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SHORT COMMUNICATION

Meta-analysis of the DRD5 VNTR in persistent ADHD

Marieke Klein[a], Stefanie Berger[a,b], Martine Hoogman[a], Janneke Dammers[a], Remco Makkinje[a], Angelien J.G.A. M. Heister[a], Tessel E. Galesloot[c], Lambertus A.L.M. Kiemeney[c], Heike Weber[b,d], Sarah Kittel-Schneider[b], Klaus-Peter Lesch[e], Andreas Reif[b], Marta Ribasés[f,g,h,i], Josep Antoni Ramos-Quiroga[f,g,h,i], Bru Cormand[j,k,l], Tetyana Zayats[m], Tor-Arne Hegvik[m,n], Kaya K. Jacobsen[m,n], Stefan Johansson[n,o], Jan Haavik[m,p], Nina R. Mota[q,r], Claiton H.D. Bau[q,r], Eugenio H. Grevet[r,s], Alysa Doyle[t,u], Stephen V. Faraone[o,v], Alejandro Arias-Vásquez[a,w,x], Barbara Franke[a,w,*]

[a]*Radboud university medical center, Donders Institute for Brain, Cognition and Behaviour, Department of Human Genetics, Nijmegen, The Netherlands*
[b]*Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital Frankfurt, Frankfurt am Main, Germany*
[c]*Radboud university medical center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands*
[d]*Department of Psychiatry, Psychosomatics and Psychotherapy, IZKF Würzburg, University Hospital of Würzburg, Würzburg, Germany*
[e]*ADHD Clinical Research Unit, Division of Molecular Psychiatry, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Würzburg, Germany*
[f]*Biomedical Network Research Center on Mental Health (CIBERSAM), Institute of Salud Carlos III, Spain*
[g]*Department of Psychiatry and Forensic Medicine, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain*
[h]*Department of Psychiatry, University Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain*
[i]*Psychiatric Genetics Unit, University Hospital, Vall d'Hebron, Barcelona, Catalonia, Spain*
[j]*Department of Genetics, Faculty of Biology, University of Barcelona, Barcelona, Catalonia, Spain*
[k]*Institute of Biomedicine, University of Barcelona (IBUB), Barcelona, Catalonia, Spain*
[l]*Instituto de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Spain*
[m]*K.G. Jebsen Centre for Neuropsychiatric Disorders, Department of Biomedicine, University of Bergen, Bergen, Norway*
[n]*Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway*
[o]*K.G. Jebsen Centre for Neuropsychiatric Disorders, Department of Clinical Science, University of Bergen, Bergen, Norway*

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[p]Department of Psychiatry, Haukeland University Hospital, Bergen, Norway
[q]Department of Genetics, Institute of Bioscience, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[r]ADHD Outpatient Program-Adult Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[s]Department of Psychiatry, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[t]Department of Psychiatry and Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[u]Stanley Center for Psychiatric Research at the Broad Institute, Cambridge, MA, USA
[v]Departments of Psychiatry and of Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY, USA
[w]Radboud university medical center, Donders Institute for Brain, Cognition and Behaviour, Department of Psychiatry, Nijmegen, The Netherlands
[x]Radboud university medical center, Donders Institute for Brain, Cognition and Behaviour, Department of Cognitive Neuroscience, Nijmegen, The Netherlands

