α- and β-Papillomavirus infection in a young patient with an unclassified primary T-cell immunodeficiency and multiple mucosal and cutaneous lesions

DOI:10.1016/j.jaad.2014.01.859

[Journal of the American Academy of Dermatology, Volume 71, Issue 1,July 2014, DOI: 10.1016/j.jaad.2014.01.859]

Elsevier Editorial System(tm) for Journal of the American Academy of Dermatology
Manuscript Draft

Manuscript Number:

Title: Alpha- and Beta-Papillomavirus infection in a young patient with an unclassified primary T-cell immunodeficiency and multiple mucosal and cutaneous lesions.

Article Type: Dermatopathology

Keywords: primary immunodeficiency; Papillomavirus; viral carcinogenesis; skin cancer; viral life cycle

Corresponding Author: Prof marisa gariglio, MD, PhD

Corresponding Author's Institution: University of Eastern Piedmont Amedeo Avogadro

First Author: Manuela M Landini, PhD

Order of Authors: Manuela M Landini, PhD; Cinzia Borgogna, PhD; Alberto Peretti; Enrico Colombo, MD; Elisa Zavattaro, MD, PhD; Renzo Boldorini, MD; Umberto Miglio; John Doorbar, PhD; Paolo Ravanini, Laboratory of Molecular Virology, "Maggiore della; Rajesh Kumar, PhD; Daniele Moratto; Raffaele Badolato, MD; Marco DeAndrea, MD, PhD; Marisa Gariglio, MD, PhD

Abstract: Background The correlation between human papillomavirus (HPV) genotype along with their histopathological and clinical features of skin lesions (from genital and non-genital sites) can present a diagnostic challenge.
Objective In this study, the correlation of HPV infection patterns with pathology and clinics was investigated in lesional and non-lesional body sites from a young patient with primary T-cell immunodeficiency.
Methods HPV infection was evaluated at both DNA and protein levels by PCR and immunohistochemistry.
Results Patient's genital lesions were exclusively caused by alpha-genotypes (high-risk type HPV51 in the anal and low-risk type HPV72 in the penile condylomas); the opposite was true for the skin lesions, which were infected by beta-genotypes only (HPV8 and 24); of which, HPV24 was the predominant type in terms of viral loads and the only one found in productive areas of infection. The patient had already developed high-grade dysplasia in the anal condylomas and showed areas of early stage dysplasia in the lesions caused by the beta-genotype HPV24.
Limitations The basic etiology of the immunodeficiency is not yet defined
Conclusion These findings provide proof of principle that both alpha and□□beta-genotypes can cause overt dysplastic lesions when immunosurveillance is lost, which is not restricted to Epidermodysplasia verruciformis.

Alpha- and Beta-Papillomavirus infection in a young patient with an unclassified primary T-cell immunodeficiency and multiple mucosal and cutaneous lesions.

Manuela M. Landini[a,d], PhD, Cinzia Borgogna[a], PhD, Alberto Peretti[a], Enrico Colombo[b], MD, Elisa Zavattaro[b], MD, PhD, Renzo Boldorini[c],MD, Umberto Miglio[c], PhD, John Doorbar[e], PhD, Paolo Ravanini[f], MD, Rajesh Kumar[g], PhD, Daniele Moratto[g], Raffaele Badolato[g], MD, Marco De Andrea[a,d] MD, PhD and Marisa Gariglio[a] MD, PhD.

[a]Virology Unit and [b]Dermatology Unit, Department of Translational Medicine, and [c]Pathology Unit, Department of Health Sciences, Medical School of Novara, Italy; [d]Department of Public Health and Pediatric Sciences, University of Turin, Italy; [e]Division of Virology, Department of Pathology, University of Cambridge, UK; Laboratory of Molecular Virology, "Maggiore della Carità" Hospital, Novara, Italy; [g]Department of Pediatrics, Institute of Molecular Medicine Angelo Nocivelli, University of Brescia, Italy

Word count: 2532 (ref. citation included)
Number of figures: 3
Number of tables: 1
Number of references: 37

CONFLICT OF INTEREST

The authors state no conflict of interest.

Correspondence: Marisa Gariglio, Virology Unit of the Department of Translational Medicine, Medical School of Novara, Via Solaroli 17, 28110 Novara, Italy. Fax: +39 0321 620421.
E-mail address: gariglio@med.unipmn.it

Alpha- and Beta-Papillomavirus infection in a young patient with an unclassified primary T-cell immunodeficiency and multiple mucosal and cutaneous lesions.

