Research Article

HPV Genotype as a Predictor of Bevacizumab Treatment Response in Recurrent Respiratory Papillomatosis Patients

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ABSTRACT

Objective: This study aimed to evaluate the clinical efficacy and safety of systemic bevacizumab in recurrent respiratory papillomatosis (RRP) and to explore the potential predictive value of human papillomavirus (HPV) genotype in this condition.

Methods: A total of 34 patients with confirmed HPV6- or HPV11-associated RRP who received at least three doses of systemic bevacizumab were enrolled. Disease burden and clinical response were evaluated based on the number of interventions, Derkay scores, and voice-related quality of life (assessed using the Voice Handicap Index-30 for adults or the pediatric Voice Handicap Index for children) before and after treatment. Adverse events were documented and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Additionally, vascular endothelial growth factor A (VEGFA) expression in RRP tissues was measured by immunohistochemistry.

Results: The overall response rate (ORR) was 70.59%. The frequency of surgical interventions was significantly reduced following treatment (median reduction from 2 to 0; $P < 0.0001$), and Derkay scores also showed a significant reduction (median reduction from 7.0 to 1.5; $P < 0.0001$). Adverse events were observed in 38.24% of patients, all of which were grade 1-2 and manageable. In juvenile-onset recurrent respiratory papillomatosis (JORRP), patients infected with HPV11 exhibited an ORR of 76.92%, with 57.58% achieving a ⩾ 50% reduction in Derkay scores. In contrast, among patients infected with HPV6, the ORR was 57.14%, and only 45.83% demonstrated a ⩾ 50% improvement in Derkay scores. Although these differences did not reach statistical significance, HPV11-positive patients with JORRP appeared to have a more favorable prognosis. Conversely, in adult-onset RRP (AORRP), patients with HPV11 infection had an ORR of 40% and a 16.67% rate of ⩾ 50% Derkay score reduction, while those infected with HPV6 demonstrated an ORR of 88.89% and a 77.78% rate of significant Derkay score improvement. Similarly, a statistically significant reduction in surgical frequency before and after treatment was observed only in HPV11-positive JORRP patients and HPV6-positive AORRP patients. All groups of RRP showed VEGFA expression, but there was no significant difference.

Conclusion: Systemic bevacizumab is effective and well-tolerated in both JORRP and AORRP. HPV genotype may influence therapeutic response and clinical outcomes in RRP patients receiving bevacizumab treatment. Specifically, HPV11-positive JORRP patients and HPV6-positive AORRP patients appear to derive greater benefit.

KEYWORDS

recurrent respiratory papillomatosis, bevacizumab, human papillomavirus, derkay score, surgical frequency

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1 Introduction

Recurrent respiratory papillomatosis (RRP) is a rare, benign epithelial neoplasm caused by human papillomavirus (HPV) infection, most commonly by HPV genotypes 11 and 6. The larynx, particularly the vocal folds, is the most frequently affected site, leading to the condition also being referred to as recurrent laryngeal papillomatosis (RLP) [1]. The predominant clinical manifestation is hoarseness, although patients may also present with stridor, abnormal crying, throat discomfort, chronic cough, persistent dyspnea, or sleep-disordered breathing. In severe cases, progressive airway obstruction can develop, potentially resulting in life-threatening complications [2].

Clinically, RRP is categorized into juvenile-onset RRP (JORRP) and adult-onset (AORRP) forms based on the age at disease onset. JORRP typically exhibits a more aggressive disease course, characterized by higher recurrence rates. Several studies have demonstrated that HPV infection is associated with earlier disease onset and increased severity in JORRP [3-5]. In contrast, the association between HPV genotype and disease severity in AORRP remains unclear [6]. Notably, the potential impact of HPV genotype on therapeutic response—particularly to systemic anti-angiogenic therapy—remains underexplored.

Bevacizumab (bev), a recombinant humanized monoclonal antibody targeting vascular endothelial growth factor A (VEGFA), has shown promising therapeutic efficacy in the management of RRP [7-10]. Since HPV genotype is an established determinant of disease severity, we hypothesized that it may also modulate the clinical response to bev in RRP. This study aimed to investigate the association between HPV11 and HPV6 genotypes and the efficacy of bev in both JORRP and AORRP. Our findings provide insights into the potential utility of HPV genotype as a predictive biomarker and could guide the development of genotype-tailored treatment strategies.

2 Materials and Methods

2.1 Study design and patients

This single-center prospective study enrolled patients with histopathologically confirmed recurrent respiratory papillomatosis (squamous papilloma on biopsy). Comprehensive clinical and pathological data were systematically collected for all patients. The study protocol strictly adhered to ethical principles outlined in the Declaration of Helsinki and received formal approval from the Institutional Review Board of Eye & ENT Hospital, Fudan University (approval number 2022046-2). Written informed consent was obtained from all subjects or their legal guardians prior to study participation.

Eligible patients were required to meet as least one of the following criteria: (1) ⩾ 2 prior surgical interventions for RRP, (2) documented rapid disease recurrence (within 6 months post-resection), or (3) distal disease extension (trachea, bronchi, or pulmonary parenchyma). Key exclusion criteria comprised: (1) presence of concurrent malignancies, (2) major bleeding episodes within 4 weeks prior to enrollment, (3) uncontrolled hypertension (⩾ 140/90 mmHg despite antihypertensive therapy), and (4) persistent non-healing wounds. All participants underwent comprehensive baseline evaluation, including complete blood count, hepatic/renal function panels, and coagulation studies, to verify adequate organ function.

