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Analyses of human papillomavirus genotypes in cervical cancer patients using real-time PCR

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MAIN POINTS

- HPV 16, 18, and 45 were identified as the most frequent genotypes in cervical cancer cases in Gaziantep.
- The relatively high prevalence of HPV 45 appears to be a distinctive regional feature compared with national and international reports.
- Regional HPV genotyping provides valuable epidemiological data for tailoring vaccination strategies and clinical management.
- The nonavalent HPV vaccine, covering the most prevalent types found in this study, should be considered the most effective option for preventing cervical cancer in this population.

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ABSTRACT

Aim: The aim of this study was to identify the most prevalent Human Papillomavirus (HPV) genotypes associated with cervical cancer in Gaziantep and to determine the most appropriate HPV vaccine for individuals who have not yet been infected.

Materials and Methods: This retrospective cross-sectional study included 74 patients diagnosed with cervical cancer at Gaziantep University Faculty of Medicine between December 2005 and June 2019. Formalin-fixed paraffin-embedded tissue samples were retrieved from the pathology archives. HPV DNA was extracted and genotyping of 14 high-risk HPV types was carried out by real-time PCR.

Results: All patients were histopathologically diagnosed with squamous cell carcinoma. Single HPV type infection was detected in six patients (8.2%) (HPV 16 in five, HPV 56 in one), whereas multiple infections were observed in 68 patients (91.8%). Among these, HPV 16 was identified in 100% of cases, HPV 45 in 82%, HPV 18 in 45%, HPV 56 in 19%, and HPV 33 in 16%. Overall, HPV 16, 45, and 18 genotypes emerged as the most frequent types contributing to cervical cancer in this region.

Conclusion: Given the genotypes identified in this study, the nonavalent HPV vaccine (9-valent; HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58) appears to be the most suitable option for our population. However, due to its limited availability in Turkey, the quadrivalent HPV vaccine (4-valent; HPV types 6, 11, 16, and 18), which may provide partial cross-protection against HPV 45, could serve as a reasonable alternative.

Keywords: Human papillomavirus, HPV vaccine, Cervical cancer, Genotype, Real-time PCR
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INTRODUCTION

Cervical cancer is the fourth most frequently diagnosed malignancy and the fourth leading cause of cancer-related mortality among women worldwide, with an estimated 604,000 new cases and 342,000 deaths reported in 2020. The disease remains a significant global health burden, particularly in sub-Saharan Africa, Melanesia, South America, and South-Eastern Asia, where it ranks as either the most common or the most fatal malignancy among women. Conversely, incidence and mortality rates are 7–10 times lower in high-income regions, such as North America and Western Asia. These geographic disparities primarily reflect inequities in access to screening and vaccination programs, as the vast majority of deaths occur in countries with limited resources and inadequate preventive strategies [1].

According to 2015 statistics from the Turkish Ministry of Health, cervical cancer is the tenth most prevalent malignancy

among women in Turkey, with an incidence rate of 4.5 per 100,000 individuals. The disease is often preceded by precursor lesions, most notably cervical intraepithelial neoplasia (CIN). Established risk factors include the early initiation of sexual activity, multiple sexual partners, sexually transmitted infections (STIs), and multiparity [2].

The strength of the association between persistent Human Papillomavirus (HPV) infection and cofactors such as smoking and oral contraceptive use remains a subject of ongoing debate; however, inconsistent findings across studies suggest these factors may significantly contribute to cervical carcinogenesis [3,4]. Notably, numerous studies indicate a more robust correlation between smoking and cervical squamous cell carcinoma (SCC) [5,6]. Cervical cancer is infrequently diagnosed in nulliparous or virginal women. Furthermore, male circumcision has been shown to reduce the risk of HPV acquisition and reinfection while increasing HPV clearance in the glans, thereby indirectly reducing the risk of cervical cancer in women [7]. Additionally, in utero exposure to diethylstilbestrol (DES) is associated with an elevated risk of clear-cell adenocarcinoma of the cervix and vagina; while this risk is most pronounced at younger ages, recent evidence indicates it may persist into midlife and older age [8].