Manuela M. Landini[a,d], PhD, Cinzia Borgogna[a], PhD, Alberto Peretti[a], Enrico Colombo[b], MD, Elisa Zavattaro[b], MD, PhD, Renzo Boldorini[c],MD, Umberto Miglio[c], PhD, John Doorbar[e], PhD, Paolo Ravanini[f], MD, Rajesh Kumar[g], PhD, Daniele Moratto[g], Raffaele Badolato[g], MD, Marco De Andrea[a,d] MD, PhD and Marisa Gariglio[a] MD, PhD.

[a]Virology Unit and [b]Dermatology Unit, Department of Translational Medicine, and [c]Pathology Unit, Department of Health Sciences, Medical School of Novara, Italy; [d]Department of Public Health and Pediatric Sciences, University of Turin, Italy; [e]Division of Virology, Department of Pathology, University of Cambridge, UK; Laboratory of Molecular Virology, "Maggiore della Carità" Hospital, Novara, Italy; [g]Department of Pediatrics, Institute of Molecular Medicine Angelo Nocivelli, University of Brescia, Italy

Capsule summary

The association between β-HPV infection and skin cancer has been established in Epidermodysplasia verruciformis.

This study provides correlations between clinics, pathology and HPV infection patterns for both α and β-genotypes in the skin lesions from a patient with an unclassified primary T-cell immunodeficiency.

Understanding of the natural history and the molecular and cellular pathogenesis of β-HPV-induced skin lesions will aid the development of new diagnostic interventions to predict skin cancer risk in the immunocompromised host, thus not restricted to Epidermodysplasia verruciformis.

Alpha- and Beta-Papillomavirus infection in a young patient with an unclassified primary T-cell immunodeficiency and multiple mucosal and cutaneous lesions.

Manuela M. Landini[a,d], PhD, Cinzia Borgogna[a], PhD, Alberto Peretti[a], Enrico Colombo[b], MD, Elisa Zavattaro[b], MD, PhD, Renzo Boldorini[c],MD, Umberto Miglio[c], PhD, John Doorbar[e], PhD, Paolo Ravanini[f], MD, Rajesh Kumar[g], PhD, Daniele Moratto[g], Raffaele Badolato[g], MD, Marco De Andrea[a,d] MD, PhD and Marisa Gariglio[a] MD, PhD.

[a]Virology Unit and [b]Dermatology Unit, Department of Translational Medicine, and [c]Pathology Unit, Department of Health Sciences, Medical School of Novara, Italy; [d]Department of Public Health and Pediatric Sciences, University of Turin, Italy; [e]Division of Virology, Department of Pathology, University of Cambridge, UK; Laboratory of Molecular Virology, "Maggiore della Carità" Hospital, Novara, Italy; [g]Department of Pediatrics, Institute of Molecular Medicine Angelo Nocivelli, University of Brescia, Italy

Word count: 2532 (ref. citation included)

Number of figures: 3

Number of tables: 1

Number of references: 37

CONFLICT OF INTEREST

The authors state no conflict of interest.

Correspondence: Marisa Gariglio, Virology Unit of the Department of Translational Medicine, Medical School of Novara, Via Solaroli 17, 28110 Novara, Italy. Fax: +39 0321 620421.

E-mail address: gariglio@med.unipmn.it

Abstract

Background. The correlation between human papillomavirus (HPV) genotype along with the histopathological and clinical features of skin lesions (from genital and non-genital sites) can present a diagnostic challenge.

Objective. In this study, the correlation of HPV infection patterns with pathology and clinics was investigated in lesional and non-lesional body sites from a young patient with a primary T-cell immunodeficiency.

Methods. HPV infection was evaluated at both DNA and protein levels by PCR and immunohistochemistry.

Results. Patient's genital lesions were exclusively caused by α-genotypes (high-risk type HPV51 in the anal and low-risk type HPV72 in the penile condylomas); the opposite was true for the skin lesions, which were infected by β-genotypes only (HPV8 and 24); of which, HPV24 was the predominant type in terms of viral loads and the only one found in productive areas of infection. The patient had already developed high-grade dysplasia in the anal condylomas and showed areas of early stage dysplasia in the lesions caused by the β-genotype HPV24.

Limitations. The basic etiology of the immunodeficiency is not yet defined.