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Abstract
Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder with a complex genetic background. *DRD5*, the gene encoding the dopamine receptor D5, was recently confirmed as a candidate gene for ADHD in children through meta-analysis. In this study, we aimed at studying the association of the ADHD-associated variable number tandem repeat (VNTR) polymorphism upstream of *DRD5* with adult ADHD. We compiled data from six sites of the International Multicentre persistent ADHD CollaboraTion (IMpACT) and reached $N=6979$ (3344 cases and 3635 healthy participants), the largest sample investigated so far. We tested the association of the common *DRD5* alleles with categorically defined ADHD and with inattentive and hyperactive/impulsive symptom counts. Our findings provide evidence that none of the common *DRD5* alleles are associated with ADHD risk or ADHD symptom counts in adults.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a multifactorial neurodevelopmental disorder with an onset before the age of 12 years. It is characterized by hyperactivity, restlessness, impulsivity, and/or inattention (American Psychiatric Association, 2013). In a considerable number of patients, the disorder persists into adulthood, with a worldwide prevalence of ADHD of 1–4% in adults (Simon et al., 2009). Although ADHD is highly heritable in both children and adults (Faraone et al., 2005), only a few genetic risk factors have consistently been associated with ADHD risk, and most variants investigated are related to genes in the dopaminergic and serotonergic neurotransmission systems (Gizer et al., 2009). The dopamine receptor D5 (*DRD5*) gene is one of these; a variable number of tandem repeat polymorphism (VNTR) with a highly polymorphic dinucleotide repeat located 18.5 kb upstream in the 5′ region of the gene produces multiple alleles, of which two have been associated with ADHD. The association was first described in 1999 (Daly et al., 1999); since then, several studies investigated the effect of the *DRD5* VNTR on ADHD. A previous meta-analysis, including 12 studies (approximately 2350 childhood cases and their parents), showed that the 148-bp allele is associated with increased ADHD risk, and that the 136-bp confers reduced ADHD risk ($n=4$ studies) (Wu et al., 2012). Mixed results for the role of the DRD5 VNTR in persistent ADHD have been reported previously (Johansson et al., 2008; Squassina et al., 2008; Langley et al., 2009; Carpentier et al., 2013). To clarify the association of the *DRD5* VNTR with ADHD in adulthood, we performed a meta-analysis of case-control cohorts from six sites (Brazil, Germany, The Netherlands, Norway, Spain, and USA; $n=6979$) of the International Multicentre persistent ADHD CollaboraTion (IMpACT, (Franke and Reif 2013)). We tested the association of the *DRD5* 148-bp and 136-bp alleles with categorically defined ADHD and also with inattentive and hyperactive/impulsive symptom counts, separately.

*Correspondence to: Radboud University Medical Center, Department of Human Genetics, (855), PO Box 9101, 6500 HB Nijmegen, The Netherlands.
E-mail address: Barbara.franke@radboudumc.nl (B. Franke).

Subsequently, we performed exploratory analyses investigating the effects of ten additional, frequent *DRD5* alleles.

2. Experimental procedures

2.1. Subjects

Within the IMpACT consortium, all patients were evaluated by experienced psychiatrists and diagnosed with persistent ADHD according to DSM-IV (Diagnostic and Statistical Manual for Mental Disorders) criteria. Ratings of ADHD symptom counts were retrieved from clinical interviews, except for the Dutch and Norwegian cohorts, where symptom counts were derived from self-report questionnaires (see (Franke et al., 2010) for detailed information on diagnostic assessments). Participants were invited to provide whole blood or saliva samples for genotyping. Studies were approved by ethics committees of the participating institutions, and written informed consent was obtained from all patients and controls prior to the study.

2.2. Genetic data

Genotyping of the *DRD5* (upstream VNTR) was performed in all six above-mentioned IMpACT cohorts by Fragment Length Analysis. The PCR reaction was performed on 30 ng genomic DNA using 0.33 μM 5′-fluorescently labeled (FAM, VIC, PET, or NED) forward primer (5′-GCTCATGAGAAGAATGGAGTG-3′) and reverse primer with PIG tail (5′-CGTGTATGATCCCTG-CAG-3′) and 1 × AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands). The cycling conditions for the polymerase chain reaction initiated with 10 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at the optimized annealing temperature (58 °C), and 1 min at 72 °C, then followed by a final step of 7 min at 72 °C. The product of the amplification was diluted 1:30 in H_2O. 1 μl of this dilution, together with 9.7 μl formamide and 0.3 μl Genescan-600 LIZ Size Standard (Applied Biosystems) was analyzed on an automated capillary sequencer (ABI3730 genetic analyzer, Applied Biosystems) according to the protocol of the manufacturer. To determine the length of the alleles, the results were analyzed with Genemapper software version 4.0 (Applied Biosystems). Output of the genotyping was the actual length in base pairs per allele, which was re-coded into three categories, i.e. homozygous of the reference allele, heterozygous, or homozygous for the non-reference allele.

