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Replication study of previous migraine genome-wide association study findings in a Spanish sample of migraine with aura

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Abstract

Background: Migraine is a common disabling condition that affects approximately 15% of the population. Several genome-wide association studies have attempted to identify susceptibility variants involved in migraine, reporting several candidate loci for the disorder.

Methods: In order to replicate findings from previous genome-wide association studies, a case–control association study was performed. Twelve single nucleotide polymorphisms were genotyped in a Spanish sample of 512 migraine with aura patients and 535 migraine-free controls.

Results: Nominal associations were found for single nucleotide polymorphisms rs2651899 (within the *PRDM16* gene), rs10166942 (near *TRPM8*), rs12134493 (close to *TSPAN2*) and rs10504861 (near *MMP16*) in our migraine with aura sample.

Conclusions: Our study provides suggestive replication, in a Spanish migraine with aura sample, of four genome-wide association study findings previously reported in common migraine. However, larger sample sets should be explored to confirm our results.

Keywords

Migraine with aura, association study, *PRDM16*, *TRPM8*, *TSPAN2*, *MMP16*

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Introduction

Migraine is a disabling complex neurological disorder that manifests with episodic and recurrent attacks, affecting around 15% of the population. It is more prevalent in women than men (three to one female:male ratio) (1). Genetic factors are thought to play an important role in common migraine, although environmental influence is also relevant (2).

Two clinical forms of the disorder are distinguished (International Classification of Headache Disorders, ICHD): migraine with aura (MA) and migraine without aura (MO) (3). Both are characterized by the presence of recurrent headaches associated with photophobia, phonophobia, nausea and aggravation by physical activity and are differentiated by the presence of aura. Aura symptoms, which often precede and accompany the headache episode, develop gradually and are most commonly visual but can be sensory,

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motor, or any combination of these, and can last from a few minutes to several days, although they typically abate in less than 60 minutes (4). MO is the most common migraine presentation, whereas MA is found in about one-third of migraine patients and is thought to have a stronger genetic load (4).

Association studies have been carried out to identify susceptibility variants underlying common migraine at two levels: analysis of candidate genes and genome-wide association studies (GWAS). Several GWAS have been performed so far, four of which reported significant results, disclosing a total of 20 associated variants (1,4–7).

The first risk variant identified, rs1835740, was revealed by a two-stage clinic-based study performed by the International Headache Genetics Consortium (IHGC) in individuals from Europe (5), the first stage consisting of a clinic-based MA sample. Although replication attempts have failed to find association in other populations (1,6,8), this variant is likely to be functional and is located close to the *MTDH* and *PGCP* genes, both related to glutamate homeostasis. Additionally, three new genome-wide significant loci were reported in a second GWAS, carried out by Chasman et al. (1) in a population-based study from the Women's Genome Health Study: *PRDM16*, *LRP1* and a variant located close to *TRPM8*, all of them identified when the discovery and the replication cohorts (the Dutch Genetic Epidemiology of Migraine study, the German Study of Health in Pomerania and the IHGC discovery stage cohort used in the first migraine GWAS) were combined.

In contrast, no genome-wide significant associations were found in a meta-analysis of population-based migraine GWAS performed by the Dutch-Icelandic Migraine Genetics Consortium and including six European cohorts (6).

More recently, the IHGC carried out a clinic-based GWAS focused on MO patients and reported 12 nominally significant signals in the discovery stage (p-value <10 e-5) (7), two of which, located in the *LRP1* gene and close to *TRPM8*, had already been reported in a previous GWAS (7).

Finally, a GWAS meta-analysis was performed on 29 clinic- and population-based studies including 23,285 migraine cases and 95,425 population-matched controls (4). Association with 12 variants within or close to the following genes were reported: *PRDM16*; *MEF2D*; *TRPM8*; *TGFBR2*; *PHACTR1*; *ASTN2*; *LRP1*; *FHL5*; *C7orf10*; *MMP16*; *TSPAN2*; *AJAP1*. The last five associations were reported in this study for the first time.