Conclusion. These findings provide proof of principle that both α and β-genotypes can cause overt dysplastic lesions when immunosurveillance is lost, which is not restricted to Epidermodysplasia verruciformis.

INTRODUCTION

Primary immunodeficiencies (PIDs) comprise a rare group of genetic disorders associated with an enhanced susceptibility to specific infections and, in certain cases, an increased incidence of malignancy.[1] Immune dysregulation leads to the reduced clearance of viruses, including human papillomavirus (HPV), which causes proliferative lesions in genital and skin sites that can also progress to cancer.[2-4]

To date, more than 150 HPV types have been completely sequenced, classified into five genera (α, β, μ, ν and γ) and a series of intragenus species, indicated by Arabic numbers, based on sequence analysis; the different types having different life-cycle characteristics and disease associations.[5,6] The most medically important HPVs belong to the genus α, which is divided into cutaneous (which cause common warts) and mucosal types; the mucosal types are further subdivided into high-risk (e.g.HPV16 and 18) and low-risk (e.g. HPV6 and 11) according to their propensity to cause cancer.[7,8] In recent years, it has become clear that many HPV types, including those contained within the β-genus, only result in asymptomatic infections in immunocompetent individuals.[9-10] However, in subjects with impaired immune function, they can cause cutaneous lesions that may become difficult to manage and in some circumstances progress to cancer.[11-13] Specific susceptibility for HPV infection has been extensively reported in patients with Epidermodysplasia Verruciformis (EV)[14-18] and warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome.[4,19,20] EV is a genodermatosis characterized by an increased susceptibility to cutaneous infections with β-genotypes. EV is thought to be an autosomal recessive disease; however, homozygous mutations in *EVER1* or *EVER2* have been identified in approximately 75% of patients clinically diagnosed with EV, leaving a considerable proportion of patients with an unexplained genetic cause. [21-23]

WHIM patients also display a specific and poorly understood susceptibility to α-HPV-induced warts.[1,19,20] Condilomas in the genital region and genital cancers, always caused by α genotypes, have also been reported in these patients. WHIM syndrome is inherited in an autosomal dominant fashion and is caused primarily by heterozygous gain-of-function mutations in the gene encoding the chemokine receptor *CXCR4* .[24,25]

More recently, patients with T cell defects associated with mutations in *RHOH* and *MST1* genes have been reported to display an increased susceptibility to β genus HPV infections.[26,27]

This study provides correlations between clinics, pathology and HPV infection patterns for both α and β genotypes in the skin lesions of a patient with an unclassified primary T-cell immunodeficiency.

MATERIALS AND METHODS

Genetic analysis

Genomic DNA was extracted from the patient's whole blood samples using the Gentra Puregene Blood Kit (Qiagen). All the coding exons and boundary introns of *EVER1* and *EVER2* genes were amplified as previously described,[12] and the products were sequenced by Primm S.r.l.. Each electropherogram was analyzed using the program Chromas Lite, version 2.01 to detect mutations.

The genetic analysis of *CXCR4, RHOH*, and *MST1* genes was carried out as previously described.[24,26,27] The PCR products were sequenced using the BigDye Terminator Kit and the sequences analyzed on a 3130 Genetic Analyzer (Applied Biosystems). Written informed consent was obtained by the patient according to the Declaration of Helsinki and approval was obtained from local ethic committee.

FACS analysis

Flow cytometry was performed as previously described.[24] Briefly, Peripheral Blood Mononuclear Cells (PBMC) ($1.5x10^6$) were resuspended in 200 μl of the appropriate medium with

CD3, CD4, CD8, CD145RA, CD45R0, CD31, CCR7, anti-HLA-DR mAbs (5 μg in 200 μl) from Beckton Dickinson..

HPV-DNA detection and Quantitative real-time PCR (Q-PCR)

Swabs and hair bulbs were taken and processed as previously described.[16-18,22,23] α-HPV-DNA genotyping was performed using the CLART® (Clinical Array Technology) Human Papillomavirus 2; Genomica, Madrid, Spain.