HPV, primarily transmitted through sexual contact, is recognized as the primary etiological agent in the development of cervical cancer [9]. High-risk types, specifically HPV-16 and HPV-18, are responsible for over 70% of cases, though other oncogenic genotypes—including HPV-31, 33, 35, 39, 45, 51, 52, 56, and 58—also play a substantial role [10]. HPV infection occurs most frequently in young women aged 18–30 years. Although approximately 90% of these infections clear spontaneously within 12–36 months, persistent infections can progress to invasive cervical cancer later in life [11].

The objective of this study was to investigate the distribution of HPV genotypes among patients with cervical cancer in Gaziantep, Turkey. By doing so, we aimed to determine the most suitable vaccine formulations for HPV-negative individuals and to highlight the critical role of genotyping in supporting early detection and clinical management for HPV-positive, unvaccinated women.

MATERIALS AND METHODS

Patient selection

Between December 1, 2005, and June 1, 2019, a total of 90 patients who histopathologically diagnosed with HPV-related cervical cancer were retrospectively identified from the electronic database of the Pathology Department. Among these, 74 patients with adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks and sufficient DNA quality were included in this retrospective cross-sectional study. Cases with tissue insufficiency or poor DNA quality were excluded. The study was approved by institutional review board in terms of scientific and ethical conduct. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Gaziantep University Clinical Research Ethics Committee (approval date: October 2, 2019; approval number: 2019/376).

Tissue processing and DNA isolation

Formalin fixed paraffin embedded (FFPE) cervicale cancer tissues were prepared for analysis. Five slices, each 10 μm thick, were excised from each block and deposited into sterile tubes using the laboratory's usual protocol. Deparaffinization was performed with xylene and graded ethanol via standard centrifugation procedures. Subsequent to drying, the tissue was incubated at 56 °C for 2 hours in a solution comprising ATL lysis buffer and Proteinase K. A 700 μL aliquot of the resultant lysate was then subjected to automated nucleic acid extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit (QIAGEN, Hilden, Germany) on the QIAsymphony SP system. Each sample was extracted a single time, according to the manufacturer's instructions. The purity of DNA and its amplification capability were evaluated using the internal control reactions included in the commercial kit. A total of 60 μL of DNA eluate was collected from each specimen.

HPV DNA analysis and genotyping

HPV DNA detection was conducted utilizing real-time PCR on the QIAGEN Rotor-Gene system. The thermal cycling protocol commenced with an initial denaturation step at 95 °C for 15 minutes, succeeded by a pre-amplification phase consisting of 5 cycles (95 °C for 5 seconds, 60 °C for 20 seconds, and 72 °C for 15 seconds). Subsequently, 40 amplification cycles were conducted at 95 °C for 5 seconds, 60 °C for 30 seconds, and 72 °C for 15 seconds. Genotyping was performed utilizing a commercial kit (NLM Diagnostici, Italy) specifically developed for the detection of 14 oncogenic HPV types. Four PCR reactions were conducted for each patient, utilizing primer sets that targeted HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Each run included positive and negative controls to ensure the assay's reliability. All reactions were performed on a microplate-based real-time PCR platform to ensure precise amplification and detection.

Evaluation of results

Amplification curves obtained from the PCR software were evaluated using the threshold value recommended by the manufacturer (threshold = 0.03). Representative examples of raw fluorescence data from the detection channels are presented in Figure 1.

Statistical analysis

Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are reported as frequencies (n) and percentages (%). Given the descriptive nature of this study, no comparative statistical tests were performed. The prevalence of each HPV genotype was expressed as a percentage of the total number of cases. To account for