Here we performed a clinic-based case–control association study in 512 MA patients and 535 non-migraine individuals from Spain aiming at replicating significant associations identified in those previous GWAS that included MA patients (1,4), although they also included MO individuals. The other three GWAS were not considered here because one of them did not render positive findings (6), another one included only MO patients (7) and the third one (5) was already subjected to replication by us in a previous report (8).

Materials and methods

Patients and controls

The original sample consisted of 517 Caucasian Spanish patients (78.13% women) and 535 ethnically matched migraine-free controls (73.83% women), recruited at Hospital Universitari Vall d'Hebron (Barcelona, Spain) and Hospital Sant Joan de Déu (Manresa, Barcelona, Spain) between 2002 and 2010. All patients were unrelated and, to the best of our knowledge, unrelated to control individuals. They were diagnosed with MA according to the ICHD 2nd edition (3) and have not been included in any previous migraine GWAS. Detailed clinical characteristics of the sample are provided in Table 1. For a complementary genetic analysis, the clinical MA sample was divided in two subgroups, 'MA only' (patients developing aura in at least 2/3 of the migraine episodes, $n = 318$) and 'Both MA and MO' (patients with less than 2/3 of the crises with aura, $n = 180$), similar to one of the two previous reports that we aim to replicate (4) and also performed in the first migraine GWAS (5). This distinction was introduced to distinguish the group of patients with

Table 1. Clinical findings in 517 patients with migraine with aura included in this study.

Feature	% of MA patients[a]
Positive family history of migraine[b]	77.2%
Age of onset (years)	13.5 ($\pm$ 12)[c]
Main features	
Pulsating quality of pain	69.9%
Unilateral pain	73.4%
Moderate-severe pain intensity	94.3%
Aggravation by physical activity	93.9%
Other features	
Photofobia	92.3%
Phonofobia	87.1%
Nausea	85.9%
Vomiting	61.8%

MA: migraine with aura.
[a]The percentage of available answers per item ranged from 88.3 to 99.2%.
[b]In first degree relatives.
[c]Mean $\pm$ SD.

rare MA episodes, but still fulfilling ICHD criteria, from those suffering an aura in most of their attacks. The control sample consisted of unrelated blood donors who lacked any personal or family history of any type of severe recurrent headaches and were approximately sex-matched with the cases. The exclusion of the disease phenotype was based on a personal interview. Controls were collected from the same small geographical area in Barcelona (Spain) as cases, and both were Spanish and Caucasian, as were their parents and grandparents. The study was approved by the local Ethics Committees and informed consent was obtained from all the participants or their legal representatives according to the Helsinki Declaration.

DNA isolation, SNP selection and genotyping

DNA was extracted from peripheral blood lymphocytes by a standard salting-out procedure (9).

Given that our clinic-based sample included only MA patients, we chose for replication those SNPs that displayed a genome-wide significant association in previous GWA studies including MA samples (1,4) (Table 2). A total of 13 SNPs were selected for genotyping: rs2651899 (*PRDM16*); rs10915437 (between *PRDM16* and *AJAP1*); rs10166942 and rs7577262 (near *TRPM8*), rs11172113 (*LRP1*); rs12134493 (near *TSPAN2*); rs2274316 (*MEF2D*); rs6790925 (near *TGFBR2*); rs9349379 (*PHACTR1*); rs13208321 (near *FHL5*); rs4379368 (*C7orf10*); rs10504861 (near *MMP16*); rs6478241 (*ASTN2*). All of them had previously been found to be associated with migraine groups, with and without aura, except for SNP rs10504861, which was only found associated with the MO group but also included in our study (4).

Genotyping of all SNPs was performed using the Sequenom technology at the National Genotyping Center (Cegen, Santiago de Compostela, Spain). Three samples from the Centre d'Étude du Polymorphisme Humain (CEPH) were included in every plate and a 100% concordance with HapMap data was observed when available. One SNP, rs2274316 in *MEF2D*, failed and it was not considered in the association study. Five patients displayed less than 85% genotyping rate and were removed from the study, so the analysis was finally carried out on 512 patients.

Statistical analysis

The analysis of minimal statistical power for the x^2 test, performed under a dominant model using the Genetic Power Calculator software (pngu.mgh.harvard.edu/~purcell/gpc) with $\alpha = 0.05$, an odds ratio (OR) of 1.25, a prevalence of 0.15 for the disorder and the highest minor allele frequency (MAF) found in controls (0.455), rendered a value of 47% in the overall sample.