β-HPV-DNA analysis was performed as previously described[16] using broad spectrum PCR (PM-PCR) in combination with a reverse hybridization system (RHA) [Skin (beta) HPV assay; Diassay BV, Rijswijk, The Netherlands].[28]

Type-specific real time Q-PCR protocols were performed on a CFX96 (Biorad) using previously described primers for HPV8, 24[16,29] or the newly designed primers for HPV16, 18, 51, 61, 72 (sequences available on request). HPV DNA copy numbers were determined using standard curves as previously described.[16]

DNA-protein (FISH) or protein-protein (IF) double detection or IHC and antibodies

The polyclonal antibodies raised against beta genus HPVE4 and L1 have been previously described (CB & MG manuscript submitted).[17] For anti-E4 and beta L1 costaining, an anti-HPV5E4 monoclonal antibody was used. Antibodies to alpha genus L1 were obtained from Dako, MCM7 from Neomarkers Fremont, and $p16^{INK4a}$ from Santa Cruz Biotechnology.

Consecutive 5-μm sections obtained from FFPE tissues were processed for the immunofluorescent detection of viral antigens coupled to DNA-FISH, or for protein-protein double detection as previously described.[16,17,30]

RESULTS

The 26-year-old Caucasian male (born 1987) revealed multiple flat, reddish papular (wart-like) lesions across his whole body (Figure 1), with the highest density on the dorsum and forearms; and numerous penile and anal condylomas were also evident (Figures 2 and 3). He is HIV negative.

Immunophenotype abnormalities are compatible with T-cell lymphocytopenia

Immunophenotype analysis of the patient's peripheral blood mononuclear cells (PBMCs) revealed marked lymphopenia with depletion of CD4 at levels as low as 250 cells/ml. In addition, analysis of CD4 subsets revealed a marked reduction of naïve CD4+ CD45RA+CCR7+ cells (3.3%) and of the recent thymic emigrant subset (RTE), (CD45RA+CCR7+CD31+: 1.3%), while central memory (CD45RA-CCR7+: 60.5 %) and effector memory T cells (CD45RA-CCR7-: 36%) were proportionally increased. Likewise, naïve CD8+ cells were decreased with a relative increase in central memory and effector CD8+ cells, indicating a depletion of the naïve compartment for both CD4 and CD8 cells. Analysis of HLA-DR expression by T cells showed that about 50% of them display an active phenotype. The patient's B cells were found to make up 1.3% of total lymphocytes; neutrophils and immunoglobulin levels were in the normal range.

Absence of mutations in genes known to be associated with similar PIDs

Genomic DNA extracted from the patient's blood was used to perform genetic analysis of genes associated with EV, such as *EVER*1 and *EVER2,*[21] or with immunodeficiencies characterized by susceptibility to HPV infections, including *CXCR4, RHOH*, and *MST1*.[24,26,27] Sequence analysis of these genes did not reveal any causative mutation.

Alpha versus Beta genotype distributions

The DNA extracted from swabs obtained from the skin of either affected or unaffected sites, and from plucked eyebrow or inguinal hair bulbs was analyzed by PCR and real time Q-PCR for α and β-HPV genotypes. As shown in Table 1, four α-genotypes were found in hair bulbs from both sites with a very low viral load. By contrast, only HPV8 and 24 β-genotypes were found in these sites with the highest load values reported for HPV24 in affected skin areas (up to $6x10^3$

copies/cell). In the swabs from the anal condylomas surface, HPV51 gave high viral loads (228 copies/cell) followed by HPV61 and 72 (both considered low-risk α-genotypes). HPV72 was also detected in the swabs from penile condylomas. Overall, the patient showed a very clear and consistent HPV signature defined by two β-genotypes, HPV8 and 24, the α-genotypes HPV51 and 72 with high viral loads and traces of HPV16, 18, and 61.

Comparison of Alpha versus Beta viral life cycle and their differential modulation of cellular markers

Biopsies from anal, penile condylomas, and two wart-like lesions of the skin were available as formalin-fixed paraffin-embedded (FFPE) blocks. To gain further insight the infection pattern and visualize viral life cycle events of α versus β-genotypes, tissue sections from these blocks were co-stained by immunofluorescence (IF) for anti-E4 and anti-L1 antibodies to characterize the expression of viral antigens and for antibodies raised against minichromosome maintenance protein 7 (MCM7), a marker of cellular proliferation.[31] Fluorescent *in situ* hybridization (FISH) was carried out for the virus genotypes detected by surface sampling.[32] As shown in Figure 2a right hand column, the anal condylomas showed areas with high-grade dysplasia that displayed $p16^{INK4a}$ staining across basal and suprabasal epithelial layers. FISH analysis for the HPV51 genome revealed many positive nuclei throughout the entire lesion, especially in the areas with lower grade of dysplasia, while HPV16, 18, 61, and 72 genomic probes gave negative results (data not shown). Expression of the late capsid protein L1 was also detected in the superficial layers. As reported for cervical cancer induced by high-risk α-genotypes (e.g. HPV16 and 18), a massive increase of E2F-activated genes was revealed, as visualized by staining for the cellular MCM7 protein, which extended throughout the entire epithelium.[7]