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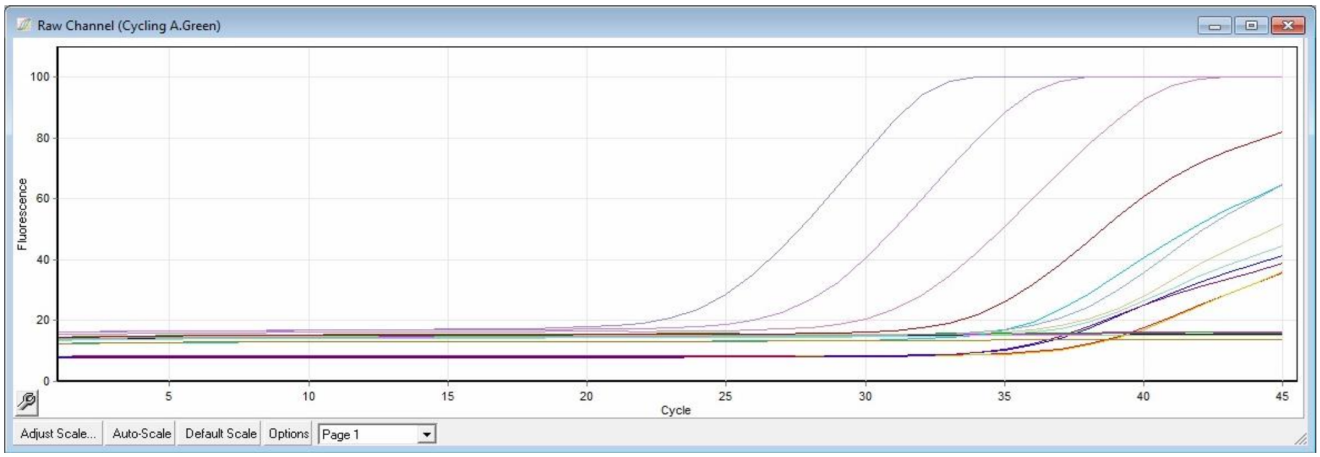

Figure 1. Raw amplification curves from the real-time PCR analysis. (Fluorescence signal curves obtained from the PCR software were evaluated according to the threshold value of 0.03. Different channels represent the HPV genotypes analyzed).

the relatively small sample size, 95% confidence intervals (CIs) were calculated using the Clopper–Pearson exact binomial method. The resulting data are summarized in the accompanying tables.

RESULTS

Seventy-four patients with pathologically confirmed cervical cancer were included in the analysis. Real-time PCR detected HPV DNA in all samples, resulting in a total positivity rate of 100%. The distribution of HPV genotypes and their 95% confidence intervals are presented in Table 1. Infections involving a single HPV type were relatively uncommon ($n = 6$, 8.2%), whereas the vast majority of patients ($n = 68$, 91.8%) harbored multiple HPV types (Table 2).

The mean age of the patients was 52.6 ± 11.8 years, with a range of 29–78. The distribution of HPV genotypes was described across three age groups (≤40, 41–50, and >50 years). HPV 16 was the most common genotype identified in all groups, followed by HPV 45 and HPV 18. Overall, the pattern of genotype distribution appeared similar across all age categories (Table 3).

The temporal distribution of cervical cancer cases based on biopsy identification years (2005–2019) is summarized in Table 4. The number of cases varied slightly from year to year, ranging between two and eight cases annually, with no clear upward or downward trend observed throughout the 14-year study period. HPV 16 remained the predominant genotype across all years, followed by HPV 45 and HPV 18, without any apparent temporal shift in genotype distribution.

The most frequently observed co-infections were HPV 16/45 and HPV 16/18/45, each of which was detected in 13 patients (17.6%). Additional combinations that appeared with notable frequency included HPV 16/33/45 (4.1%), HPV 16/39/45 (5.4%), and HPV 16/56 (5.4%). Across all patterns, HPV 16 was consistently the dominant genotype and was present in nearly every combination.

Table 1. Variation of HPV genotypes in our study.