Statistical analysis for the association study was carried out using the SNPassoc R library (10). Hardy–Weinberg equilibrium (HWE) was tested in our control sample, and *P*-values were all over 0.05, except for rs10915437 ($P = 0.0470$), which was not further considered in our analysis. We compared allele and genotype frequencies using x^2 tests and considering co-dominant, dominant, recessive, overdominant and log-additive models. For the log-additive model the Mantel–Haenszel x^2 test was implemented. All the results were adjusted by sex using a likelihood ratio test. Corrections for multiple testing were applied using 10,000 permutations with PLINK (11) and Bonferroni's correction. For the latter, the threshold for significance was established as $0.0003 = 0.05/(11 \times 3 \times 5)$, with 11 SNP tests, three clinical groups and five genetic models.

Association between case–control status and the different genetic variants was also estimated using logistic regression and including sex as a predictive variable ($\text{logit} = \beta_o + \beta_1 * \text{SNP} + \beta_2 * \text{sex}$). Analyses assuming additive, dominant, recessive and co-dominant models for each SNP were performed separately and then compared using Akaike Information Criterion (AIC). All the analyses were performed with the R software (http://CRAN.R-project.org).

Results

We successfully genotyped 12 SNP markers selected from two previous GWA studies (1,4) in 512 MA patients and 535 gender and ethnically matched controls from Spain. One of them was not considered in our analysis as it was not in HWE. Our study included both allele and genotype comparisons (Table 3).

We achieved nominal associations for two out of three genome-wide significant findings from the study by Chasman et al. (1): rs2651899 in the *PRDM16* gene (allelic model, $P = 0.0056$) and rs10166942, located near *TRPM8* (recessive, $P = 0.0442$) (Table 3). However, none of the SNPs survived Bonferroni's correction for multiple testing (all *P*-values > 0.0003) or permutation corrections (all permuted *P*-values > 0.05, data not shown). The evaluation of the remaining nine SNPs, selected from the meta-analysis study performed by Anttila et al. (4), failed to replicate any of the associations in our MA sample.

We subsequently split the sample into the subclinical groups 'MA only' and 'Both MA and MO' to gain clinical homogeneity at the expense of reducing the size of the sample, following the subdivision reported in Anttila et al. (4). Only one of the previous association signals remained significant (rs2651899, 'MA only', allelic model, $P = 0.0034$) but novel nominal associations were obtained with 'Both MA and MO'

Table 2. Selected SNPs for replication of GWAS findings. Comparison of the direction of the effect for the nominally associated SNPs in our study.

					Reference study			Replication study		
Associated SNP	Selected study	Position[a]	Gene	Distance	Minor allele	OR	Associated migraine group	Minor allele	OR	Associated migraine group, model
rs12134493	Anttila et al. (4)	chr1:115677946	near *TSPAN2*	45, 825 bp downstream	A	1.14	All samples	A	1.52	MA + MO, dominant
rs2274316[b]	Anttila et al. (4)	chr1:156446242	*MEF2D*	Intragenic	C	1.07	All samples			
rs2651899	Chasman et al. (1)/ Anttila et al. (4)	chr1:3083712	*PRDM16*	Intragenic	C / C	1.07–1.25/1.09	Migraine/ All samples	C	1.27 / 1.34	All samples / MA only, allelic
rs10915437[c]	Anttila et al. (4)	chr1:4183006	near *AJAP1*	532,099 bp upstream	G	0.86	Clinics only (MA and MO)			
rs11172113	Chasman et al. (1)/ Anttila et al. (4)	chr12:57527283	*LRP1*	Intragenic	C / C	0.81–0.90/0.90	Migraine/ All samples[d]			
rs7577262	Anttila et al. (4)	chr2:234818869	near *TRPM8*	7,174 bp upstream	A	0.87	All samples[d,e]			
rs10166942	Chasman et al. (1)	chr2:234825093	near *TRPM8*	950 bp upstream	C	0.80–0.86	Migraine	C	0.48	All samples, recessive
rs6790925	Anttila et al. (4)	chr3:30480085	near *TGFBR2*	167,909 bp upstream	T	1.15	Clinics only (MA and MO)			
rs9349379	Anttila et al. (4)	chr6:12903957	*PHACTR1*	Intragenic	G	0.86	MO only[f]			
rs13208321	Anttila et al. (4)	chr6:96860354	near *FHL5*	150,070 bp	A	1.18	All samples[d]			
rs4379368	Anttila et al. (4)	chr7:40466200	*C7orf10*	Intragenic	T	1.11	All samples[d]			
rs10504861	Anttila et al. (4)	chr8:89547932	near *MMP16*	208,215 bp upstream	T	0.86	MO only	T	0.26	MA + MO, recessive
rs6478241	Anttila et al. (4)	chr9:119252629	*ASTN2*	Intragenic	A	1.16	Clinics only (MA and MO)[f]			

