RESEARCH ARTICLE

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Evaluation of Clinically Overlapping Prader-Willi and Prader-Willi-Like Syndromes Using MS-MLPA and aCGH

Klinik Olarak Örtüşen Prader-Willi ve Prader-Willi Benzeri Sendromları'nın MS-MLPA ve aCGH ile Değerlendirilmesi

ABSTRACT

Objective

Prader-Willi Syndrome (PWS) is a multisystemic disorder associated with paternal deletions, maternal uniparental disomy (matUPD), and mutations in the 15q11-q13 region. Prader-Willi-Like Syndrome (PWLS) cases, although lacking alterations in this region, display similar clinical features. Genetic variations in chromosomes 1, 2, 6, 10, 12, 14 and X have been suggested to contribute to PWLS, complicating diagnosis. This study aimed to analyze methylation profiles and identify possible genetic variations in all chromosomes to differentiate PWS from PWLS.

Material and Methods

Eleven cases with PWS phenotypes normal cytogenetic karyotypes, and no 15q11.2-q13 deletions were included. Methylation-Specific-Multiplex-Ligation-Dependent-Probe-Amplification (MS-MLPA) was used for methylation analysis. In cases with normal methylation, Array Comparative Genomic Hybridization (aCGH) was used to evaluate entire regions of the genome for copy number variants.

Results

An abnormal methylation pattern was detected in one case. The remaining nine cases showed no changes in the 15q11.2-q13 region and were classified as PWLS. Based on aCGH analysis of these patients, we found some alterations that might be important in PWLS pathogenesis.

Conclusions

PWS was identified in only one case, while the others were classified as PWLS. Some alterations that can be involved in pathogenesis of PWLS were detected by aCGH in the PWLS group but to make a clinically significant interpretation and to better understand the etiology of PWLS, all PWLS patients' parents have to be examined by aCGH too.

Key Words

PWS, PWLS, MS-MLPA, aCGH

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ÖZ

Amaç

Prader-Willi Sendromu (PWS), 15q11-q13 bölgesindeki paternal delesyonlar, maternal uniparental disomi (matUPD) ve gen mutasyonları ile ilişkili multisistemik bir hastalıktır. Prader-Willi Benzeri Sendrom (PWLS) olarak sınıflandırılan bazı olgular, 15q11-q13 bölgesinde değişiklik olmamasına rağmen PWS'ye benzer klinik özellikler gösterir. 1, 2, 6, 10, 12, 14 ve X kromozomlarındaki genetik değişikliklerin PWLS ile ilişkili olduğu öne sürülmekte olup, bu durum tanıyı zorlaştırmaktadır. Bu çalışmada PWS ile PWLS'nin ayrımını gösterebilmek için kriterlere uygun on bir olgunun metilasyon profillerinin belirlenmesi ve tüm kromozomlardaki olası değişimlerin tespiti amaçlanmıştır.

Gereç ve Yöntemler

PWS fenotipine ve sitogenetik olarak normal karyotipe sahip, 15q11.2-q13 delesyonu bulunmayan on bir olgu çalışmaya dahil edilmiştir. Metilasyon-Spesifik-Multipleks-Ligasyon-Prob-Amplifikasyonu (MS-MLPA) ile bu bölgenin metilasyon analizi yapılmıştır. Normal metilasyon gösteren olgularda Array-Karşılaştırmalı-Genomik-Hibridizasyon (aCGH) ile genomdaki tüm bölgelerin kopya sayısı değişiklikleri incelenmiştir.

Bulgular

On olgunun yalnız birinde anormal metilasyon paterni tespit edilmiştir. Dokuz olguda ise 15q11.2-q13 bölgesinde değişim gözlenmediğinden, bu olgular PWLS olarak değerlendirilmiştir. aCGH analizine dayanarak, PWLS (Prader Willi-benzeri sendrom) patogenezinde önemli olabilecek bazı değişiklikler tespit ettik.

Sonuç

Yalnızca bir olguda PWS tespit edilmiş, diğer olgular ise PWLS kapsamında değerlendirilmiştir. PWLS grubunda aCGH ile, sendromun patogenezinde yer alabilecek bazı değişiklikler tespit edilmiştir. Ancak, klinik olarak anlamlı bir yorum yapılabilmesi ve PWLS etyolojisinin daha iyi anlaşılabilmesi için, tüm PWLS hastalarının ebeveynlerinin de aCGH ile incelenmesi gerekmektedir.