2.2 Treatment

Patients received intravenous bevacizumab administered at a dose of 7.5–10 mg per kilogram of body weight every 3–4 weeks. This treatment regimen was implemented in accordance with our previously published study [11]. Dose adjustments with this range were permitted based on individual patient tolerance and treatment response.

2.3 Clinical assessment

Clinical response to systemic bevacizumab was evaluated by comparing the number of required interventions during the 12-month period following three bevacizumab doses against the 12-month pre-treatment baseline, consistent with published criteria [12,13]. Response categories were defined as follows: complete response (CR): No surgical or pharmacological interventions required during the 12-month after three doses of bev. Partial response (PR): 50%–99% reduction in the number of clinically indicated surgical and pharmacological interventions over the same period. Stable disease (SD): < 50% reduction in interventions compared with the baseline year. Overall response rate (ORR): combined CR and PR rates. Continuous administration of medication upon meeting treatment indication was considered a single medication intervention, regardless of the number of doses administrated during that episode. Anatomic disease burden was assessed via Derkay scores based on standardized laryngoscopy and narrow band imaging (NBI). Voice-related quality of life was evaluated using the Vocal Handicap Index-30 (VHI-30) or pediatric VHI (pVHI), based on patient or guardian questionnaire. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.

2.4 HPV detection

For each formalin-fixed, paraffin-embedded (FFPE)

tissue sample, 6 to 12 consecutive sections with a thickness of 4 μm were prepared. Genomic DNA was extracted using standard protocols, followed by assessment of DNA concentration and purity. HPV genotyping, covering 23 types, was performed using a PCR-based reverse dot blot hybridization assay (Yaneng Biotechnology Co., Ltd., China). The assay detected 6 low-risk HPV types (6, 11, 42, 43, 81, 83) and 17 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82). Samples testing positive for high-risk types were re-analyzed to confirm the findings.

2.5 Immunohistochemistry (IHC)

The FFPE tissue sections (4 μm thick) were baked at 64 °C for 90 minutes, followed by deparaffinization in xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed using a Tris-EDTA-based buffer. Endogenous peroxidase activity was quenched, and nonspecific binding was blocked by incubating sections at room temperature (RT) for 30 minutes. Sections were then incubated with a rabbit anti-human VEGFA primary antibody (Anti-VEGFA polyclonal antibody, atlas, HPA069116) for 1 hour at RT in a humidified chamber. After repeated washing, sections were incubated with a polymer horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody for 30 minutes at RT. Color development was achieved using a chromogenic substrate solution for 2–5 minutes and terminated by rinsing with buffer under microscopic observation. Slides were then counterstained, dehydrated, cleared, mounted, and subjected to image acquisition.

2.6 Statistical analysis

All statistical analyses were performed using SPSS software (version 23.0, IBM Corp., Armonk, NK, USA). For independent continuous variables, the Student's *t*-test or one-way analysis of variance (ANOVA) was used when data were normally distributed. If the normality assumption was violated, non-parametric tests (Wilcoxon rank-sum test or Kruskal-Wallis test) were applied. The chi-square test or Fisher's exact test was used for categorical data. For paired samples, a paired *t*-test was used if the differences followed a normal distribution; otherwise, the Wilcoxon signed-rank test was applied. Graphical data representations were generated using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A two-sided P value of < 0.05 was considered indicative of statistical significance.

3 Results

3.1 Bevacizumab improves interventions, severity scores, and voice function in RRP

A total of 34 HPV11- or HPV6-positive RRP patients who received at least three doses of systemic bevacizumab were enrolled (Figure 1). The cohort included 20 JORRP (58.82%) and 14 AORRP (41.18%) cases, with a median follow-up duration of 22.5 months (range, 16–32). Baseline demographics and clinical characteristics are presented in Table 1.

To assess the treatment response to systemic bev, the number of clinically indicated interventions (surgical and medical) during the 12 months after three doses of bev was compared with that during the 12 months before bev. All 34 patients received at least three doses of systemic bev. Among them, 14 (41.18%) achieved CR, and 10 (29.41%) achieved PR, yielding an ORR of 70.59% (24/34). SD was observed in the remaining 10 patients (29.41%). No cases developed progressive disease (PD) (Figure 2a).

A significant reduction in surgical frequency was observed during the 12 months following three doses of bev compared to the 12 months prior to bev (median reduction from 2 to 0, $P < 0.0001$) (Figure 2b). To further illustrate changes in lesion burden, Derkay scores were measured at baseline and after three doses of bev. A significant decrease in Derkay scores was noted post-treatment of bev (median decrease from 7.0 to 1.5, $P < 0.0001$) (Figure 2c).

The vocal fold is the most commonly affected site in

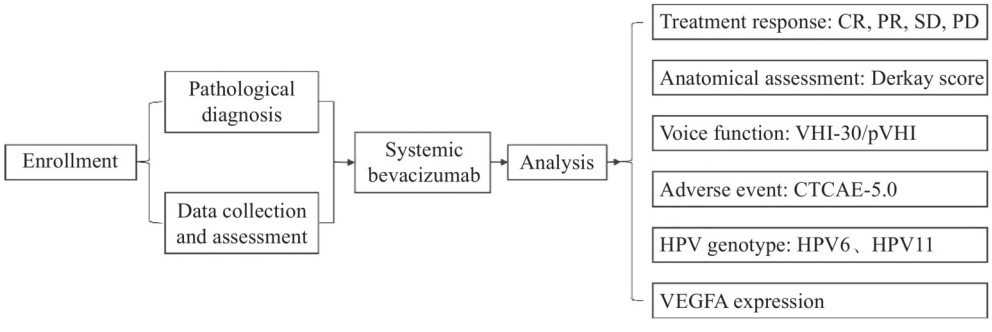

Fig. 1 Flowchart of patient enrollment, treatment, and assessment. CR, complete response, PR, partial response, SD, stable disease, PD, progressive disease. VHI, Voice Handicap Index. CTCAE, Common Terminology Criteria for Adverse Events. HPV, human papillomavirus. VEGFA, Vascular Endothelial Growth Factor A.