MA: migraine with aura; MO: migraine without aura; SNP: single nucleotide polymorphism.
[a]Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly.
[b]Genotyping failed in our study.
[c]Excluded from our study (controls not in Hardy–Weinberg equilibrium).
[d]Additional association with the 'MO only' group.
[e]Additional association with the 'clinics only (MA and MO)' group.
[f]Additional association with the 'All samples' group.
Minor allele frequencies (MAFs) and odds ratios (ORs) are expressed as a range for the study of Chasman (performed in different population groups).
Minor allele in (+) strand.

Table 3. Case–controls association study performed in our sample of 512 MA patients and 535 control individuals.

				Genotypes															Alleles	
				Co-dominant							Recessive		Dominant		Overdominant		Additive		Allele 2 vs. 1	
			MAF	Cases (%)			Controls (%)				**22 vs. 21 + 11**		**22 + 21 vs. 11**		**11 + 22 vs. 12**					
SNP	Position[a]	Gene	Ca-Co	11	12	22	11	12	22	*p*-value	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)
rs2651899	chr1:3083712	*PRDM16*	0.516–0.455	124 (24.22)	248 (48.44)	140 (27.34)	158 (29.53)	267 (49.91)	110 (20.56)	**0.0198**	**0.0113**	1.45 (1.09–1.93)	**0.0474**	1.32 (1.00–1.74)	0.6867	0.95 (0.75–1.21)	**0.0059**	1.27 (1.07–1.51)	**0.0056**	1.27 (1.07–1.51)
rs10166942	chr2:234825093	near *TRPM8*	0.183–0.181	336 (65.62)	165 (32.23)	11 (2.15)	364 (68.04)	148 (27.66)	23 (4.30)	0.0519	**0.0442**	0.48 (0.23–1.00)	0.4221	1.11 (0.86–1.44)	0.1105	1.24 (0.95–1.62)	0.9629	1.01 (0.80–1.26)	0.963	0.99 (0.8–1.24)
rs11172113	chr12:57527283	*LRP1*	0.336–0.347	235 (45.90)	210 (41.02)	67 (13.09)	227 (42.43)	245 (45.79)	63 (11.78)	0.3254	0.5437	1.12 (0.78–1.62)	0.2773	0.87 (0.68–1.12)	0.1353	0.83 (0.65–1.06)	0.6191	0.96 (0.80–1.14)	0.6132	1.05 (0.87–1.26)
rs12134493	chr1:115677946	near *TSPAN2*	0.127–0.110	389 (75.98)	116 (22.66)	7 (1.37)	422 (78.88)	108 (20.19)	5 (0.93)	0.4926	0.5073	1.47 (0.46–4.68)	0.2712	1.18 (0.88–1.57)	0.3424	1.15 (0.86–1.55)	0.2400	1.18 (0.90–1.54)	0.2459	0.85 (0.66–1.11)
rs7577262	chr2:234818869	*TRPM8*	0.098–0.097	415 (81.05)	94 (18.36)	3 (0.59)	438 (81.87)	90 (16.82)	7 (1.31)	0.4182	0.2301	0.45 (0.12–1.75)	0.7675	1.05 (0.77–1.43)	0.5461	1.10 (0.80–1.52)	1.0000	1.00 (0.75–1.34)	1.0000	1 (0.75–1.34)
rs6790925	chr3:30480085	near *TGFBR2*	0.380–0.371	205 (40.04)	225 (43.95)	82 (16.02)	209 (39.07)	255 (47.66)	71 (13.27)	0.3060	0.1753	1.27 (0.90–1.79)	0.8132	0.97 (0.76–1.24)	0.2337	0.86 (0.68–1.10)	0.5998	1.05 (0.88–1.25)	0.5962	1.05 (0.88–1.25)