HPV Genotype	n (%) [95% CI]
HPV 16	5 (6.8) [2.2--15.2]
HPV 16/45	13 (17.6) [9.8--28.5]
HPV 16/18/45	13 (17.6) [9.8--28.5]
HPV 56	1 (1.4) [0.0--7.3]
HPV 16/31/45	2 (2.7) [0.3--9.4]
HPV 16/18/39/45	2 (2.7) [0.3--9.4]
HPV 16/18/33/45/56	1 (1.4) [0.0--7.3]
HPV 16/45/66	2 (2.7) [0.3--9.4]
HPV 16/18/45/56/66	1 (1.4) [0.0--7.3]
HPV 16/45/56	2 (2.7%) [0.3--9.4]
HPV 16/39/45	4 (5.4) [1.5--13.2]
HPV 16/58	1 (1.4) [0.0--7.3]
HPV 16/18/45/59	1 (1.4) [0.0--7.3]
HPV 16/33/52	1 (1.4) [0.0--7.3]
HPV 16/18/33/39/45	1 (1.4) [0.0--7.3]
HPV 16/18/33/45	2 (2.7) [0.3--9.4]
HPV 16/56	4 (5.4) [1.5--13.2]
HPV 16/45/59	1 (1.4) [0.0--7.3]
HPV 16/68	1 (1.4) [0.0--7.3]
HPV 16/18/45/51	2 (2.7) [0.3--9.4]
HPV 16/33/39/45	1 (1.4) [0.0--7.3]
HPV 16/33/45	3 (4.1) [0.9--11.5]
HPV 16/18/31/45	2 (2.7) [0.3--9.4]
HPV 16/18	1 (1.4) [0.0--7.3]
HPV 16/18/45/56	2 (2.7) [0.3--9.4]
HPV 16/18/33/35/56	1 (1.4) [0.0--7.3]
HPV 16/31/56/68	1 (1.4) [0.0--7.3]
HPV 16/39	1 (1.4) [0.0--7.3]
HPV 16/18/31	1 (1.4) [0.0--7.3]
HPV 16/18/33/35/45/52/68	1 (1.4) [0.0--7.3]

Values are presented as number (percentage) with 95% confidence intervals (Clopper–Pearson exact method). The table summarizes the distribution of HPV genotypes detected in 74 cervical cancer cases.

DISCUSSION

In this study, the most common HPV genotypes among cervical cancer cases in a tertiary hospital in Gaziantep were identified as genotypes 16, 18, and 45. The predominance of HPV 16 and 18 is consistent with both national and international reports, while the relatively high prevalence of HPV 45 indi-

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Table 2. Descriptive statistical data of the patients infected with a single or multiple HPV types.

Infection type	N	%
Single HPV type	6	8.2
Multiple HPV types	68	91.8
Total	74	100

Table 3. Distribution of HPV genotypes according to age groups.

HPV Genotype	≤40 years (%)	41–50 years (%)	>50 years (%)
HPV 16	28.6	32.1	31.8
HPV 18	28.6	17.0	13.0
HPV 31	0.0	0.0	3.9
HPV 33	0.0	5.7	5.2
HPV 35	0.0	3.8	0.0
HPV 39	0.0	5.7	3.9
HPV 45	28.6	24.5	26.6
HPV 51	0.0	0.0	1.3
HPV 52	0.0	1.9	0.6
HPV 56	14.3	5.7	5.2
HPV 58	0.0	0.0	0.6
HPV 59	0.0	1.9	0.6
HPV 66	0.0	0.0	1.9
HPV 68	0.0	1.9	1.3

Values represent the percentage of patients in each age group positive for the indicated genotype. Multiple infections were possible. Median age: 52 years, IQR: 44–60.

Table 4. Annual distribution of cervical cancer cases (2005--2019).

Year	Number of cases (n)
2005	3
2006	4
2007	6
2008	5
2009	7
2010	6
2011	5
2012	8
2013	6
2014	5
2015	4
2016	4
2017	5
2018	4
2019	2

cates a high prevalence in the region. These findings highlight the regional variation in HPV genotype distribution and emphasize the importance of incorporating local epidemiological data into cervical cancer prevention and vaccination strategies.

The highest frequency of HPV infection occurs in sexually active young women, particularly those aged 25–29 years, after which the prevalence tends to stabilise or slightly decline [12]. Recent Turkish data also support the high prevalence of HPV infection even among women with normal cytology. In a study by Görür et al. [13], high-risk HPV genotypes were detected in 43% of 270 women aged 19–69 years using multiplex polymerase chain reaction (PCR).

