Human Papillomavirus-type distribution in anogenital lesions of prepubertal children

S.A. Braun,1,2,† S. Silling,3,† S.M. Schloer,4 S.C. Hofmann,5 B. Fritzen,6 F. Oellig,7 P. Lehmann,5 B. Homey,2 C. Assaf,6 S. Emmert,6 R. Fölster-Holst,9 C. Tigges,10 U. Wieland,3,† A. Kreuter10,*,†

1Department of Dermatology, University Hospital Muenster, Muenster, Germany
2Department of Dermatology, Medical Faculty, Heinrich-Heine University, Duesseldorf, Germany
3Institute of Virology, National Reference Center for Papilloma and Polyomaviruses, University of Cologne, Cologne, Germany
4Center for Molecular Biology of Inflammation, Institute of Medical Biochemistry, University of Muenster, Muenster, Germany
5Department of Dermatology, Allergology, and Dermatosurgery, HELIOS University Hospital Wuppertal, University of Witten-Herdecke, Wuppertal, Germany
6Department of Dermatology and Venereology, HELIOS Hospital Krefeld, Krefeld, Germany
7Institute of Pathology, Mülheim a.d.R., Germany
8Clinic and Policlinic for Dermatology and Venereology, University Medical Center Rostock, Rostock, Germany
9Department of Dermatology, Christian-Albrechts-University, University Medical Center Schleswig-Holstein, Kiel, Germany
10Department of Dermatology, Venereology, and Allergology, HELIOS St. Elisabeth Hospital Oberhausen, University of Witten-Herdecke, Oberhausen, Germany
*Correspondence: A. Kreuter. E-mail: alexander.kreuter@helios-gesundheit.de

Abstract

Background In contrast to adults, only limited data are available on the human papillomavirus (HPV)-type spectrum in anogenital warts (AGW) of children.

Objective This study aimed to evaluate the HPV-type spectrum in AGW of prepubertal children.

Materials & methods In a retrospective German multicentre study, HPV genotyping was performed in AGW biopsies of 55 1- to 12-year-old children using HPV group-specific PCRs followed by hybridization with type-specific probes or sequence analysis.

Results Human papillomavirus-DNA was found in 53 of the 55 AGW. In 58.5% (31/53) of the HPV-positive AGW, mucosal HPV types were detected. HPV6 (27/53, 50.9%) was the predominant type. 43.4% (23/53) of the lesions were induced by cutaneous HPV types (HPV2, HPV27, HPV57). Mucosal HPV types were significantly more common in children under 5 years of age than in children 5 years of age and older (22/25, 88.0% [95% CI: 70.0–95.8] vs. 9/28, 32.1% [95% CI: 17.9–50.7], P < 0.001). In contrast, cutaneous HPV types were significantly more prevalent in the 5- to 12-year age group (4/25, 16.0% [95% CI 6.4–34.7] vs. 19/28, 67.9% [95% CI 49.3–82.1], P < 0.001).

Conclusion Anogenital warts in 5- to 12-year-old children are frequently associated with cutaneous HPV types, possibly due to horizontal transmission. HPV typing, in addition to comprehensive clinical and psychosocial evaluation, can potentially help in the assessment of these cases.

Conflict of interest All authors declare no conflicts of interest.