Table 1 Demographics and characteristics of patients

Patient	Sex	HPV type	Age at bev	Age at onset	Surgeries before bev	Prior adjuvant therapy	Number of laryngeal subsites*	Extra laryngeal RRP lesions	Derkay at bev	Tracheotomy
J1	M	11	198 m	25 m	37	None	3	None	3	No
J6	F	11	23 m	14 m	4	None	4	None	8	No
J8	F	6	29 m	17 m	5	None	6	None	8	No
J9	M	11	114 m	97 m	5	None	8	None	14	No
J10	M	6	244 m	72 m	14	None	9	trachea	18	Yes (capping)
J11	M	6	46 m	14 m	5	Local BLM	1	None	1	No
J13	F	11	299 m	192 m	12	None	4	None	4	No
J14	F	11	37 m	12 m	3	IFN spray	8	None	24	No
J16	F	6	46 m	28 m	2	None	6	None	18	No
J18	M	11	260 m	24 m	70	Zadaxin	6	Trachea, pulmonary	13	Yes (decannulation)
J20	F	11	190 m	36 m	50	Local BLM, 9vHPV	1	pulmonary	2	No
J21	M	11	101 m	10 m	8	None	3	None	9	No
J23	F	6	213 m	92 m	10	9vHPV	1	None	1	No
J24	M	11	139 m	134 m	2	None	2	None	4	No
J25	F	6	110 m	11 m	5	None	5	Pyriform sinus	8	No
J26	M	11	280 m	128 m	10	None	6	None	7	No
J27	M	11	119 m	69 m	17	Local BLM	0	None	0	No
J29	F	11	28 m	6 m	4	Systemic TCM	6	None	11	No
J30	M	11	72 m	67 m	2	None	1	None	2	No
J32	F	6	275 m	84 m	12	9vHPV	4	None	4	No
A3	M	11	29 y	24 y	18	Local BLM	2	trachea	6	No
A4	M	6	34 y	32 y	7	Systemic TCM	4	None	4	No
A5	M	11	42 y	36 y	19	None	5	None	7	No
A6	F	11	50 y	30 y	12	Systemic IFN	2	None	2	No
A7	M	6	36 y	35 y	2	Local BLM	5	None	5	No
A8	F	6	23 y	22 y	4	Local BLM	3	None	4	No
A9	M	11	21 y	21 y	2	None	9	None	11	No
A10	M	6	53 y	46 y	2	None	6	None	9	No
A11	M	6	63 y	56 y	9	IFN IH	7	None	7	No
A12	M	6	38 y	33 y	6	9vHPV	6	None	8	No
A14	M	6	41 y	36 y	14	None	7	None	8	No
A15	F	11	63 y	44 y	22	None	8	None	14	Yes (capping)
A16	F	6	39 y	30 y	4	9vHPV	1	None	1	No
A17	M	6	44 y	32 y	9	None	3	None	3	No

J, juvenile-onset recurrent respiratory papillomatosis. A, adult-onset recurrent respiratory papillomatosis. M, male. F, female. Bev, bevacizumab. TCM, Traditional Chinese Medicine. BLM, bleomycin. IFN, interferon. HPV, human papillomavirus. IH, inhalation. Surgeries before bev, cumulative lifetime number of surgical interventions prior to bevacizumab initiation.

RRP. In our study cohort, vocal fold involvement was present in all patients except one AORRP case (33/34, 97.1%). To eliminate the confounding effects of surgical intervention on voice outcomes, voice assessments were conducted before and after each bev treatment course (comprising three doses), using VHI-30 for adults and pVHI for children. The results demonstrated clinically meaningful improvements in voice-related quality of life across all measured domains—functional, physical, and emotional—for both adults and pediatric patient populations (Figures 2d and 2e).