The infection rate was significantly higher in patients with abnormal cytology (77%) compared to those with normal cytology (37%). Consistent with this age-related pattern, a multicenter Turkish study involving only women with normal cytology reported the highest HPV prevalence in the 25–29 age group, with HPV 16 and HPV 45 identified as the most frequent genotypes [14]. These findings underscore the fact that a substantial proportion of women may harbor high-risk HPV types despite having normal cytologic results, emphasizing the critical value of molecular HPV testing in cervical cancer screening and risk assessment. Consequently, detailed HPV genotyping is essential for mapping regional epidemiological patterns and tailoring preventive strategies. Genotyping further serves to refine regional data, guide national vaccination programs, and direct clinical follow-up for infected individuals. Accordingly, this research aims to determine the distribution of HPV subtypes most commonly associated with cervical cancer in Gaziantep and its surrounding regions. Based on these findings, we intend to identify the optimal vaccine formulations for preventive use in HPV-negative individuals aged 11–12 years, while highlighting the necessity of early detection and effective treatment for previously infected, unvaccinated patients.

Several investigations conducted across Turkey have documented varying HPV prevalence rates, which are largely influenced by the specific study populations and diagnostic techniques employed. Güney et al. (1997) used PCR and DNA in situ hybridization (DISH) to detect HPV DNA in 9.5% of pregnant women and 45% of women presenting with condyloma or dysplasia [15]. In a large screening cohort of 22,488 tests, abnormal cytology and HPV positivity were detected in 7.5% and 7.4% of cases, respectively. Among the HPV-positive samples, non-16/18 genotypes were most frequent (62.2%), followed by HPV 16 (22.9%) and HPV 18 (7.3%), while multiple-type infections occurred in 7.7% of cases. In this cohort, HPV testing demonstrated higher sensitivity but lower specificity than cytology in detecting high-grade cervical lesions (CIN2–CIN3) [16]. Similarly, Özçelik et al. found a prevalence of 6.1% among 230 participants [17]. In another study, HPV DNA was detected in 40.4% (150/371) of cervical samples; among these positive cases, cytological abnormalities were present in 12.9%, while 25.3% showed no cytological changes. The most common genotype was HPV 16 (45.3%), followed by HPV 56 (17.3%), HPV 18 (12.0%), and HPV 51 (12.0%) [18].

Onan et al. reported HPV DNA detection rates of 4.2% in CIN I, 14.8% in CIN II, and 45% in CIN III [19]. In a screening series of 1,353 women, İnal et al. identified HPV DNA in 1.5% of cytologically normal individuals, whereas all patients with abnormal cytology tested positive [20]. Ergünay et al. found HPV DNA in 80% of 35 cytologically abnormal cases, with high-risk genotypes present in 78.6% of those samples [21]. Yıldız et al. observed that p16 expression was positive

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in all high-grade squamous intraepithelial lesion (HSIL) cases and 80% of low-grade squamous intraepithelial lesion (LSIL) cases, with an overall HPV DNA positivity rate of 48.6% [22]. Furthermore, Işıklı et al. reported LSIL or HSIL in 11.7% of women undergoing colposcopy following Pap smear screening [23], while Özgül et al. demonstrated p16INK4a positivity in all HSIL and cervical cancer cases, compared to 46.2% in LSIL cases [24]. Similarly, Müderris et al. reported that HPV 16 was the most common genotype in both cytologically normal and abnormal cases. They also noted that the frequencies of ASC-US and HSIL were significantly higher in women infected with HPV 16 than in those infected with other genotypes, further supporting the strong association between specific high-risk HPV types and cytological abnormalities in the Turkish population [25].