Anahtar Sözcükler

PWS, PWLS, MS-MLPA, aCGH

INTRODUCTION

Prader-Willi Syndrome (PWS, OMIM #176270) is a multisystemic disorder characterized by phenotypic abnormalities, cognitive impairment, and neurometabolic changes that affect the metabolic, endocrine and neurological systems (1-3). The primary features of this rare, complex disease include severe infantile hypotonia, poor sucking, hypogonadism, hyperphagia, early-onset obesity in childhood, small hands and feet, developmental delay, behavioral problems (such as skin picking, stubbornness) and typical facial features (e.g., small upturned nose, almond-shaped eyes) (4-6). Short stature in PWS is often caused by growth hormone deficiency (GHD). Without strict management of excessive behaviors, many patients face a significant risk of developing life-threatening obesity, which is frequently associated with type 2 diabetes mellitus (T2DM) (7). Therefore, obesity control is the primary goal in the early diagnosis and management of PWS (8).

The prevalence of PWS is estimated to be 1 in 20,000 to 30,000 individuals (9). Worldwide, PWS affects approximately 400,000 individuals, with no significant racial or ethnic differences. However, some studies have reported a higher prevalence among Caucasians (3, 4, 10, 11).

PWS, a genomic imprinting disorder, arises from specific genomic and epigenetic expression patterns of alleles inherited from the parents. PWS-related genes (MKRN3, MAGEL2, NDN, NPAP1, SNURF-SNRPN, and non-coding RNAs) are located in the q11-q13 region of chromosome 15, known as the Prader-Willi Critical Region (PWCR). Among these genes, the small ribonucleolar protein (SNRPN) plays a central role in the development of PWS. In healthy individuals, the maternal alleles of the PWCR are imprinted (methylated), while the paternal alleles are unimprinted (unmethylated). This means that only the paternally inherited alleles are expressed, with no alternative alleles to compensate for any errors in the paternal imprinting mechanism (12-15). There are three main mechanisms that cause PWS: paternal deletions, maternal uniparental disomy (mat UPD), and imprinting center defects, such as microdeletions or epimutations on chromosome 15 (3). In most PWS patients (approximately 70%), paternal deletions result in genomic imprinting errors, while maternal UPD accounts for 25% of patients, and imprinting center defects account for the remaining 2.5%. Balanced translocations, on the other hand, are considered to be rare compared to the other mechanisms and are observed in less than 1% of patients (16-18). A thorough review of the literature shows that the clinical findings of classic PWS are caused not only by genetic alterations observed in the 15q11-13 region but also by alterations in specific regions of other chromosomes that can be related to PWLS including 1,2,6,10,12,14 and X (19-24). Patients exhibiting phenotypic features of PWS without mutations in the 15q11-q13 region are classified as Prader Willi-Like Syndrome (PWLS) (21). In the literature, deletion of 6q16 has been identified as the most common chromosomal abnormality observed in PWLS (20, 25). The most frequent

clinical features of PWLS are obesity (84%), hyperphagia (72.7%), cognitive disability (54.5%), psychomotor delay (50%) and hypotonia (43.18%) (19). These overlapping clinical features make accurate diagnosis and appropriate treatment challenging, particularly in the context of genetic counseling. As a result, clinicians have difficulty in using clinical presentation alone to guide optimal diagnostic and treatment strategies, highlighting the importance of distinguishing PWS from PWLS. This study aims to identify the methylation profiles of PWS patients and to investigate the potential alterations in all chromosome regions to help differentiate PWS from PWLS.