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transmitted through direct skin-to-skin contact during sexual intercourse. Therefore, the occurrence of AGW in prepubertal children raises the suspicion of sexual abuse. However, the interpretation of anogenital HPV-induced lesions in children as a clinical sign of sexual abuse is controversial, as non-sexual transmission is possible and epidemiological data on HPV in children are still limited. Besides vertical HPV transmission from mother to child during or shortly after birth, HPV can also be transmitted horizontally. Hereby, HPV is distributed via smear infection either by the child itself (autoinoculation) or by the parents/caregivers (heteroinoculation) from an infected body site, e.g. the hands, to a non-infected site as the anogenital skin. Today, over 200 different HPV genotypes have been classified that are phylogenetically divided into five genera called alpha, beta, gamma, mu and nu. The genus alpha comprises both mucosal and cutaneous HPV types, while the genera beta, gamma, mu and nu contain cutaneous HPV types. More than 40 HPV types of the genus alpha are found in the anogenital tract and can be divided into low-risk (LR; e.g. HPV6, HPV11) and high-risk (HR) types (e.g. HPV16, HPV18) according to their oncogenic potential. Approximately 90% of AGW found in adults is associated with the LR alpha-HPV types HPV6 and HPV11. Cutaneous warts such as common or plantar warts are induced by alpha (e.g. HPV2, 27, 57), gamma (e.g. HPV4) or mu/nu (e.g. HPV1, HPV41) HPV types. Studies in adults have shown that certain HPV types appear to be largely sitespecific for warts at mucosal or cutaneous sites. In children, horizontal transmission allows that cutaneous HPV types, which typically cause extra-genital common warts, are transferred to the genital area, where they can cause AGW caused by mucosal HPV types. The main objective of this retrospective multicentre study was to evaluate the HPV-type spectrum of AGW in prepubertal children.

Materials and methods
All documented AGW biopsies of children from six German dermatology departments (in the cities of Düsseldorf, Oberhausen, Krefeld, Wuppertal, Rostock and Kiel) with a focus on paediatric dermatology were retrospectively collected from the respective archives of dermatopathology. The patients (n = 55) were diagnosed and treated between 2007 and 2018. Only prepubertal children (30 girls, 25 boys) younger than 13 years of age were included in the study (age range 1–12 years, and see Table 1 for details). As far as available, additional clinical information about AGW localization, the additional presence of cutaneous warts on hands, arms or feet, and the level of suspicion of child abuse were collected (Table 1). The criteria used to underpin the suspicion of sexual abuse are listed in the German child protection guideline, see appendix 2: Pediatric Sexual Assault Nurse Examiner (P-SANE) questionnaire (https://www.awmf.org/leitlinien/detail/ll/027-069.html).

Human papillomavirus-DNA detection and typing were performed as previously described. Briefly, DNA was isolated from formalin-fixed paraffin-embedded tissue (a total of 10 3-µm sections per sample) using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). HPV-DNA detection and typing were performed by group-specific polymerase chain reaction (PCR) assays (Ani/A8 PCR and BSGP5+/6 PCR) followed by hybridization with type-specific probes using a bead-based multiplex assay that covers 42 alpha-HPV types (LR or unclassified mucosal types: HPV6, HPV11, HPV40, HPV42–44, HPV54, HPV55, HPV61, HPV71, HPV72, HPV81, HPV83, HPV84, HPV89; probable/possible HR types: HPV26, HPV30, HPV34, HPV53, HPV66–69, HPV70, HPV71, HPV73, HPV82, HPV85, HPV97; HR types: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59; cutaneous types: HPV27, HPV57). Samples that were HPV-negative in both PCR assays were additionally analysed with a short-fragment group-specific HPV-PCR (SPF10-PCR yielding 65 bp PCR products) followed by HPV typing with a reverse line-blot assay that covers 32 alpha-HPV types (HPV6, HPV11, HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV40, HPV42–45, HPV51–54, HPV56, HPV58, HPV59, HPV61, HPV62, HPV66–68, HPV70, HPV73, HPV81–83, HPV89; INNO-LiPA HPV Genotyping Extra II, Fujirebio, Gent, Belgium). For the detection of cutaneous wart-associated HPV types, two PCR protocols were performed as