In Samsun Province, Taşkın et al. reported a high-risk HPV positivity rate of 9.17% among 5,406 women, with HPV 16 and HPV 18 accounting for 28.62% and 9.67% of infections, respectively; other high-risk types constituted 78.83% of cases. HPV 16 positivity peaked in women aged 30–39 years, while HPV 18 and other high-risk types were more frequent in the 40–49 age group. Notably, the highest prevalence was observed during the summer months, particularly in June 2021 [26]. Recent region-specific studies further demonstrate marked geographical variability in HPV distribution. In Mersin province, Yaman et al. reported a high-risk HPV prevalence of 12.6% among 12,641 women screened between 2019 and 2022, with significantly higher rates among those with abnormal cytology and HPV 16 as the dominant genotype [27]. A hospital-based study from Ankara involving 4,267 women reported an overall HPV positivity rate of 14.2%, with HPV 16 and HPV 18 detected in 2.4% and 0.7% of samples, respectively, and pooled high-risk HPV types identified in 8.8% [28]. Similarly, an Istanbul-based analysis of 2,285 cervical samples found a high-risk HPV positivity rate of 36.3%. The highest prevalence was seen in women aged 17–34 years, with HPV 16 (30.9%), HPV 39 (14.6%), and HPV 51 (14.2%) as the most frequent genotypes; multiple high-risk infections were observed in 40.7% of the positive cases [29].

In a multicenter hospital-based analysis across Turkey, Dursun et al. assessed a cohort of 6,388 women from 12 gynecologic oncology centers, reporting an overall HPV positivity rate of 25%. HPV DNA positivity was significantly higher among women with abnormal cytology compared to those with normal findings (52% vs. 27%, $p < 0.05$). The most frequently detected genotypes included HPV 16 (32%), HPV 6 (17%), HPV 11 (9%), HPV 18 (8%), HPV 31 (6%), HPV 51 (5%), and HPV 33 (3%). This indicates that while HPV 16 remains the predominant genotype, a considerable proportion of infections are attributable to non-16/18 types [30]. Finally, Usubütün et al. identified HPV DNA in 93.5% of 248 cervical cancer specimens, with HPV 16 being the most prevalent, followed by HPV 18 and HPV 45; together, HPV 16 and 18 comprised 75.4% of all invasive cases [31]. Avcı et al. documented HPV DNA in 61% of 77 women undergoing colposcopy, noting that all HSIL cases tested positive [32]. These findings, alongside large-scale clinical trials like the PATRICIA study, demonstrate that HPV vaccines may confer partial cross-protection against non-vaccine oncogenic types such as HPV 31, 33, and 45 [33].

Limitations and Strengths

This study has certain limitations. Due to its retrospective design, individual risk factors for HPV infection, including sexual history, HIV status, sociodemographic characteristics, and vaccination history, could not be assessed. Additionally, the analysis relied exclusively on paraffin-embedded tissue samples, precluding the determination of viable virus presence. The limited sample size and the study's confinement to a single institution restrict the generalizability of the results. A further limitation is that only the high-risk HPV types present in the commercial kit were evaluated; low-risk and less prevalent types were excluded from assessment. Furthermore, real-time PCR did not facilitate the evaluation of viral load or the integration of viral genomes. The lack of a control group hindered direct comparison, as the primary emphasis of the study was on the distribution of HPV genotypes in cervical cancer patients.

Notwithstanding these limitations, the research had significant merits. Genotyping was performed by real-time PCR on archival paraffin-embedded tissue specimens from patients with histologically confirmed cervical cancer who were treated at a tertiary referral facility in southeastern Turkey. These data provide significant epidemiological insight into the regional distribution of HPV types. This information may guide public health efforts, especially in customizing immunization campaigns to meet regional requirements.

CONCLUSION

In summary, HPV genotypes 16, 18, and 45 were found to be the predominant types associated with cervical cancer in our region. The findings emphasize the importance of continuous surveillance of genotype distribution to better tailor regional prevention and vaccination strategies. Expanding access to the nonavalent vaccine and strengthening screening programs may significantly reduce the burden of HPV-related cervical cancer in the future.

Ethics Committee Approval: This study was approved by the Gaziantep University Clinical Research Ethics Committee with decision no: 2019/376 on October 2, 2019.

Informed Consent: We did not need an informed consent for the data we obtained were from the archived specimens of the patients.

Peer-review: Externally peer-reviewed.

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Data Availability Statement: All data obtained or analyzed during this study are included in this published article. Further information is available from the corresponding author upon reasonable request.

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