Figure 2b, shows the histological features of the penile condylomas which revealed hyperplasia and low-grade dysplasia with many HPV72-FISH-positive nuclei, while FISH analysis for HPV16, 18, 51, and 61 genomes was negative as was p16^{INK4a} staining (data not shown). The MCM7 signal was only being apparent in the upper epithelial layers.[7,31]

A different staining pattern was visualized in the cutaneous lesions. As shown in Figure 3, the epithelium of the flat wart-like lesions displayed the unequivocal histological features associated with HPV infection by cutaneous genotypes. In these areas, co-immunostaining of HPV24-DNA by FISH and E4 by immunofluorescence revealed the presence of many cells exhibiting intense HPV24 DNA-positive nuclei and cytoplasmic E4 staining. In contrast, viral genome amplification was no longer detected in the central dysplastic area by FISH, while cytoplasmic E4 expression was still present in the more superficial layers. FISH analysis for HPV8 was negative, as it was also for HPV51 and 72 (data not shown). MCM7 expression was increased in the lesion in comparison with the adjacent normal epithelium, and was well evident in the basal layers, extending into the suprabasal layers in the productive areas and to a higher extent in the dysplastic central area. p16^{INK4a} staining was negative throughout the entire lesion (data not shown). Expression of the major coat protein L1 occurred in a subset of E4-positive cells in the upper epithelial layers in the areas displaying FISH-positive nuclei, while it showed an aberrant cytoplasmic expression in the mid-superficial layers in the central dysplastic area.

DISCUSSION

The present study describes a case of primary T-cell immunodeficiency with a remarkable and specific susceptibility to HPV infections, who does not carry any of the genetic mutations currently associated with EV,[21] and WHIM syndrome.[25] He has a T-cell defect characterized by abnormally low numbers of naïve T cells (affecting both the CD4+ and CD8+ compartments), very likely due to a developmental defect of T lymphocytes, and high numbers of memory T cells presenting an exhausted phenotype that probably results from chronic viral infection. Despite some commonalities with PIDs harboring mutations in *RHOH* and *MST1* genes, sequence analysis of these genes did not reveal any causative mutation.[26,27] The lack of primary lymphedema also excludes any correlation with WILD syndrome.[33] The patient suffered some recurrent bacterial infections during his childhood; but since his teenage years, he has not had any major health problems other than those resulting from the HPV infection. Another interesting feature of this patient is that his susceptibility to HPV infection involves both cutaneous and genital sites. This is very different from the situation in EV patients where susceptibility is considered to be restricted to the β genus, as genital lesions caused by α genus have never been reported in this setting.[11,14,34]

Characterization of the HPV infection pattern by both PCR and immunohistochemistry (for the viral proteins E4 and L1) in a number of lesional and non-lesional body sites revealed that the patient's genital lesions were exclusively caused by α-genotypes (high-risk type HPV51 in the anal condylomas and low-risk type HPV72 in the penile condylomas); the opposite was true for the skin lesions, which were infected by β-genotypes only. Two β-genotypes were found, namely HPV8 and 24; of which, HPV24 was always the predominant type in terms of viral loads and the only one found in productive areas of infection. These HPV24-induced skin lesions provide a good example of the cytopathic effect caused by the β-genotypes, which display unique features compared with those reported for the α-types: the lesions are characterized by enlarged cells with prominent blue-grey pallor, perinuclear halos, and cytoplasmic granuli. Visualization of the E4 viral protein was also confirmed as an invaluable marker for the detection of areas of productive infection for the β-genotypes, as its expression consistently overlaps with areas of viral genome amplification as detected by FISH.[35] Consistent with the data reported for cervical lesions caused by high-risk genotypes (e.g. HPV16 and 18),[35,36] stimulation of cell cycle entry was very apparent in the basal and above layers in the HPV51-positive condyloma (high-risk α-type), with many cells being driven through mitosis. In the lesion caused by HPV72 (low-risk α-type), the stimulation of cell cycle entry in the basal layers was much less obvious, and the MCM7 signal indicating cell cycle re-entry (but not cell division) was only apparent in cells of the mid-epithelial layers where viral genome amplification was shown to take place. The lower ability of low-risk HPV types to drive cell proliferation is currently correlated with a lower incidence in neoplasia.[7] Consistent with this finding, $p16^{INK4a}$ overexpression was only observed in the anal condylomas caused by HPV51.