Table 1 Patient characteristics of 55 children with anogenital wart

| Variable                          | Value            |
|-----------------------------------|------------------|
| Number of patients, n             | 55               |
| Mean (range)/median (IQR) age at diagnosis, years | 5.4 (1–12)/5 (3–8) |
| 1- to 4-year-old, n (%)           | 25 (45.5)        |
| 5- to 12-year-old, n (%)          | 30 (54.5)        |
| Sex                               |                  |
| Female, n (%)                     | 30 (54.5)        |
| Male, n (%)                       | 25 (45.5)        |
| Localization of AGW              |                  |
| Perianal, n (%)                   | 43 (78.2)        |
| Genital, n (%)                    | 7 (12.7)         |
| Perianal and genital, n (%)       | 3 (5.5)          |
| Intranaal, n (%)                  | 1 (1.8)          |
| Not specified, n (%)              | 1 (1.8)          |
| Presence of cutaneous warts on upper or lower extremity, n (%) | 9 (16.4)†       |
| Sexual abuse                      |                  |
| Strong suspicion of sexual abuse, n (%) | 3 (5.5)†       |
| No evidence of sexual abuse, n (%) | 22 (40.0)†      |
| No data on sexual abuse available, n (%) | 30 (54.5)        |
| HPV-DNA detected in AGW          | 53 (96.4)        |

AGW, anogenital wart; HPV, human papillomavirus; IQR, interquartile range. †Six patients had hand warts, one patient had a wart on the forearm and on a finger, one patient had a foot wart, and one patient had warts on hands and feet. ‡Evaluated by a specialist experienced in child protection.

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previously published.18,19 Briefly, primer pairs CP61M/CP70M and CP62M/CP69M18 as well as HVP2/B5 and CN1F/CN1R19 were used to detect HPV types of species alpha2/alpha4 and genera mu/mu, respectively. PCR-products were submitted to agarose gel electrophoresis. For sequencing, amplimers were purified using the QIAQuick PCR Purification Kit (Qiagen). Sequencing was performed with the respective primer using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany) and DyeEx 2.0 Spin Kit (Qiagen) according to the manufacturer’s instructions. Sequence analyses were run on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems GmbH). The obtained sequences were analysed with MacVector (MacVector Inc., Apex, NC, USA) and aligned online using the NCBI Blast tool (https://blast.ncbi.nlm.nih.gov) for HPV type determination.

Each PCR run included HPV-reference samples as positive controls and extracted DNA of HPV-negative RTS3b cells as negative controls. Additionally, all samples were analysed with a beta-globin gene PCR to determine the quantity and quality of the extracted DNA and to demonstrate that the samples were free of inhibitory substances.20,22

The study was approved by the ethics review board of the University of Witten/Herdecke (no. 166/2017).

Statistical analyses were performed with SPSS Statistics, version 26 (IBM Corporation, New York, NY, USA) and Prism 6.00 (GraphPad Software, La Jolla, CA, USA). Qualitative data were cross-tabulated and tested for association by Pearson chi-square tests (two-sided). Distribution of patient age was analysed using the Mann–Whitney U-test for two independent samples. P values <0.05 were considered statistically significant.

Results

Overall, 55 AGW of 55 children between 1 and 12 years of age (median 5, mean 5.4 years) were included in the study. The proportion of girls and boys was similar (30 girls, 54.5%). In 78.2% of the cases, the AGW were only present perianally (n = 43; 20 girls, 23 boys), two girls and one boy had both genital AGW and perianal AGW, and in seven girls the genital area (labia majora) was affected. One 2-year-old girl had intraanal AGW and in one 1-year-old boy, information on AGW localization was missing (Table 1). Only in three cases, a strong suspicion of sexual abuse was documented, but follow-up data on these cases were not existing. In 22 children, no evidence of sexual abuse was found after evaluation by specialists experienced in child protection. In the remaining cases, information on sexual abuse was not available. The presence of common warts on hands, arms or feet was documented concomitant cutaneous warts, HPV-typing results were significant more frequently in 5- to 12-year-old children than in children between 1 and 4 years of age (67.9% [95% CI: 49.3–82.1] vs. 16.0% [95% CI: 6.4–34.7], P < 0.001, Chi-square test). In contrast, mucosal HPV types occurred more frequently in the younger age group of 1- to 4-year-old children than in the older children between 5 and 12 years of age (88.0% [95% CI: 70.0–95.8] vs. 32.1% [95% CI: 17.9–50.7], P < 0.001, chi-square test; Table 2). HPV6 was the most frequent HPV type in AGW of children 4 years of age and younger (20/25, 80.0%) and HPV57 was the most common type in children 5 years of age and older (11/28, 39.3%; Table 2, Fig. 1a). The median age of children with mucosal HPV types was 3 years [interquartile range (IQR) 2.0–6.0, n = 30], compared to 8 years in children with cutaneous types (IQR 5.8–10.0, n = 22; P < 0.0001, Mann–Whitney U-Test; Fig. 1b). The child with the HPV16 + 57 double infection was excluded from the latter analysis.