3.2 Bevacizumab is well tolerated in juveniles and adults with RRP

No grade 3 or higher adverse events (AEs) were observed

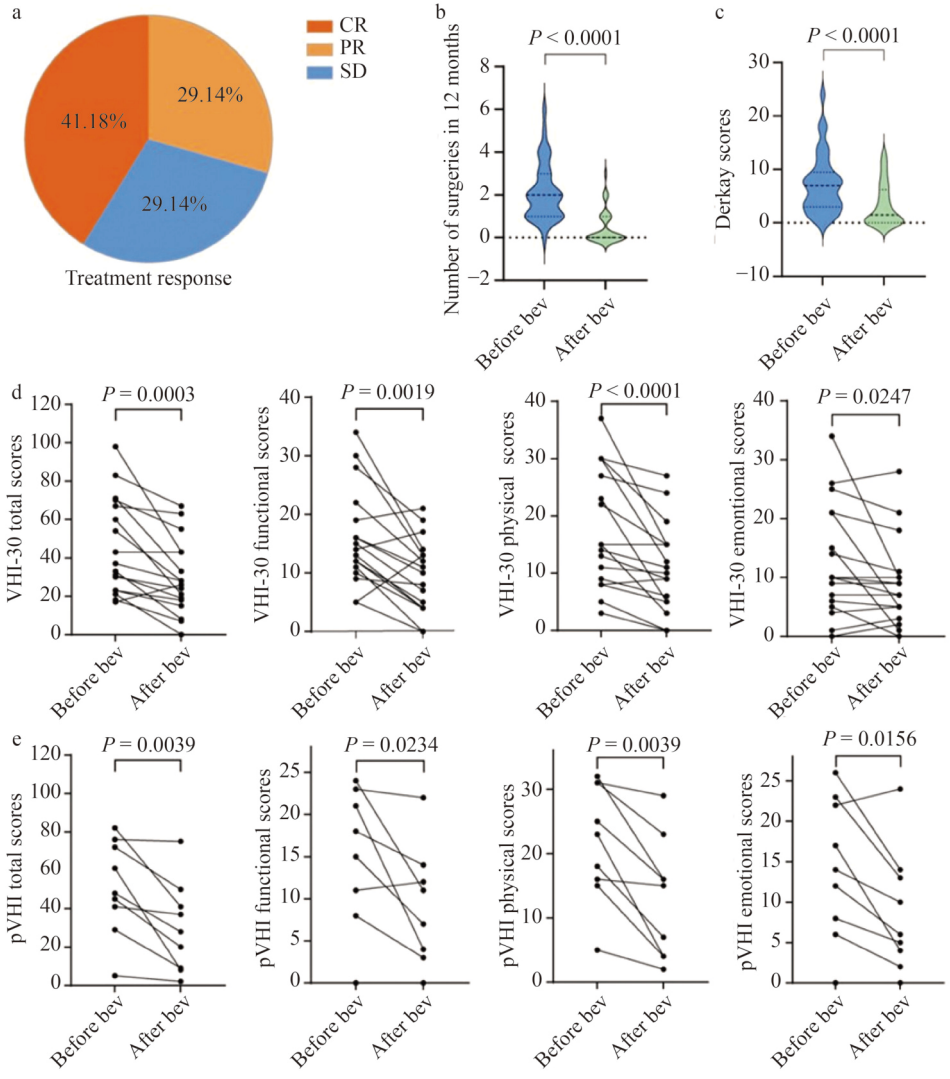

Fig. 2 Clinical efficacy of bevacizumab. (a) Proportion of patients with different treatment responses among all RRP patients, including CR (complete response), PR (partial response), and SD (stable disease). (b) Changes in the number of surgical interventions during the 12 months before and after three doses of bev. (c) Changes in Derkay scores before and after three doses of treatment. (d) Changes in the Voice Handicap Index-30 (VHI-30) and (e) pediatric VHI (pVHI) before and after three doses of treatment.

(CTCAE 5.0). A summary of AEs occurring during and after systemic bev treatment is presented in Table 2. Among the 34 patients, 13 (38.24%) experienced AEs, including 5 JORRP cases (38.46% of total AEs, representing 25% of all JORRP patients) and 8 AORRP cases (61.54% of total AEs, representing 57.14% of all AORRP patients). The most frequent AEs were grade 1 proteinuria (8.82%, 3/34), grade 1 menstrual irregularity (8.82%, 3/34), grade 1 fever (8.82%, 3/34), and grade 2 hypertension (8.82%, 3/34).

In the JORRP group, AEs included grade 1 proteinuria, epistaxis, menstrual irregularity, fever, and nausea. Menstrual irregularity resolved within 2 weeks to 1 month after discontinuation of bev treatment. Fever was relieved with antipyretic medication. All other AEs resolved spontaneously without specific treatment.

In the AORRP group, AEs included grade 1 protein-

Table 2 Adverse events of RRP treated with systemic bevacizumab

Adverse event (CTCAE5.0)	JORRP (n = 20)	AORRP (n =14)	Sum (n = 34)
Patients, n (%)	5 (25.00%)	8 (57.14%)	13 (38.24%)
Proteinuria	1 (5.00%)	2 (14.29%)	3 (8.82%)
Gingival bleeding	0	2 (14.29%)	2 (5.88%)
Epistaxis	1 (5.00%)	1 (7.14%)	2 (5.88%)
Menstrual irregularities	2 (10.00%)	1 (7.14%)	3 (8.82%)
Gingival swelling	0	2 (14.29%)	2 (5.88%)
Fever	1 (5.00%)	2 (14.29 %)	3 (8.82%)
Hypertension	0	3 (21.43%)	3 (8.82%)
Nausea	1 (5.00%)	0	1 (2.94%)
Muscle soreness	0	1 (7.14%)	1 (2.94%)
Finger paraesthesia	0	1 (7.14%)	1 (2.94%)
Hematuria	0	1 (7.14%)	1 (2.94%)

CTCAE, Common Terminology Criteria for Adverse Events.

uria, gingival bleeding, epistaxis, menstrual disorder, gingival swelling, fever (< 39 °C), nausea, muscle pain, finger numbness, and hematuria, as well as grade 2 hypertension. Three patients with hypertension consulted cardiology, received oral antihypertensive medication, and temporarily discontinued bev. One elderly postmenopausal patient experienced asymptomatic hematuria with urine occult blood (+++), and discontinued treatment due to hypertension. Menstrual irregularity resolved within 1 month after discontinuation of bev. Fever was relieved with antipyretics. All other AEs resolved spontaneously without specific intervention.

3.3 HPV genotype as a potential predictor for clinical response to bevacizumab in juveniles and adults with RRP

All patients were tested for HPV genotype (Figure 3). Among them, eighteen (18/34, 52.94%) patients were infected with HPV11, and sixteen (16/34, 47.06%) were infected with HPV6. In JORRP, HPV11 and HPV6 were detected in 65.00% and 35.00% of cases, respectively, in AORRP, these figures were 35.71% and 64.29%, respectively. Although the difference was not statistically significant, HPV11 was more predominant in JORRP, while HPV6 was more common in AORRP (Table 3).