In the HPV24-positive lesions, cytoplasmic E4 expression was constantly found in the areas displaying clear-cut cytopathic effects that coincided with viral genome amplification and expression of the late structural protein L1 in dying superficial cells, as has been reported for many Papillomaviruses.[37] Of interest, in these productive areas, MCM7 expression was very strong and always present in the basal and some of the above layers, indicating that cells were stimulated to entry the cell cycle. In addition, a dysplastic area was found where MCM7 expression extended throughout the epithelium in the absence of detectable viral genome amplification, but with E4 expression maintained in the superficial layers. This MCM7 staining pattern was closer to that of the high-risk α−genotypes rather than low-risk types, indicating that β-HPV replication drives the cells above the basal layer to enter the cell cycle in order to facilitate the amplification of its genome.[31] This observed stimulation of basal cell proliferation may contribute, in association with other transforming agents, such as UVB irradiation, to the transformation process.[11,12,14]

Although the patient was very young (26 years), he had already developed high-grade dysplasia in some genital condylomas and also showed areas of early stage dysplasia in the skin lesions caused by the β-genotype HPV24. These findings prompt us to propose the following affirmations: i) symptomatic β-HPV infection of the skin is not restricted to patients harboring EVER gene deficiencies, which are thought to be compromised at the keratinocyte level; ii) β-HPV susceptibility is primarily associated with loss of immunosurveillance, rather than with alteration of the infected keratinocytes, as demonstrated in this patient and all the other reported PIDs without EVER genes mutations; iii) the patient's inability to clear HPV infections has led to the uncontrolled replication of a few genotypes from both the α and β genera with a clear-cut tropism;

iii) both genera are causing proliferative lesions with a high probability of progressing to invasive cancer. It is indeed very likely that he will develop skin cancer with a more aggressive phenotype in the future, as can be envisaged from the dysplastic area already found in a skin wart-like lesion and the clinical picture of his forehead.

Overall, our findings provide further compelling evidence that in the immunocompromised host, regardless of his EVER gene genetic status, persistence of high rate replication of β-genotypes causes skin proliferative lesions with a documented risk of progression to skin cancer.

REFERENCES

1. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. J Allergy Clin Immunol 2011;127:1329-41 e1322; quiz 1342-23.
2. Leiding JW, Holland SM. Warts and all: human papillomavirus in primary immunodeficiencies. J Allergy Clin Immunol 2012;130:1030-48.
3. Cubie HA. Diseases associated with human papillomavirus infection. Virology 2013;445:21-34.
4. Sri JC, Dubina MI, Kao GF, Rady PL, Tyring SK, Gaspari AA. Generalized verrucosis: a review of the associated diseases, evaluation, and treatments. J Am Acad Dermatol 2012;66:292-311.
5. Bravo IG, de Sanjose S, Gottschling M. The clinical importance of understanding the evolution of papillomaviruses. Trends Microbiol 2010;18:432-38.
6. Bernard HU. Taxonomy and phylogeny of papillomaviruses: an overview and recent developments. Infect Genet Evol 2013;18:357-61.
7. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine 2012;30 Suppl 5:F55-70.
8. McLaughlin-Drubin ME, Meyers J, Munger K. Cancer associated human papillomaviruses. Curr Opin Virol 2012;2:459-66.
9. Feltkamp MC, de Koning MN, Bavinck JN, Ter Schegget J. Betapapillomaviruses: innocent bystanders or causes of skin cancer. J Clin Virol 2008;43:353-60.
10. Foulongne V, Sauvage V, Hebert C, Dereure O, Cheval J, Gouilh MA, et al. Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing. PLoS One 2012;7:e38499.
11. Akgul B, Cooke JC, Storey A. HPV-associated skin disease. J Pathol 2006;208:165-75.

12. Nindl I, Gottschling M, Stockfleth E. Human papillomaviruses and non-melanoma skin cancer: basic virology and clinical manifestations. Dis Markers 2007;23:247-59.

13. Bouwes Bavinck JN, Plasmeijer EI, Feltkamp MC. Beta-papillomavirus infection and skin cancer. J Invest Dermatol 2008;128:1355-58.

