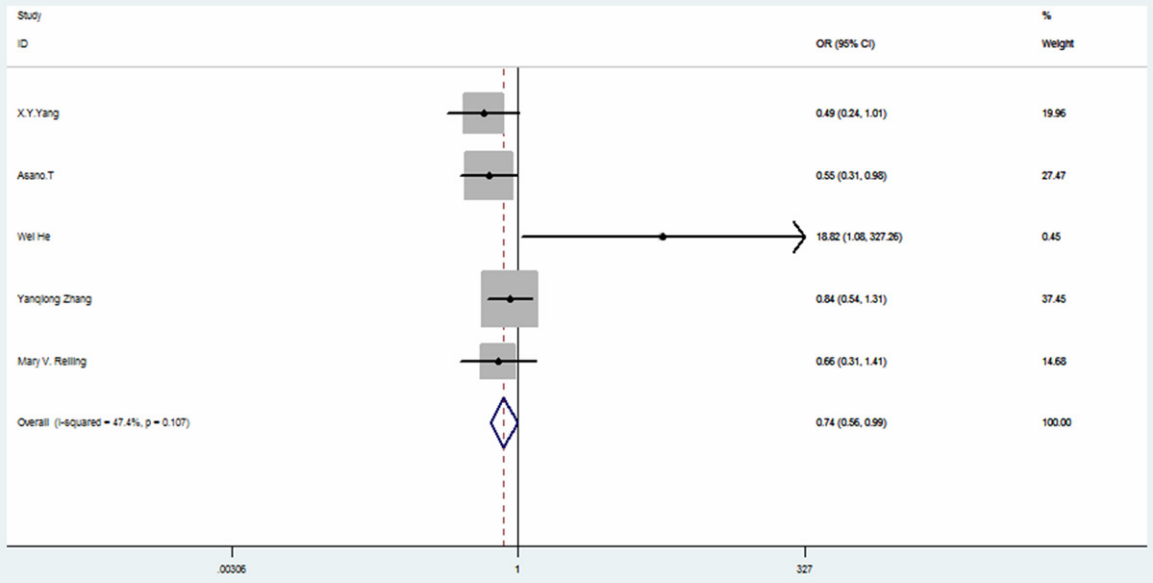

Figure 2. Forest plot for T/A vs. G of the association between *ABCB1* rs2032582 and ONFH risk.

the recessive model analysis. Through sensitivity analysis, we excluded one study (Yanqiong Zhang et al., 2014) because its significant impact on the pooled ORs and heterogeneity only in this model. Overall, rs1045642 was associated with a decreased ONFH risk in the

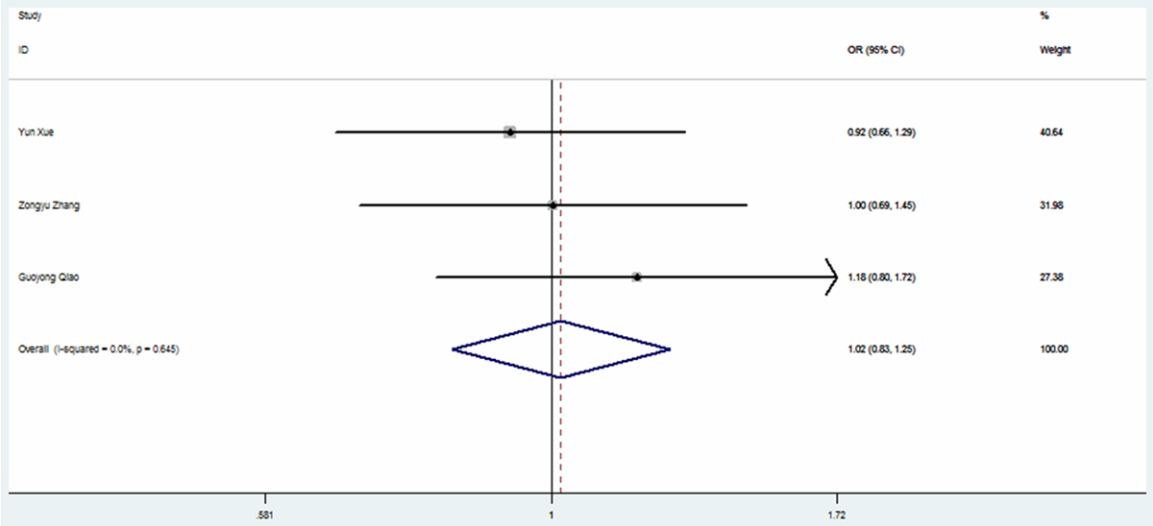

Figure 3. Forest plot for T vs. C of the association between *ABCB1* rs1128503 and ONFH risk.