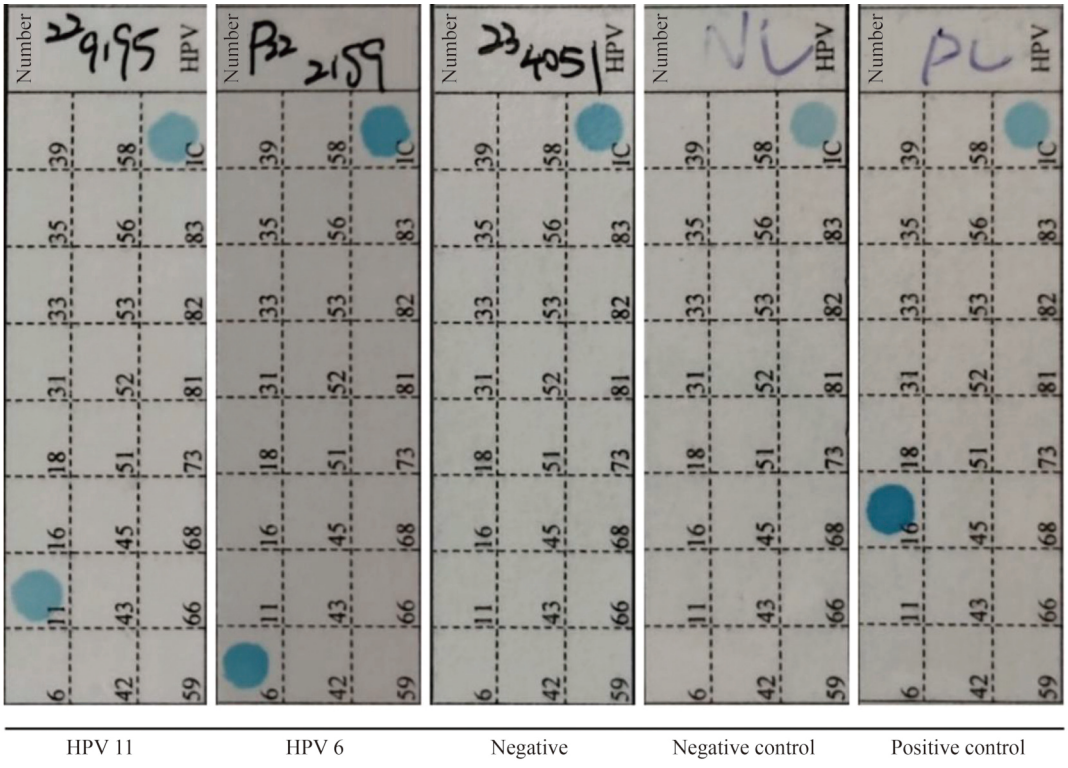

Fig. 3 Representative results of HPV genotyping by PCR and chip hybridization assay

Table 3 Baseline characteristics of the patients

Parameter	JORRP (n = 20)	AORRP (n = 14)	P
Age at onset	2.65 (0.5−16.0)	32.50 (21−56)	< 0.001[a]
Age at bev	9.70 (1.9−24.9)	40.00 (21−63)	< 0.001[a]
Sex (M:F)	10:10	10:4	0.296[b]
HPV type			0.091[b]
11	13.00 (65.00%)	5.00 (35.71%)	
6	7.00 (35.00%)	9.00 (64.29%)	
No. laryngeal subsites	4.00 (0–9)	5.00 (1–9)	0.478[a]
Derkay score	7.50 (0–24)	6.50 (1–14)	0.769[a]
No. surgeries in prior year	6.50 (2–70)	8.00 (2–22)	0.931[a]

Data are median (range), n (%). HPV, human papillomavirus. [a] Wilcoxon rank-sum test. [b] Fisher's exact test. $P < 0.05$ was considered statistically significant.

To evaluate the predictive value of HPV genotypes on the therapeutic efficacy of bev in RRP, we further analyzed treatment outcomes across different HPV genotypes in JORRP and AORRP. Figures 4a and 4b depict the number of clinically indicated interventions for each patient during the 12 months before and after three doses of bev in JORRP and AORRP, respectively. Figures 4c and 4d illustrate changes in Derkay scores before and after each treatment course (three doses of bev) in JORRP and AORRP, excluding the influence of surgery.

Among all patients, those infected with HPV11 and HPV6 exhibited comparable ORR (66.67% vs 75.00%, P = 0.715). In JORRP patients, the objective response rate (ORR) was 76.92% in those infected with HPV11, compared to 57.14% in those with HPV6 (P = 0.613; Figures 4a and 4e). The proportion of patients achieving a ⩾ 50% improvement in Derkay score was 57.58% in the

HPV11 group and 45.83% in the HPV6 group ($P = 0.381$; Figures 4c and 4f). Although the differences were not statistically significant, HPV11-positive JORRP patients appeared to have better clinical outcomes. In contrast, among AORRP patients, the ORR for HPV11 was 40%, with 16.67% achieving a ⩾ 50% reduction in Derkay score, whereas in the HPV6 group, these values were 88.89% and 77.78%, respectively ($P = 0.095$ and $P = 0.001$ respectively; Figures 4b, 4d, 4e, and 4f).