MATERIALS and METHODS

Patient Selection and Ethical Regulations

This research complies with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee of the Faculty of Medicine, Akdeniz University (approval number: 2020/426 and 2017/320). All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration. This work is based on the author's master's thesis titled 'Investigation of Methylation and Uniparental Disomy (UPD) Profiles in Individuals with Normal Karyotypes Showing Prader-Willi Syndrome Features' and is supported by Akdeniz University Scientific Research Projects Coordination Department (Project IDs: TSA-2022-5664 and TYL-2017-2815). Eleven patients under the age of 18 who visited the Pediatric Genetics Department of Akdeniz University Medical Faculty and exhibited the phenotypic features of PWS (hypotonia, hyperphagia, obesity, hypogonadism, almond-shaped eyes, short hands and feet, poor sucking, developmental delay) were included in this study. All patients had a normal karyotype and were determined not to have a deletion in the 15q11-q13 region using Fluorescence In Situ Hybridization (FISH) method. One participant voluntarily withdrew from the study, leaving ten patients for further analysis.

DNA Isolation and Sample Preparation

Genomic DNA (gDNA) isolation was performed using the Invitrogen PureLink TM Genomic DNA Mini Kit (K1820-01). The quality and quantity of all gDNA samples were measured using 2 µL of each sample on a Nanodrop 1000 spectrophotometer (V.3.7). The DNA samples were then diluted to a concentration of 10 ng/µL for MS-MLPA analysis, using the SALSA MLPA ME028 Prader-Willi / Angelman Probemix (C1-0118) kit, according to the manufacturer's instructions. The results were analyzed with the CRM Coffalyser (V4)(MRC Holland).

Array Comparative Genomic Hybridization (aCGH)

aCGH was applied to PWLS group patients' gDNAs obtained from their peripheral blood at an external center using ISCAN device and InfiniumTM Global Screening Array-24v3.0 BeadChip-Illumina kit. This method was used to detect potential alterations in all regions including copy number variations and deletions, even at the single-base level, which could explain clinical features in patients with a normal methylation profile.

RESULT

This study includes eleven patients under the age of 18 years who met the diagnostic criteria for PWS. Among them, one patient exhibited an abnormal methylation pattern based on MS-MLPA results. This patient, a 14-month-old boy, demonstrated clinical features including hypotonia, hyperphagia related obesity, cognitive impairment, almond-shaped eyes, small hands and feet, hypogonadism, anger related stubbornness and strabismus with myopia. There is no consanguineous marriage between the parents of this patient. The phenotypic characteristics of the patient with the abnormal methylation profile are illustrated in Figure 1.

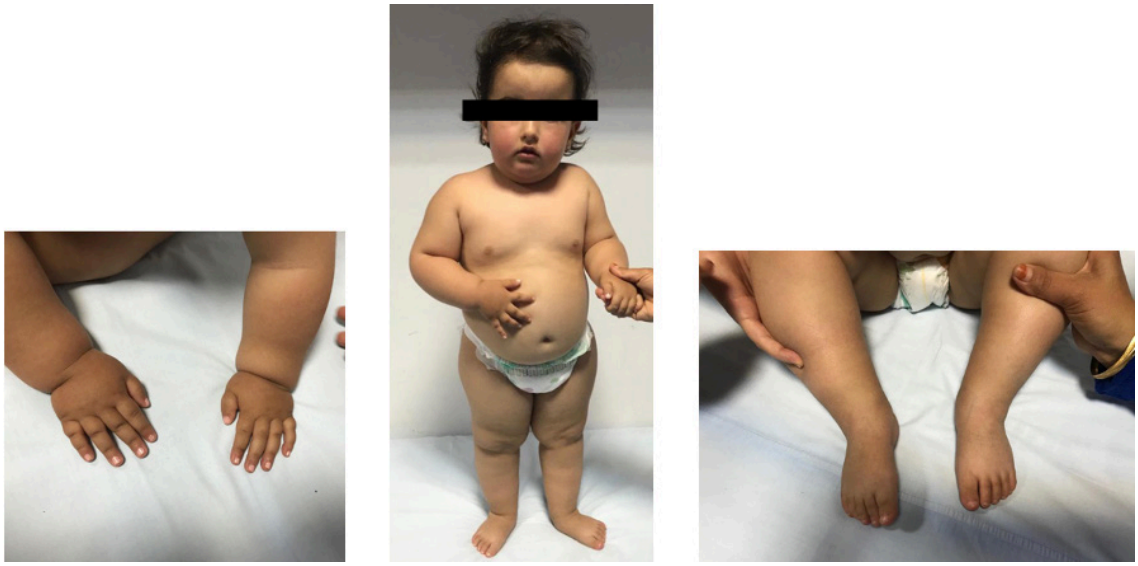

Figure 1. The clinical examinations of the patient. Small hands and feet (left and right picture), hypotonia and hyperphagia related obesity, almond shaped (middle picture) are clinical features of patient related with PWS.