The frequency distribution of the *DRD5* alleles (with a frequency >1%) across the different cohorts is shown in Figure 1. A total of 3344 adult ADHD patients (44.4% female, average age=34.9 years [range 18–75]) and 3635 healthy individuals (51.7% female, average age=39.1 years [range 18–92]) were available for the study. In all cohorts, allele frequencies were in Hardy–Weinberg equilibrium (HWE, $P>0.05$); the smallest cohort (USA, $n=147$) showed non-significant deviation from HWE ($P=0.021$, 12 *DRD5* alleles tested), which might be due to sampling variance in the underpowered cohort.

2.3. Statistical analyses

Output of the genotyping was the actual length in base pairs per allele, which was re-coded into three categories, i.e. homozygous of the reference allele, heterozygous, or homozygous for the non-reference allele. To prevent biases due to ethnic discrepancies, calculations were performed in each sample separately; subsequently, samples were subjected to meta-analysis (see below). A trend test was used to evaluate the ADHD risk conferred by carrying one of the *DRD5* allelic variants using logistic regression (additive model) for each individual IMpACT cohort. Analyses of symptom counts were performed separately for inattentive and hyperactive/impulsive symptoms in cases only. For this analysis, symptom count distributions were normalized and standardized using the Blom transformation. Impact of genotypes on inattentive and hyperactive/impulsive symptom scores was determined with linear regression using genotype dosages (additive model) as independent and the trait of interest as dependent variable. Age and gender were included as covariates in all analyses. Twelve alleles of the *DRD5* VNTR were selected for analysis (Figure 1) and all data analyses were performed separately for each *DRD5* allele using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.).

All six cohorts were used to run a fixed effects meta-analysis model using the “rma” command implemented in

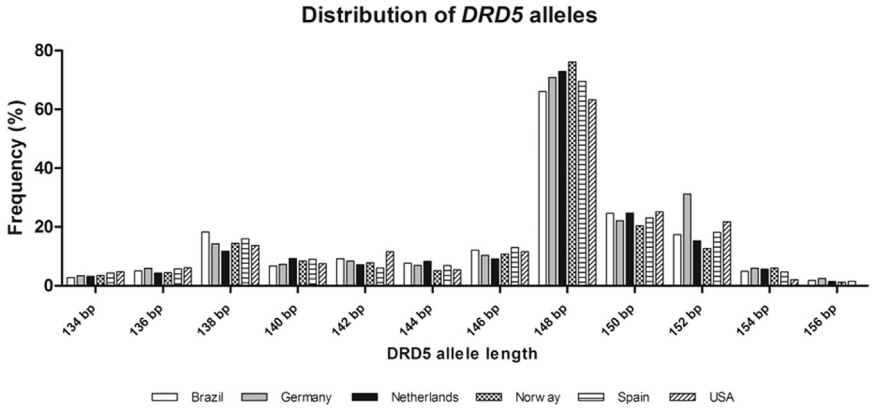

Figure 1 Distribution of *DRD5* VNTR alleles across all IMpACT cohorts included in the study. Only alleles with a minor allele frequency (MAF) >1% are shown.

the R package "metafor" v1.9-4 (Viechtbauer, 2010). As a measure for effect size either the odds ratios (ORs) or betas were calculated. Q-statistic and I^2-metric were used to test and discard heterogeneity. When no heterogeneity was present, the pooled OR/beta was estimated using fixed effects model. The results of the association tests are indicated as pooled ORs/betas with the corresponding 95% confidence intervals (CIs) of the allele-induced risk of persistent ADHD or ADHD symptom counts.

2.4. Power analysis

Power analysis using the Genetic Power Calculator (GPC) (Purcell et al., 2003) and previously reported effect estimates showed that we had 64.1% power to detect effect sizes similar to the ones reported previously (OR $\geq$ 1.26 for the 148-bp allele), 80% power to detect OR > 1.4, and 100% power for effects of OR $\leq$ 0.58 (for the 136-bp allele) (Wu et al., 2012). Also, our study had 90.1% power to detect associations explaining $\geq$ 0.5% of variance in ADHD symptom counts.