Bevacizumab significantly reduced the overall interventions required, with a particularly notable decrease in surgical procedures in both JORRP and AORRP. Paired analyses of the number of surgical interventions within 12 months prior to and after three doses of bev demonstrated significant reductions in both JORRP and AORRP ($P < 0.0001$ and $P = 0.0020$, paired t-test). As expected, when comparing the number of surgeries before and after treatment, a statistically significant reduction was observed in HPV11-positive JORRP patients and HPV6-positive AORRP patients ($P = 0.0020$ and $P = 0.0039$ respecti-

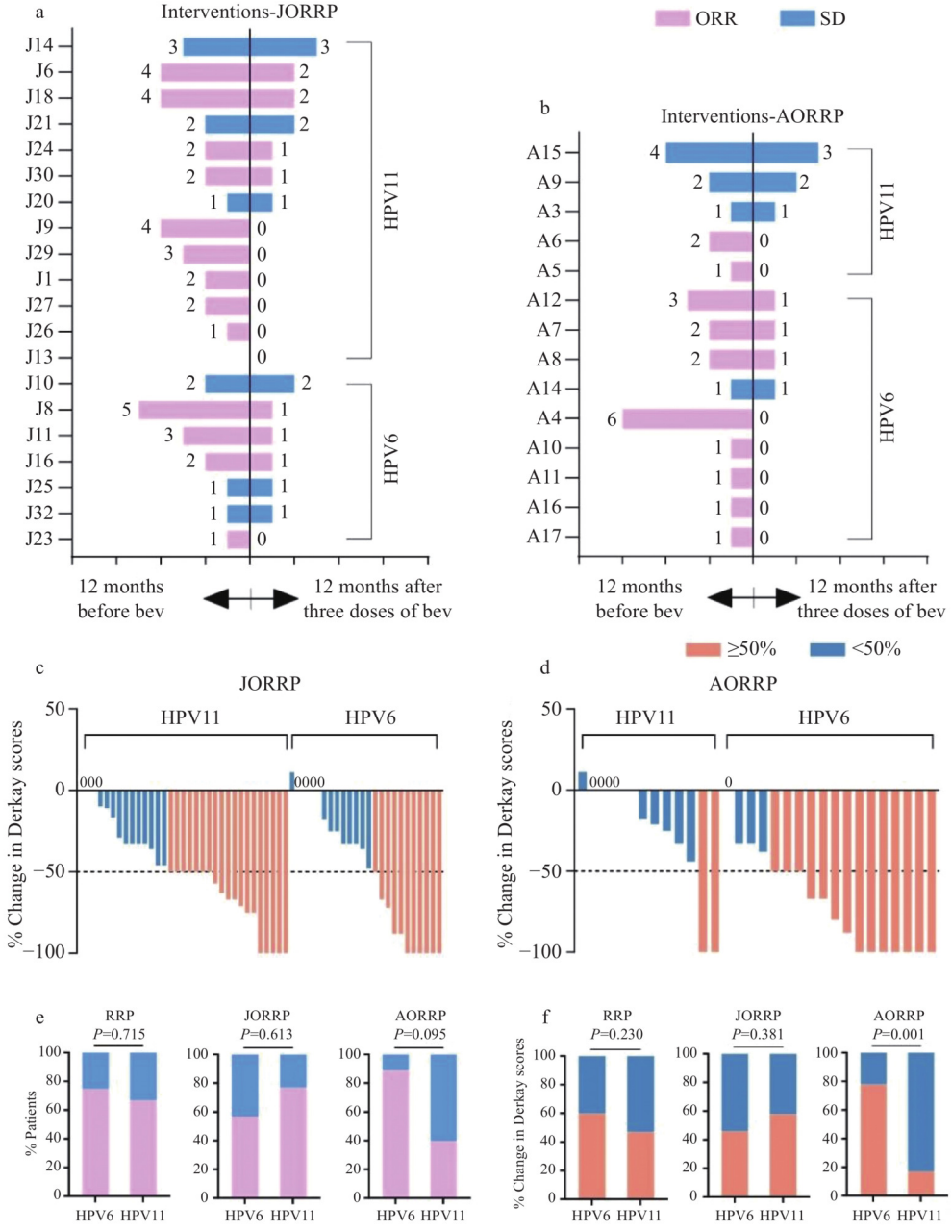

Fig. 4 Clinical efficacy and disease burden. Clinical indicated interventions in the 12 months (a) before and (b) after three doses of bev in JORRP and AORRP. Percentage change in Derkay scores (c) pre- and (d) post-treatment for each course (three doses of bev). A value a -100 indicates a 100% improvement, meaning the Derkay score decreased to 0; a value of -50 indicates a 50% improvement. (e) Proportion of patients with overall response rate (ORR) across different HPV genotypes. (f) Proportion of patients with ⩾ 50% improvement in Derkay scores across different HPV genotypes.

vely, Wilcoxon signed-rank test, Figure 5). In contrast, no statistically significant reduction in surgical frequency was observed in HPV6-positive JORRP or HPV11-positive AORRP patients (P = 0.0625 and P = 0.0890, respectively, Wilcoxon signed-rank test, Figure 5).