rs9349379	chr6:12903957	*PHACTR1*	0.348–0.368	218 (42.58)	232 (45.31)	62 (12.11)	217 (40.56)	242 (45.23)	76 (14.21)	0.5579	0.3038	0.83 (0.58–1.19)	0.5154	0.92 (0.72–1.18)	0.9567	1.01 (0.79–1.28)	0.3281	0.92 (0.77–1.09)	0.3244	0.91 (0.76–1.09)
rs13208321	chr6:96860354	*FHL5*	0.267–0.244	274 (53.52)	203 (39.65)	35 (6.84)	307 (57.38)	195 (36.45)	33 (6.17)	0.4569	0.6222	1.13 (0.69–1.85)	0.2133	1.17 (0.91–1.49)	0.3056	1.14 (0.89–1.46)	0.2299	1.13 (0.93–1.37)	0.2301	0.89 (0.73–1.08)
rs4379368	chr7:40466200	*C7orf10*	0.096–0.081	419 (82.00)	86 (16.83)	6 (1.17)	451 (84.30)	81 (15.14)	3 (0.56)	0.3845	0.2633	2.16 (0.54–8.71)	0.3055	1.18 (0.86–1.64)	0.4433	1.14 (0.82–1.59)	0.2284	1.20 (0.89–1.63)	0.2263	1.21 (0.89–1.63)
rs10504861	chr8:89547932	near *MMP16*	0.192–0.192	333 (65.04)	161 (31.45)	18 (3.52)	352 (65.79)	161 (30.09)	22 (4.11)	0.8182	0.6189	0.85 (0.45–1.61)	0.8096	1.03 (0.80–1.33)	0.6493	1.06 (0.82–1.38)	0.9727	1.00 (0.81–1.25)	0.9723	1 (0.8–1.24)
rs6478241	chr9:119252629	*ASTN2*	0.423–0.434	161 (31.45)	269 (52.54)	82 (16.02)	172 (32.15)	262 (48.97)	101 (18.88)	0.4095	0.2362	0.82 (0.60–1.14)	0.8298	1.03 (0.79–1.34)	0.2715	1.15 (0.90–1.46)	0.6113	0.96 (0.80–1.14)	0.6177	1.05 (0.88–1.24)

MAF: minor allele frequency; Ca: cases; Co: controls; OR: odds ratio; CI: confidence interval.

[a]Genome Browser on Human Feb. 2009 (GRCh37/hg19) assembly.

In bold, nominal *P*-values ($P < 0.05$).

for SNPs rs12134493, located near *TSPAN2* (dominant model, $P = 0.0362$) and rs10504861, near *MMP16* (recessive model, $P = 0.0288$) (see Table 1 in online Supplementary Material).

We also tested association between the SNPs and the different phenotypes using logistic regression modelling, that rendered significant results for SNPs rs2651899 ('All MA', additive, $P = 0.0061$; 'MA only', additive, $P = 0.0038$) and rs12134493 ('Both MA and MO', additive, $P = 0.0417$), in all cases with the additive model as the most suitable one for predicting the disease status (AIC values of 1446.7, 1123.8 and 804.14, respectively). However, non-significant results were obtained for rs10166942 and rs10504861 (see Table 2 in online Supplementary Material).

In all cases the direction of the effect was consistent with the previous reports (Table 2). Specifically, for rs2651899 and rs12134493 the risk allele is the minor one both in the original report and in our replication study, C and A in the (+) DNA strand, respectively. In contrast, for rs10166942 and rs10504861, the associated risk alleles, T and C, are the most frequent ones in all studies.