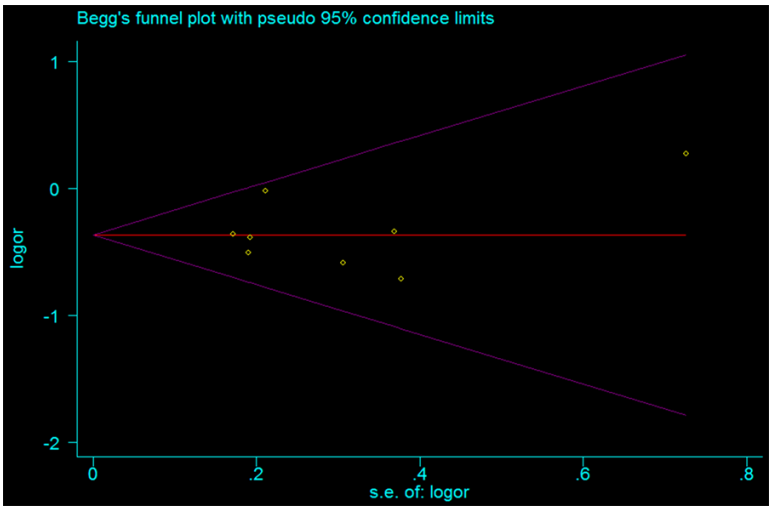

Figure 4. Begg's funnel plot for T vs. C to detect publication bias.

included population under the allelic model (OR=0.69, 95% CI: 0.59-0.82, *P*=0.000; **Figure 1**), homozygous model (OR=0.44, 95% CI: 0.31-0.64, *P*=0.000), heterozygous model (OR=0.72, 95% CI: 0.56-0.92, *P*=0.010), dominant model (OR=0.65, 95% CI: 0.51-0.82, *P*=0.000), and recessive model (OR=0.46, 95% CI: 0.32-0.67, *P*=0.000). In the stratified analysis, correlations remained significant under the allelic model (OR=0.73, 95% CI: 0.59-0.91, *P*=0.004), homozygous model (OR=0.50, 95% CI: 0.31-0.80, *P*=0.004), dominant model (OR=0.70, 95% CI: 0.52-0.94, *P*=0.018), and recessive model (OR=0.41, 95% CI: 0.23-0.72, *P*=0.002) in the GC-reduced ONFH subgroup.

Six articles reported the role of rs2032582 in ONFH risk. Significant heterogeneity among included studies was present in each of the genetic models. Thus, we carried out a sensitivity analysis to estimate the effects of each investigation on the pooled results and observed that the pooled ORs and heterogeneity were affected obviously by omitting one study (Yun Xue et al., 2014). Finally, we removed this study and synthesized the remaining five articles in which the cases were all GC-reduced ONFH. The pooled data showed that rs2032582 was significantly associated with a decreased GC-reduced ONFH risk under the allelic model (OR=0.74, 95% CI: 0.56-0.99, *P*=0.040; **Figure 2**), and homozygous model (OR=0.46, 95% CI: 0.25-0.83, *P*=0.010).

Three articles assessed the association of rs1128503 and ONFH risk. When the three studies were pooled into analysis, however, no significant association was observed between rs1128503 and ONFH susceptibility under the five genetic models analysis (**Table 2**; **Figure 3**).

Publication bias

The sharp of the Begg's funnel plot did not show any obvious asymmetry (**Figure 4**), and all

the *P* values of the Egger's linear regression test were greater than 0.05, which revealed that there was no statistical evidence of publication bias among included studies under the five genetic models analysis.

Discussion

Although non-traumatic ONFH has clearly been correlated with alcohol abuse and long-term use of high doses of GC, some populations with these conditions have a low incidence of this disease [19]. Thus, genetic factors are important in identifying susceptible individuals for early alternative to these risk conditions and primary prevention of ONFH. The present analysis indicated that the T allele of rs1045642 and the T/A allele of rs2032582 were associated with a decreased risk of ONFH. Furthermore, stratification analysis by ONFH type also suggested that the T allele of rs1045642 was a protective factor for GC-reduced ONFH.

The *ABCB1* rs2032582, presents in exon 21, was reported to be related to an amino acid substitution from Ala to Thr (rs2032582 G>A) and to Ser (rs2032582 G>T), respectively, leading to an enhanced efflux transporting activity of P-glycoprotein. The *ABCB1* rs1045642, locates in exon 26, does not alter the amino acid sequence, however, has a strong association with rs2032582 [20, 21]. Karssen et al. found that P-glycoprotein at the level of the blood-brain barrier could effectively transport GC and reduce the intake to brain [22]. Han et al. suggested that enhanced P-glycoprotein activity could decrease the risk of GC-reduced ONFH by inhibiting apoptosis and adipogenesis in bone marrow cells [23]. These evidences are in accordance with our current results that the *ABCB1* gene polymorphismsrs 2032582 and rs1045642 were protective factors for ONFH.

Several potential limitations in our present analysis need to be addressed. First, the included studies in the present analysis were moderate and the small sample size may not enough to show the definite conclusions. Second, there are significant associations between rs2032582 and rs1045642 and ONFH risk mainly in Asians, more studies are still required to confirm the relationship in non-Asians. Third, cases in the included studies had various basic diseases, such as systemic lupus erythematosus, organ transplantation, and neoplastic diseases, which were not analyzed due to lack of enough data to conducted a subgroup analysis.

In conclusion, our present meta-analysis indicated that *ABCB1* polymorphisms rs2032582 and rs1045642 are protective factors for ONFH. When stratifying by ONFH type, the rs1045642 was also correlated with decreased risk of GC-reduced ONFH. It is possible that these polymorphisms can provide evidences for others in this field to further confirm the correlations and become promising targets to predict ONFH risk. However, a well-designed case-control study considered the limitations above with a larger sample size is needed in the future.

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Disclosure of conflict of interest

None.

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