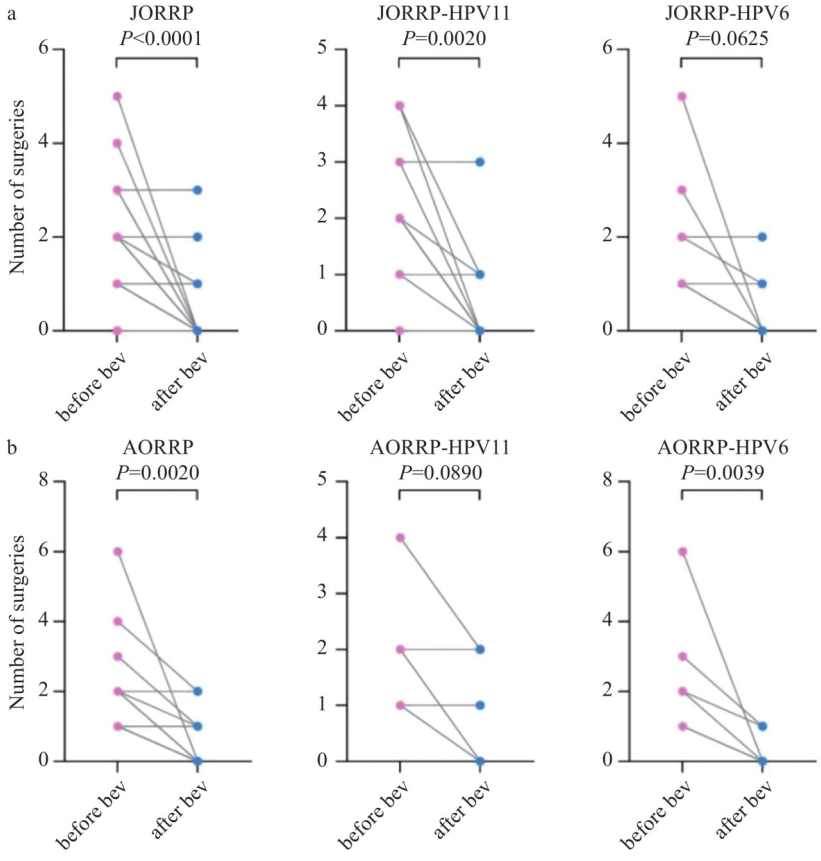

Fig. 5 Therapeutic effects of patients with different HPV types. (a) Change in surgical interventions in the 12 months before and after three doses of bev in the JORRP group. (b) Change in surgical interventions in the 12 months before and after three doses of bev in the AORRP group.

3.4 VEGFA is expressed in JORRP and AORRP with HPV6 and HPV11

To explore the mechanism underlying bevacizumab efficacy in RRP, we measured VEGFA expression in HPV11- and HPV6-positive JORRP and AORRP tissue samples. VEGFA was present in lesions of both genotypes (Figure 6), supporting the rationale for anti-angiogenic therapy in RRP. However, VEGFA staining intensity did not differ significantly between HPV11- and HPV6-positive lesions in either the JORRP or AORRP cohorts. This finding suggests that additional mechanisms may contribute to the genotype-specific treatment response and warrant further investigation.

4 Discussion

This study demonstrated that bevacizumab significantly improved clinical outcomes in both juvenile-onset and adult-onset RRP patients, including reduced intervention frequency, decreased disease severity scores, and improved voice-related quality of life. The treatment was well tolerated across all age groups. In addition, HPV genotypes, including HPV11 and HPV6, may serve as predictors for treatment response in RRP.

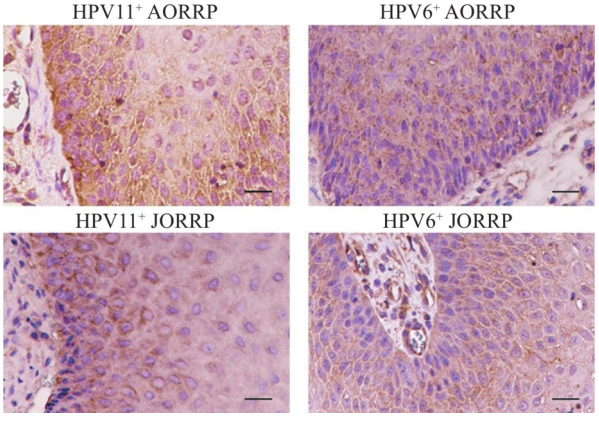

Fig. 6 Expression of VEGFA in HPV6- and HPV11-positive adult and juvenile RRP tissues.

In the evaluation of treatment response, we included not only surgical procedures but also interventions where systemic therapy was administered in lieu of surgery, reflecting cases in which disease activity persisted but could be managed pharmacologically without immediate surgical intervention. A total of 70.59% of patients demonstrated a treatment response to bev, with 41.18% achieving CR. These findings align with previously published data on systemic bev treatment for RRP [7-10]. Subsequently, we evaluated changes in surgical frequency and observed a significant reduction in the number of surgical procedures following bev treatment. Repeated surgical debulking, while necessary for symptom management, is associated with cumulative iatrogenic laryngeal injuries including anterior commissure synechiae and decreased vocal fold pliability[14]. Thus, reducing surgical interventions not only alleviates the physical and psychological burden on patients but also contributes to the preservation of long-term vocal function.

A significant reduction in Derkay scores was observed, indicating substantial regression of lesions. Previous studies have highlighted the correlation between Derkay scores and disease severity, including symptom burden and surgical intervention requirements [15,16]. Therefore, improvement in Derkay scores further supports the clinical efficacy of bev in managing RRP. Additionally, anatomical regression may contribute to improved voice quality and a reduced risk of airway obstruction.

Post-treatment voice assessments revealed significant improvements across all three subscales —functional, physical, and emotional —highlighting both anatomical resolution and patient-centered benefits. Improvements in voice-related quality of life suggest that bev may help preserve or restore vocal function, which is crucial for both pediatric and adult patients, as voice plays a central role in communication, education, and social development [17].

Although bev demonstrates overall efficacy in treating RRP, a subset of patients exhibited suboptimal response. Identifying potential predictors of therapeutic outcomes is therefore of considerable clinical importance.