Discussion

In summary, four out of 12 tested SNPs revealed a nominal association with MA in our sample, tentatively replicating previous GWAS findings: rs2651899 (*PRDM16*), rs12134493 (near *TSPAN2*), rs10166942 (near *TRPM8*) and rs10504861 (near *MMP16*). The first two associations are the most robust replication signals in our study, as they emerge in the x^2 testing under different genetic models and in the logistic regression analysis. In contrast, rs10166942 and rs10504861 show association only under the recessive model in the x^2 comparisons and not in the logistic regressions.

SNP rs2651899 was found to be associated with migraine in the two previous studies that we aimed to replicate (1,4) and more recently in a replication study carried out in a Chinese sample (12) and thus emerges as the most consistent hit across this set of GWAS studies. rs2651899 is located in the first intron of the *PRDM16* gene and its role in the pathophysiology of migraine remains still unclear. However, it has recently been associated with cardiac disease (non-syndromic left ventricular non-compaction cardiomyopathy and dilated cardiomyopathy) (13). Given the known comorbidity between cardiovascular disease and migraine, *PRDM16* may be considered as a candidate gene in future studies. The association with SNP rs10166942 has previously been replicated in a Danish population (2). This variant is located close to *TRPM8*, a gene that has been consistently associated with migraine in several studies, supporting a connection between migraine and neuropathic pain (1). Our study is the first to replicate the association of rs12134493 and rs10504861 with migraine, with nominal associations found in our 'Both MA and MO' subgroup. rs12134493 lies next to *TSPAN2*, a gene encoding a cell surface protein, which mediates signal transduction. rs10504861 is located near *MMP16*, encoding a matrix metalloproteinase (MT-MMP2/MMP16) responsible for the cleavage of LRP1, a protein known to increase axonal and synaptic plasticity in cortical neurons. A SNP in the *LRP1* gene, rs11172113, also showed association with migraine in a GWAS (1,4) and in a subsequent replication study (2), but displayed negative results in our study.

There are coincidences and discrepancies between our results and the associations reported previously, which may be explained by several methodological issues: (i) the sample size of our cohort (about 500 patients and 500 controls) is smaller than those of the previous GWAS. Thus, the study is underpowered and the likelihood of false negative findings is high. Although we found nominal findings, none of them survived multiple testing corrections; (ii) our patients' group consists of subjects clinically diagnosed with MA, while the previous studies that we are replicating include MA and also MO patients. Although MA and MO share clinical features, their genetic background may differ, at least in part, as shown by previous studies (4). To shed light on the shared and specific genetic risk factors for MA and MO, it would be interesting to systematically cross-test any migraine-associated SNP in the two clinical entities. Moreover, the fact that we are comparing clinic- and population-based studies may be another reason for the discordant results, as diagnosis of aura is usually more reliable in a clinical setting; (iii) in our sample, the proportion of pure MA differs from that reported in previous studies. Likewise to the MO vs. MA paradigm, it is conceivable that subphenotypes of MA (i.e. 'MA-only' vs. 'Both MA and MO') may conceal specific genetic backgrounds; (iv) the small number of positive replications in our study may also be related to the underlying architecture of MA vs. MO. It is conceivable that rare variation plays a more important role than common variation in MA, whereas frequent variants would be more relevant in MO. In favour of this hypothesis is that most association findings from GWA studies have been identified in MO or in 'all migraine' samples.

In conclusion, although our results do not attain statistical significance, the nominal findings obtained here suggest that previously reported loci may be involved in the genetic architecture of MA. However, additional replication studies in larger MO and MA sets are warranted to weigh up the relevance of these susceptibility loci to both common migraine phenotypes.

Article highlights

- Case–control association study of 512 migraine with aura patients and 535 migraine-free controls aimed at replicating previously reported migraine GWAS findings.
- Suggestive association with four out of 12 loci studied: one SNP in the *PRDM16* gene and three SNPs located close to *TRPM8*, *TSPAN2* and *MMP16*.
- These genes have been related to cardiac disease, neuropathic pain, signal transduction or axonal/synaptic plasticity.

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Conflict of interest

P.P.R. reports support for conference visits from MSD and Almirall, and consultancy for Allergan. A.M. received honorary fees for lectures and advisory boards from Novartis Europe.

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