In the three cases with a high suspicion of sexual abuse (one 3- and one 4-year-old girl with AGW on the labia, and one 3-year-old boy with perianal AGW), HPV6 was found in the AGW.

For nine children (five girls, four boys, 3–11 years, median age 8), all of whom had perianal AGW, clinical information on concomitant cutaneous warts on hands (n = 6), arm and hand (n = 1), foot (n = 1), or feet and hands (n = 1) was available. In six of these children, there was no evidence of sexual abuse, for three of them information on sexual abuse was not available. Cutaneous types HPV2 (n = 4) or HPV57 (n = 3) were identified in seven of these children’s AGW; one child’s AGW was HPV6-positive and one child’s AGW was HPV-negative. Figure 2 shows a 10-year-old boy with HPV2-induced perianal AGW. He also had a HPV2-positive common wart on his left forearm and a wart on one of his fingers. However, in an 8-year-old girl with a HPV57-positive finger wart, HPV6 was detected in the AGW. For the remaining seven children with documented concomitant cutaneous warts, HPV-typing results of the cutaneous warts were not available.

Discussion

In contrast to adults, only limited data are available on the HPV-type spectrum of AGW in children. The results of studies on AGW in children are partly contradictory. Possibly, this can be explained by differences in study populations, selection bias,
different age ranges, limited information on confirmed sexual abuse, low numbers of cases examined, different types of samples (biopsies, swabs) and differences in sensitivity and covered type spectrum of the HPV-typing assays used.\textsuperscript{16,17,23–27} The present study is one of the few studies with a larger number of children (55 children) in a multicentre setting. For comprehensive HPV typing, AGW biopsies were analysed with multiple PCR protocols that cover more than 40 mucosal and cutaneous HPV types. If necessary, HPV sequence analyses were performed.

The main finding of the present study is a high prevalence of cutaneous HPV types in AGW of prepubertal children. In 43.4% of cases, the AGW were induced by mono-infections with HPV2, HPV27 or HPV57, which all belong to the species 4 of the genus alpha-papillomavirus and are typically found in common warts.\textsuperscript{14,24} In contrast, over 90% of AGW of adults in the general population are induced by HPV6 and HPV11, with the majority of lesions associated with HPV6.\textsuperscript{11,12} Horizontal HPV transmission via smear infection might explain the high percentage of cutaneous HPV types in the childrens’ AGW, especially in those with concomitant digital warts. On the other hand, wart-inducing HPV types of the species alpha 4 such as HPV27 and HPV57 have also been found in the anogenital tract, and sexual abuse can also occur through the fingers of adults.\textsuperscript{2,17}