HPV genotype has long been recognized as a critical determinant of disease severity in JORRP. Specifically, HPV11 infection has been associated with earlier disease onset, more aggressive disease courses, higher Derkay scores, and increased surgical intervention frequency compared to HPV6 in JORRP [18,19]. Given this established association between genotype and clinical phenotype, we hypothesized that HPV subtype might also influence the therapeutic response to systemic bev. Our findings suggest a potential interaction between HPV genotype and response to bev in both JORRP and AORRP, with distinct genotype-specific clinical outcomes. In JORRP, patients with HPV11 showed a higher ORR, significant reductions in surgical interventions, and improved Derkay scores. In AORRP, patients with HPV6 demonstrated a higher ORR, a more pronounced improvement in Derkay scores, and a significant decrease in the number of surgical interventions required. This concordance across multiple clinical endpoints supports the hypothesis that HPV genotype may influence not only the baseline disease severity, as previously reported, but also the therapeutic response to anti-angiogenic therapy. Notably, the divergence in genotype-associated response patterns between JORRP and AORRP may reflect fundamental differences in host immune maturity, viral-host interactions, and angiogenic microenvironment at different stages of life.

Previous studies have demonstrated that the E6 and E7 oncoproteins of high-risk HPV16 promote angiogenesis by upregulating HIF-1α and VEGF expression [20,21]. Although HPV11 and HPV6 are classified as low-risk genotypes and lack the same oncogenic potential, we speculate that they may differentially regulate angiogenic pathways in the context of JORRP and AORRP. While low-risk HPV types are not traditionally associated with strong angiogenic activity, some studies have suggested that HPV11 may exhibit higher transcriptional activity compared to HPV6 [22,23], potentially inducing localized hypoxia and secondary VEGF upregulation. Additionally, host factors such as age-dependent immune responses, chronic inflammation, and epithelial remodeling may further modulate the angiogenic environment [24,25] in a genotype-specific manner. Such differences in the angiogenic profile could underlie the observed variations in clinical responses to anti-angiogenic therapy and warrant further mechanistic investigation.

VEGFA was expressed in both JORRP and AORRP infected with either HPV11 or HPV6, providing a molecular rationale for the application of bev in RRP. Bev binds all isoforms of VEGFA with high specificity, blocking its bioactivity, and thereby inhibiting angiogenesis and promoting normalization of the vasculature[26]. However, some patients showed suboptimal responses despite VEGFA expression, suggesting additional resistance mechanisms. Bev may exert immunomodulatory effects by attenuating VEGF-mediated signaling pathway [27]. Moreover, our observation that patients with different HPV genotype exhibit comparable VEGFA expression but divergent treatment outcomes suggests that genotype-specific factors —such as immune modulation or alternative angiogenic pathways—may contribute to differential therapeutic response. High VEGFA expression impair the effects of TH1 cells and cytotoxic T cells by downregulating interferon regulatory factor 1 (*IRF1*) and granulysin (*GNLY)* [28], and it also inhibits dendritic cell maturation, thereby weakening anti-tumor immunity, promoting the accumulation of immunosuppressive cells, and disrupting the immune balance within

the tumor. Anti-VEGFA therapy can reduce Tregs and MDSCs [29], thus improving the immune microenvironment. We hypothesize that the immune microenvironment of RRP differs between JORRP and AORRP patients infected with different HPV genotypes, which may account for the variability in the clinical efficacy of bev. These findings warrant further mechanistic studies to delineate the molecular pathways driving genotype- and age-specific disease phenotypes and to identify potential biomarkers for stratifying patients most likely to benefit from anti-angiogenic therapy.

This study has several limitations. First, the modest sample size may have limited statistical power to detect subtle but clinically meaningful effects, therefore, these non-significant *P* values are more likely to reflect insufficient power due to the small sample size rather than definitive absence of an effect. For the statistical results with *P* values between 0.05 and 0.10 in this study, we consider them to indicate a trend toward significant, with suggestive and exploratory value, warranting confirmation in larger-sample, multicenter, or pooled analyses. Second, while treatment strategies were tailored to individual disease severity, the cohort size precluded meaningful subgroup analyses by specific intervention types. Furthermore, the study did not include mechanistic exploration of VEGF signaling pathways or potential resistance mechanisms; future research incorporating serial biomarker assessments could provide valuable insights into treatment response heterogeneity.

In conclusion, systemic bevacizumab demonstrates a promising clinical benefit in both JORRP and AORRP patients. The treatment was well tolerated, with manageable adverse events limited to grade 1-2 in 38.4% of patients. Subgroup analyses suggest that HPV genotype may influence treatment response and clinical outcomes. In JORRP, HPV11-positive patients exhibited a higher ORR and greater reduction in both surgical intervention frequency and Derkay scores. Conversely, in AORRP, patients with HPV6 demonstrated superior outcomes. These findings underscore the potential value of HPV genotyping in optimizing bevacizumab treatment strategies for RRP.

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Ethical statement

Not applicable.

Conflicts of interest

The author has no conflicts of interest to disclose.

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Data availability statement

The datasets analyzed during the current study and de-identified individual patient clinical data are available from the corresponding author on reasonable request. The raw individual patient data were protected and are not available due to data privacy laws.

Author contributions

J. Chen contributed to study design, conceptual development, and manuscript editing. C. Wei contributed to study conception and manuscript editing. J. Shao contributed to study conception and supervised the completion of the study. X. Zhao contributed to study design, data collection and manuscript drafting. J. Sun prepared paraffin-embedded tissue sections and performed HPV genotyping. H. Yu and X. Zhao conducted immunohistochemistry and analysis. R. Fang, P. He, L. Cheng, H. Wu, and L. Lin conducted clinical investigations and pathological diagnoses. All authors reviewed the results and approved the final manuscript.

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