The Coffalyser.net Ratio Chart outputs of MS-MLPA analysis showing copy number changes and abnormal methylation profiles of the patient are shown in Figure 2.

When the readings of the patient were analyzed according to the SALSA MLPA Probemix ME028-C1 PWS/AS (C1-0118) instructions, no copy number alterations were detected with a ratio of 1 observed for all regions tested. However, after digestion of the DNA to obtain methylation profile, PWS-related regions exhibited a ratio of 1, indicating an abnormal methylation profile. This abnormal methylation profile can be due to either UPD or an imprinting center (IC) mutation, which both can cause PWS (26).

The clinical phenotypes suggestive of PWLS in the nine patients with normal methylation pattern are hypotonia, feeding issues, neurodevelopmental and behavioral problems, sexual developmental dysfunction. Since copy number variations may be associated with PWLS, we performed an aCGH to analyze the possible genetic alterations in all chromosomes to differentiate PWS from PWLS. Alterations observed in all chromosomes by aCGH were listed in Table I. All data were analyzed using the Franklin by Genoox (https://franklin.genoox.com/clinical-db/home, accessed December 5th, 2024), ClinVar and DECIPHER databases (27, 28). Most of the alterations observed in PWLS patients were located on chromosome X. A copy number gain in the Xp22.31 region containing Anosmin 1 (ANOS1) gene, was observed in 6 of the 9 patients examined, giving a frequency of 66.6%. This frequency is two times higher than the mixed-patient pool that we studied in our clinical laboratory showing 31.52%. Apart from these, in one patient, a copy number loss for the 4q12 region spanning 410.1 kb containing the Sarcoglycan beta (SGCB) gene, was detected, which can be pathogenic when homozygous, according to Franklin by Genoox database.