3. Results

In total, 12 alleles of the *DRD5* VNTR were selected for analysis (Figure 1). Neither the 148-bp childhood ADHD risk allele nor the 136-bp childhood ADHD protective allele were associated with ADHD risk in adults (OR=0.97, 95% CI 0.90-1.04, P=0.425 and OR=0.92, 95% CI 0.73-1.15, P=0.464, respectively; Figure 2A). The same was found in the analysis of the separate ADHD symptom domains ($N \geq 2415$; $P > 0.05$, Figure 2B and C). An exploratory analysis investigating the ten additional *DRD5* alleles did not reveal any significant association with ADHD risk or ADHD symptom counts either (best p-value was P=0.07 for the 146-bp allele and ADHD risk). Overall results did not change using a random-effects model.

4. Discussion

While evidence for an association of *DRD5* VNTR alleles with ADHD in children had been strengthened by meta-analysis (Wu et al., 2012), we did not find similar effects in the current meta-analysis of persistent ADHD in adults. These differences are consistent with twin data that suggest the risk alleles contributing to ADHD symptoms partly differ by age (Chang et al., 2013; Pingault et al., 2015). Differential association in childhood and adulthood has also been reported for the dopamine transporter gene (*DAT1/SLC6A3*) (Franke et al., 2010). The potential age-dependent effect of *DRD5* on ADHD could be further investigated in longitudinal study designs. However, we cannot entirely exclude the chance of a false negative finding for the 148-bp allele, for which we had limited power of 64.1%. Notably, earlier meta-analytic studies included fewer participants than ours (e.g. the previous meta-analysis included around 2350 children with ADHD for analysis of the 148-bp allele, and less than 1000 patients for analysis of the 136-bp allele (Wu et al., 2012)), which could explain the discrepancy between findings. Only three earlier studies investigating the association between the *DRD5* VNTR and persistent ADHD have been published (Johansson et al., 2008; Squassina et al., 2008; Carpentier et al., 2013), and one additional study used a childhood cohort that was followed-up for five years (Langley et al., 2009). The results were inconsistent across studies, with only two studies reporting some evidence of positive association (Johansson et al., 2008; Langley et al., 2009); one of those two was a subsample of the Norwegian sample included in the current study, and the originally reported association was no longer present in this larger sample. In conclusion, based on the results of the current meta-analysis, it seems unlikely that the *DRD5* VNTR contributes substantially, if at all, to ADHD persistence into adulthood.

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None of the funding sources had further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

MK, AAV, and BF conceived and designed the study. MH, JD, RM, AJGAMH, TEG, HW, SKS, MR, TZ, TAH, KKJ, NRM, EHG, and AD contributed data. MK and SB conducted statistical analyses and wrote the first draft of the manuscript. BF, LALMK, KPL, AR, JAR, BC, SJ, JH, CHDB, and SVF provided funding for the project. All co-authors provided critical feedback on the manuscript, suggested additional analyses and critical revisions, and edited the manuscript for clarity and precision. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Ramos-Quiroga has served on the speakers' bureau and acted as consultant for Eli Lilly and Co., Janssen-Cilag, Novartis, Lundbeck, Shire, Ferrer, and Laboratorios Rubió. He has received travel awards from Eli Lilly and Co., Janssen-Cilag, and Shire for participating in psychiatric meetings. The ADHD Program chaired by Dr. Ramos-Quiroga has received unrestricted educational and research support from Eli Lilly and Co., Janssen-Cilag, Shire, Rovi, and Laboratorios Rubió in the past two years.