Our results are in line with findings from previous studies.\textsuperscript{16,17,24} For example, Handley 	extit{et al.} have identified HPV2 in 41.9% of 31 cases using in situ hybridization for five cutaneous and six mucosal HPV types and Marcoux 	extit{et al.} have found the cutaneous HPV types 2, 3, 7 and 57 in 65.5% of 35 children with AGW using PCR and sequencing.\textsuperscript{16,17} However, other studies have shown a higher prevalence of mucosal HPV types in AGW of prepubertal children. This could be due to the use of PCR protocols that mainly amplify mucosal HPV-types.\textsuperscript{23–25,29–31} For example, in a recent study from Brazil, 60% of 19 children with AGW carried mucosal HPV types.\textsuperscript{23} In that study, five children (26%) additionally had high-risk HPV types 16 or 33.\textsuperscript{23} In a recent single-centre study from Germany, HPV typing of 17 AGW of children and adolescents between 2 and 18 years of age was performed using a HPV-PCR assay that covers 32 mucosal alpha-HPV types.\textsuperscript{24} In all seven children under the age of 12 years, HPV6 was identified, and three of these children additionally carried HPV16.\textsuperscript{24} Information about suspected sexual abuse was not reported in that study. The authors concluded that the coexistence of oncogenic HPV types in AGW of children and adolescents may be more frequent than previously assumed. In our study population, only three of 53 children (5.7%) carried mucosal high-risk HPV types.\textsuperscript{23} If possible, the likelihood of possible abuse as a source of HPV infection increases with age. In their study, the positive predictive value of

### Table 2: HPV-type distribution in 53 HPV-positive AGW of prepubertal children

| HPV types       | All children, \(n\) (\% [95% CI]) | Children 1–4 years, \(n\) (\% [95% CI]) | Children 5–12 years, \(n\) (\% [95% CI]) | \(P\)-value\footnote{§} |
|-----------------|-----------------------------------|----------------------------------------|----------------------------------------|------------------------|
| All             | 53                                | 25                                     | 28                                     |                        |
| HPV2            | 6 (11.3) [5.3–22.6]               | 0 (0) [0.0–13.3]                       | 6 (21.4) [10.2–39.5]                  | 0.014                  |
| HPV6            | 27 (50.9) [37.9–63.9]             | 20 (80.0) [60.9–91.1]                  | 7 (25.0) [12.7–43.4]                  | <0.001                 |
| HPV11           | 1 (1.9) [0.3–9.9]                 | 1 (4.0) [0.7–19.5]                     | 0 (0) [0.0–12.1]                      | 0.285                  |
| HPV16           | 2 (3.8) [1.0–12.8]                | 1 (4.0) [0.7–19.5]                     | 1 (3.6) [0.6–17.7]                    | 0.935                  |
| HPV27           | 2 (3.8) [1.0–12.8]                | 0 (0) [0.0–13.3]                       | 2 (7.1) [2.0–22.7]                    | 0.173                  |
| HPV33           | 1 (1.9) [0.3–9.9]                 | 1 (4.0) [0.7–19.5]                     | 0 (0) [0.0–12.1]                      | 0.285                  |
| HPV57           | 15 (28.3) [18.0–41.6]             | 4 (16.0) [6.4–34.7]                    | 11 (39.3) [23.6–57.6]                 | 0.060                  |
| HPV61           | 1 (1.9) [0.3–9.9]                 | 0 (0) [0.0–13.3]                       | 1 (3.6) [0.6–17.7]                    | 0.340                  |
| Mono-infection  | 2 (3.8) [1.0–12.8]                | 2 (8.0) [2.2–25.9]                     | 0 (0) [0.0–12.1]                      | 0.127                  |
| Mucosal types\footnote{†} | 31 (58.5) [45.1–70.7]          | 22 (88.0) [70.0–95.8]                  | 9 (32.1) [17.9–50.7]                  | <0.001                 |
| Mucosal LR types\footnote{‡} | 29 (54.7) [41.5–67.3]          | 21 (84.0) [65.4–93.6]                  | 8 (28.6) [15.3–47.1]                  | <0.001                 |
| Mucosal HR types\footnote{‡} | 3 (5.7) [1.9–15.4]               | 2 (8.0) [2.2–25.0]                     | 1 (3.6) [0.6–17.7]                    | 0.486                  |
| Cutaneous types\footnote{‡} | 23 (43.4) [31.0–56.7]          | 4 (16.0) [6.4–34.7]                    | 19 (67.9) [49.3–82.1]                 | <0.001                 |

AGW, anogenital warts; CI, confidence interval; HPV, human papillomavirus; HR, high-risk; LR, low risk.