14. Pfister H. Chapter 8: Human papillomavirus and skin cancer. J Natl Cancer Inst Monogr 2003:52-6.

15. Patel T, Morrison LK, Rady P, Tyring S. Epidermodysplasia verruciformis and susceptibility to HPV. Dis Markers 2010;29:199-206.

16. Dell'Oste V, Azzimonti B, De Andrea M, Mondini M, Zavattaro E, Leigheb G, et al. High beta-HPV DNA loads and strong seroreactivity are present in epidermodysplasia verruciformis. J Invest Dermatol 2009;129:1026-34.

17. Borgogna C, Zavattaro E, De Andrea M, Griffin HM, Dell'Oste V, Azzimonti B, et al. Characterization of beta papillomavirus E4 expression in tumours from Epidermodysplasia Verruciformis patients and in experimental models. Virology 2012;423:195-204.

18. Landini MM, Zavattaro E, Borgogna C, Azzimonti B, De Andrea M, Colombo E, et al. Lack of EVER2 protein in two epidermodysplasia verruciformis patients with skin cancer presenting previously unreported homozygous genetic deletions in the EVER2 gene. J Invest Dermatol 2012;132:1305-08.

19. Palm MD, Tyring SK, Rady PL, Tharp MD. Human papillomavirus typing of verrucae in a patient with WHIM syndrome. Arch Dermatol 2010;146:931-32.

20. Dotta L, Tassone L, Badolato R. Clinical and genetic features of Warts, Hypogammaglobulinemia, Infections and Myelokathexis (WHIM) syndrome. Curr Mol Med 2011;11:317-25.

21. Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet 2002;32:579-81.

22. Azzimonti B, Mondini M, De Andrea M, Gioia D, Dianzani U, Mesturini R, et al. CD8+ T-cell lymphocytopenia and lack of EVER mutations in a patient with clinically and virologically typical epidermodysplasia verruciformis. Arch Dermatol 2005;141:1323-25.

23. Zavattaro E, Azzimonti B, Mondini M, De Andrea M, Borgogna C, Dell'Oste V, et al. Identification of defective Fas function and variation of the perforin gene in an epidermodysplasia verruciformis patient lacking EVER1 and EVER2 mutations. J Invest Dermatol 2008;128:732-35.

24. Gulino AV, Moratto D, Sozzani S, Cavadini P, Otero K, Tassone L, et al. Altered leukocyte response to CXCL12 in patients with warts hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome. Blood 2004;104:444-52.

25. Tassone L, Notarangelo LD, Bonomi V, Savoldi G, Sensi A, Soresina A, et al. Clinical and genetic diagnosis of warts, hypogammaglobulinemia, infections, and myelokathexis syndrome in 10 patients. J Allergy Clin Immunol 2009;123:1170-73, 1173 e1171-73.

26. Crequer A, Picard C, Patin E, D'Amico A, Abhyankar A, Munzer M, et al. Inherited MST1 deficiency underlies susceptibility to EV-HPV infections. PLoS One 2012;7:e4401 0.

27. Crequer A, Troeger A, Patin E, Ma CS, Picard C, Pedergnana V, et al. Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. J Clin Invest 2012;122:3239-47.

28. de Koning M, Quint W, Struijk L, Kleter B, Wanningen P, van Doorn LJ, et al. Evaluation of a novel highly sensitive, broad-spectrum PCR-reverse hybridization assay for detection and identification of beta-papillomavirus DNA. J Clin Microbiol 2006;44:1792-1800.

29. Schaper ID, Marcuzzi GP, Weissenborn SJ, Kasper HU, Dries V, Smyth N, et al. Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. Cancer Res 2005;65:1394-1400.

30. Peh WL, Doorbar J. Detection of papillomavirus proteins and DNA in paraffin-embedded tissue sections. Methods Mol Med 2005;119:49-59.

31. Middleton K, Peh W, Southern S, Griffin H, Sotlar K, Nakahara T, et al. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. J Virol 2003;77:10186-201.

32. de Koning MN, Khoe LV, Eekhof JA, Kamp M, Gussekloo J, Ter Schegget J, et al. Lesional HPV types of cutaneous warts can be reliably identified by surface swabs. J Clin Virol 2011;52:84-7.