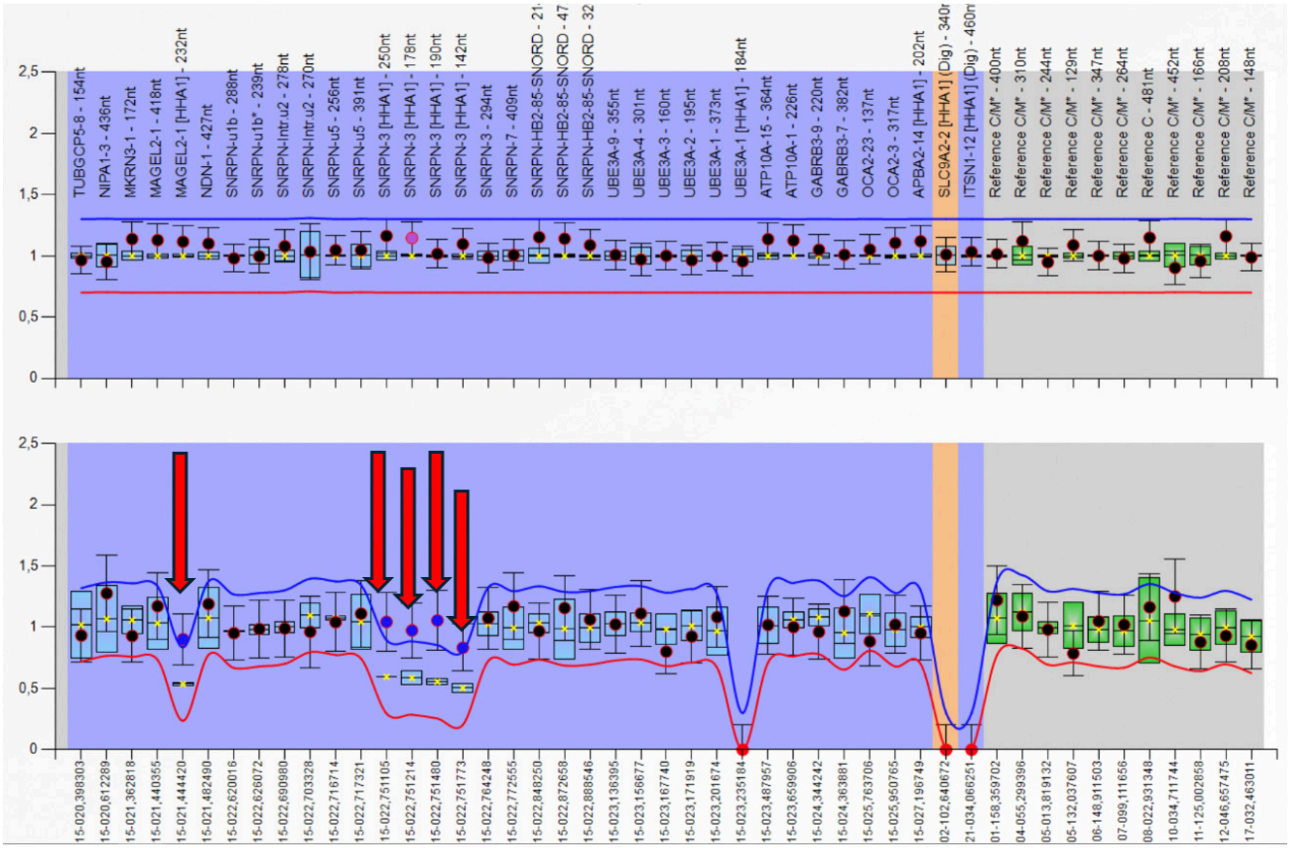

Figure 2. The Coffalyser.net Ratio Chart outputs showing the copy number and methylation ratio of regions with an abnormal methylation pattern in patient gDNA. The top chart displays the copy number ratio of undigested DNA, while the bottom chart shows the copy number variation and methylation profile changes of digested DNA. Red arrows indicate abnormal methylation profile.

Table I. aCGH analysis of 9 patients without abnormal methylation pattern.

Genes	Patient sex	Frequency in our patients	Chromosomal region	Event	Cytoband	Classification (Franklin by Genoox)
ANOS1	M	6/9 (66.6%)	chrX:8.533.915-8.540.253	CN Gain	Xp22.31	Uncertain (VUS)
PCDH19	M	3/9 (33.3%)	chrX:100.397.489-100.408.228	CN Gain	Xq22.1	Uncertain (VUS)
MECP2	M	2/9 (22.2%)	chrX:154.026.729-154.031.467	CN Gain	Xq28	Uncertain (VUS)
ABCD1	M	1/9 (11.1%)	chrX:153.740.618-153.743.575	CN Gain	Xq28	Uncertain (VUS)
LRRC66, SGCB, LINC02480, SPATA18	M	1/9 (11.1%)	chr4:51.963.334-52.373.437	CN Loss	4q12	PATHOGENIC when homozygous

DISCUSSION

Prader-Willi Syndrome (PWS) is a complex genetic imprinting disorder characterized by neonatal hypotonia, feeding problems, developmental delay, and later-onset hyperphagia, which can lead to severe obesity, short stature, and other characteristic features. In healthy individuals, the maternally inherited allele in the 15q11-q13 region is imprinted (i.e. methylated), while only the paternally inherited allele remains unmethylated. When the expression of the paternal allele is disrupted, no other active region compensates for the loss (29).

The three main mechanisms causing PWS are paternal deletion, maternal UPD, and IC defects, with balanced translocations being rare (12). There are different diagnostic methods to detect these mechanisms. Since the paternal deletion is the most common cause, clinicians often use FISH as the first test following cytogenetic analysis. However, FISH can only detect deletions and is unable to identify other mechanisms (12, 17). Therefore, more comprehensive diagnostic tests are needed.