\*In two cases, two different HPV types were found in the AGW biopsies (HPV6 + HPV53 and HPV57 + HPV16), with one type being a typical AGW (HPV6) or cutaneous wart (HPV57) inducing type and the other type being a mucosal HR type (HPV53, HPV16), respectively.

\|HPV53, HPV61 were considered low-risk mucosal, HPV16 and HPV33 high-risk mucosal, and HPV2, HPV27 and HPV57 cutaneous HPV types.\|

\(\chi^2\)-test, squared (children 1–4 years vs. 5–12 years).
mucosal HPV detection for possible sexual abuse was 36% for children 4–8 years of age and 70% for children older than 8 years. In our study, all archived AGW of children younger than 13 years of age seen between 2007 and 2018 at six referral centres for paediatric dermatology were retrospectively analysed, irrespective of the medical history and the suspicion of sexual abuse. Therefore, horizontal transmission from cutaneous hand warts might be more prevalent. Age distribution of children might influence the HPV-type spectrum found in their AGW. In our study, the median age of children with cutaneous HPV types was significantly higher than that of children with mucosal HPV types (8 vs. 3 years). It seems that older children are more likely to have AGW induced by HPV types found in common warts. In contrast, children under the age of 5 years might have acquired the detected mucosal HPV types from their mothers antenatally, perinatally, and postnatally. However, HPV transmission by sexual abuse can occur in all age groups.

This study might have some practical relevance for the management of children with AGW. The presence of cutaneous HPV types in AGW of children without any indications of sexual abuse, as in some of our cases, might be suggestive of horizontal transmission as a possible way of AGW acquisition in prepubertal children. As shown here, this seems to be particularly the case in children of 5 years and older. Therefore, a thorough anamnesis and examination for cutaneous warts are of particular importance in children with AGW of that age group and should include their family members. Existing cutaneous warts on hands and feet should be treated at the same time as the AGW to prevent retransmission.

As shown by us and others, AGW of children 4 years of age and younger are frequently associated with mucosal HPV types. In these cases, medical history and physical examination should include HPV-related diseases of all caregivers.

Our data show that AGW in prepubertal children primarily occur in the perianal area. This might be explained by anatomical (larger skin area) and local cofactors such as eczema and macerations, frequently seen in the perianal area, especially in children wearing diapers.

Prophylactic HPV vaccination is available for more than a decade. Both the quadrivalent vaccine and its successor, the nona-valent vaccine, cover HPV6 and HPV11. In countries with high HPV vaccination rates (>70–80%) such as Australia or Denmark, a significant decline of AGW has been observed shortly after the introduction of HPV vaccination. Even in countries with moderate vaccination rates, some decrease in AGW has been observed. This development will most likely influence the HPV-type spectrum found in AGW of children in the future. In our study, 52.8% of the analysed AGW was associated with HPV6 or HPV11, 84% in children 1–4 years of age and 25% in children above the age of four. With increasing HPV vaccination rates, HPV6/11-induced AGW of prepubertal children will probably decrease in both sexually abused and non-abused children of all ages.
The results of the present study should be interpreted in the light of its limitations. Although a large number of AGW biopsies was analysed, this was a retrospective study, with limited information on childhood sexual abuse. For example, in less than half of the cases, an examination by a child protection specialist was documented. Moreover, information on the presence of hand or foot warts was not available for the majority of children, and information on the clinical findings and HPV status of adults with access to the children of this study is lacking. Nevertheless, this is one of the largest studies on AGW in prepubertal children in which comprehensive HPV-typing methods have been used, covering both mucosal and cutaneous HPV types.

In summary, this retrospective study demonstrates that the majority of AGW in children between 5 and 12 years of age is caused by cutaneous HPV types. In none of these cases, suspicion of sexual abuse was documented. In prepubertal children aged 5 years and older, HPV typing, in addition to comprehensive clinical and psychosocial evaluation, could potentially help in the assessment of cases.

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