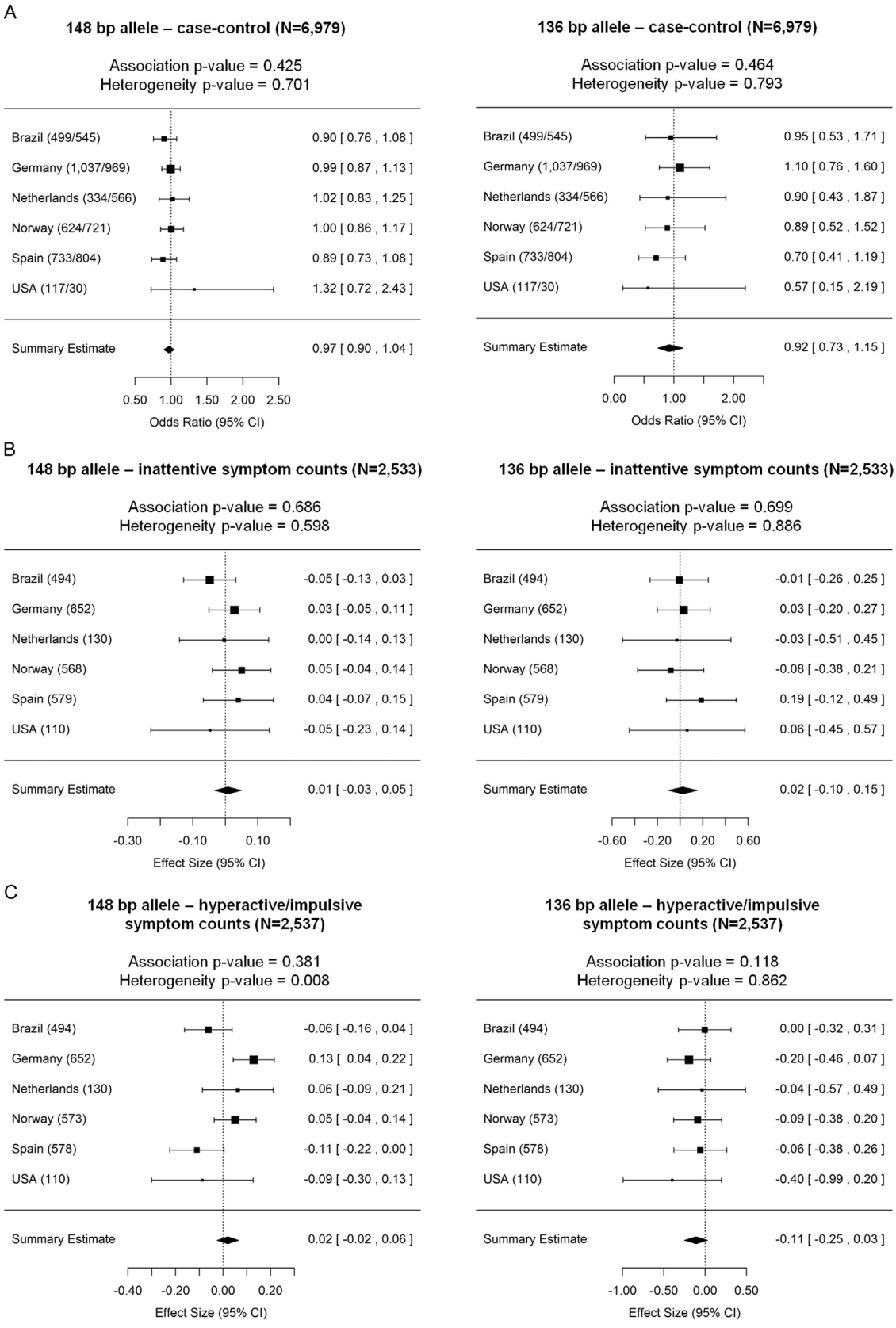

Figure 2 (A) Forest plots showing the association analysis of the *DRD5* 148-bp and 136-bp alleles with ADHD risk. (B) Forest plots showing the association analysis of the *DRD5* 148-bp and 136-bp alleles with ADHD inattentive symptom counts in patients with adult ADHD. (C) Forest plots showing the association analysis of the *DRD5* 148-bp and 136-bp alleles with ADHD hyperactive/impulsive symptom counts in patients with adult ADHD. For each, cohort the number of cases and controls is presented in brackets.

Dr. Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Dr. SV Faraone has received research support from, served as consultant or adviser to or participated in CME programs sponsored by Alcobra, Janssen, Eli Lilly, NIH, Novartis, McNeil, Pfizer and Shire; he receives royalties from Guilford Press and Oxford University Press. In the past year, Dr. SV Faraone received income, potential income, travel expenses and/or research support from Pfizer, Ironshore, Shire, Akili Interactive Labs, CogCubed, Alcobra, VAYA Pharma, Neurovance, Impax and NeuroLifeSciences. With his institution, he has US patent US20130217707 A1 for the use of sodium-hydrogen exchange inhibitors in the treatment of ADHD. All other authors declare that they have no conflicts of interest.

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