Recently, MS-MLPA, which detects both copy number variations and abnormal methylation profiles has emerged as a more advanced and efficient method for PWS diagnosis (30). In this study, we used the MS-MLPA method to screen eleven patients for PWS and found an abnormal methylation profile in only one patient. One of the eleven patients voluntarily withdrew from the study. In the remaining nine patients, no deletions or abnormal methylation patterns were observed, although the clinical findings overlapped with both major and minor features of PWS In the literature, deletion of 6q16 has been reported in some patients with PWLS and is the most common chromosomal abnormality observed in PWLS (19, 25, 31-33). Other chromosomal abnormalities associated with regions on chromosomes 1p, 2p, 3p, 6q, and 9q, maternal uniparental disomy (mat UPD)14 and fragile X syndrome have also been reported. (19-24, 34). Therefore, in this study, we performed aCGH analysis in nine patients, excluding the patient with abnormal methylation profile, to determine copy number alterations throughout the genome. aCGH is a comprehensive method for identifying chromosomal alterations and copy number variations throughout the genome, but cannot detect point mutations or balanced chromosomal abnormalities, which is considered to be the limitation of the method (35). We found four different copy number gains classified as a Variant of Uncertain Significance (VUS) according to the Franklin Genoox database, and one copy number loss classified as pathogenic by the same database. Six out of nine patients exhibited a gain of Xp22.31 region, which contains the ANOS1 gene, resulting in a frequency of 66.6% for these alterations. The deletion of the ANOS1 gene is responsible for X-linked form of Kallmann Syndrome (KS1), which is characterized by hypogonadotropic hypogonadism (36). According to the Franklin by Genoox and American College of Medical Genetics and Genomics (ACMG) Classification, this gain variation is classified as a VUS. To our knowledge, ANOS1 copy number gains have not been associated with a disease, and gains in the Xp22.31 region are not considered significant as they occur in more than 1% of the general population. In order to better understand whether ANOS1 gain, which was observed in six of the nine patients in our study, increases the risk of PWLS, the parents of the patients should also be screened.

In one of the nine patients, we observed a loss of the 4q12 region, spanning 410.1 kb which consists of four genes: LRRC66, SGCB, LINC02480, SPATA18. Loss of SPATA18 and SGCB genes are linked to autosomal recessive Limb Girdle Muscular Dystrophies (LGMD) and is characterized by progressive muscle wasting and weakness (37). The homozygous loss of this region is classified as pathogenic according to Franklin by Genoox and ACMG Classification. Of note, if there are point mutations in this gene, we can miss it due to the inability of aCGH to identify point mutations. In order to make a meaningful interpretation of all the variants that we mentioned above, the parents of PWLS patients have to be examined by aCGH. Therefore, in patients such as our patient, where aCGH indicates a loss of a chromosomal region but does not explain the full spectrum of clinical features, so additional screening such as whole genome sequencing is necessary.

CONCLUSION

In this study, we performed MS-MLPA and aCGH to investigate the putative mechanisms causing PWLS in ten patients. We have observed an altered methylation profile in one patient, indicating that this patient had PWS caused by either UPD or IC mutation. None of the nine PWLS patients exhibited copy number variations in the 6q16 region although this locus is commonly associated with PWLS. On the other hand, we have observed some alterations in the patients we studied, most of which are reported to be VUS. In order to better understand whether the alterations we observed can be associated with PWLS, further studies are needed to analyze both the patients and their parents in a larger cohort of patients. Additionally, employing advanced molecular techniques such as whole genome analysis, may provide more comprehensive insights and contribute to a more accurate diagnosis of these conditions.

Ethics Committee Approval

This research complies with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the Medical Faculty Ethical Committee, Akdeniz University (approval number: 2012/1590-1251).

Informed Consent

All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration.

Author Contributions

Concept - P.B., S.Y.U., S.B., B.N., E.M., F.Z.H.Z. ; Design - P.B., S.Y.U., S.B., B.N., E.M., F.Z.H.Z..; Supervision - P.B., S.Y.U., F.Z.H.Z.; Resources - S.Y.U.; Materials - S.Y.U., B.N., E.M.; Data Collection and/or Processing – P.B.; Analysis and/ or Interpretation - P.B., S.Y.U., S.B., B.N., E.M., F.Z.H.Z.; Literature Search – P.B. ; Writing Manuscript - P.B., S.Y.U., S.B., B.N., E.M., F.Z.H.Z. ; Critical Review - P.B., S.Y.U., S.B., B.N., E.M., F.Z.H.Z.

Conflict of Interest

The authors have no conflict of interest to declare.

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