33. Kreuter A, Hochdorfer B, Brockmeyer NH, Altmeyer P, Pfister H, Wieland U. A human papillomavirus-associated disease with disseminated warts, depressed cell-mediated immunity, primary lymphedema, and anogenital dysplasia: WILD syndrome. Arch Dermatol 2008;144:366-72.

34. Gul U, Kilic A, Gonul M, Cakmak SK, Bayis SS. Clinical aspects of epidermodysplasia verruciformis and review of the literature. Int J Dermatol 2007;46:1069-72.

35. Doorbar J. The E4 protein; structure, function and patterns of expression. Virology 2013;445:80-98.

36. Griffin H, Wu Z, Marnane R, Dewar V, Molijn A, Quint W, et al. E4 antibodies facilitate detection and type-assignment of active HPV infection in cervical disease. PLoS One 2012;7:e49974.

37. Yemelyanova A, Gravitt PE, Ronnett BM, Rositch AF, Ogurtsova A, Seidman J, et al. Immunohistochemical detection of human papillomavirus capsid proteins L1 and L2 in squamous intraepithelial lesions: potential utility in diagnosis and management. Mod Pathol 2012;26:268-74.

FIGURE LEGENDS

Figure 1. Photographs of affected skin areas in the study patient. The top picture shows the flat, reddish papular lesions (wart-like) on the back. The inset is a higher magnification of these papular lesions; the bottom picture shows the forehead with many red, flat-topped, small papular lesions.

Figure 2. Distribution of the viral L1 protein, HPV DNA, and cellular markers (MCM7 and p16^{INK4a}) in biopsies from anal (a) and penile (b) condylomas. (**a**) The top pictures show a biopsy tissue section stained using H&E corresponding to areas of low-grade (left column) and high-grade dysplasia (right column). The panels in the second row display the same section stained for HPV51 DNA using FISH (red) to visualize the cells in which viral genome amplification was occurring. In the third row, a serial section was stained for the cellular proliferation marker MCM7 (red). The image of the fourth row left column shows a serial section stained with antibodies to the late capsid protein L1 (green). The white dotted line indicates the basal layer. All sections were counterstained with DAPI (blue) to visualize cell nuclei. The lower left picture and the image in fourth row right column show a serial section stained for the cellular protein p16^{INK4a} by immunoenzymatic staining. The bottom right picture presents a photograph of the anal condylomas. (**b**) The top picture shows the H&E staining pattern in a biopsy section of the penile condylomas. In the lower panel, the same section was stained for HPV72 DNA using FISH to detect viral genome amplification (red). A serial section was double stained with antibodies to the cellular proliferation marker MCM7 (red), third image from the top, and the late capsid protein L1 (fourth image from the top) (green). The white dotted line indicates the basal layer. All sections were counterstained with DAPI (blue) to visualize cell nuclei. The bottom picture shows a photograph of the penile condylomas. Scale Bar = 100 µm.

Figure 3. Distribution of viral proteins E4 and L1, HPV DNA, and MCM7 (marker of cell proliferation) in biopsies from a papular wart-like lesion of the neck shown in Figure 1. The top pictures show H&E staining in biopsy sections. The panels below in the left hand column correspond to the region indicated by the red rectangle in the H&E image, showing a dysplastic area; while panels in the central column correspond to the region indicated by the red square, showing a productive area with the classical β-HPV-induced cytopathic effects. The right hand column shows the edge of the lesion at its interface with the normal epithelium. In the upper panels, sections first stained with H&E were then double stained for the early viral protein E4 expression (green) and viral genome amplification by HPV24 DNA-FISH (red). The central panels show serial sections double stained with antibodies to the late viral capsid protein L1 (red) and E4 (green). The lower panels show serial sections immunostained for the cell proliferation marker MCM7. All sections were counterstained with DAPI (blue) to visualize cell nuclei. The white dotted line indicates the basal layer. Scale bar = 50 μm.

Abbreviations

HPV, Human Papillomavirus; PID, primary immunodeficiency;EV, Epidermodysplasia Verruciformis; WHIM, warts hypogammaglobulinemia infections and myelokathexis; PBMC, peripheral blood mononuclear cells; RTE, recent thymic emigrant; FFPE, formalin-fixed paraffin embedded; MCM, minichromosome maintenance protein; FISH, Fluorescent *in situ* hybridization; HSIL, high grade squamous intraepithelial lesion; WILD, Warts, depressed cell-mediated Immunity, primary Lymphedema, and anogenital Dysplasia.

ACKNOWLEDGMENTS

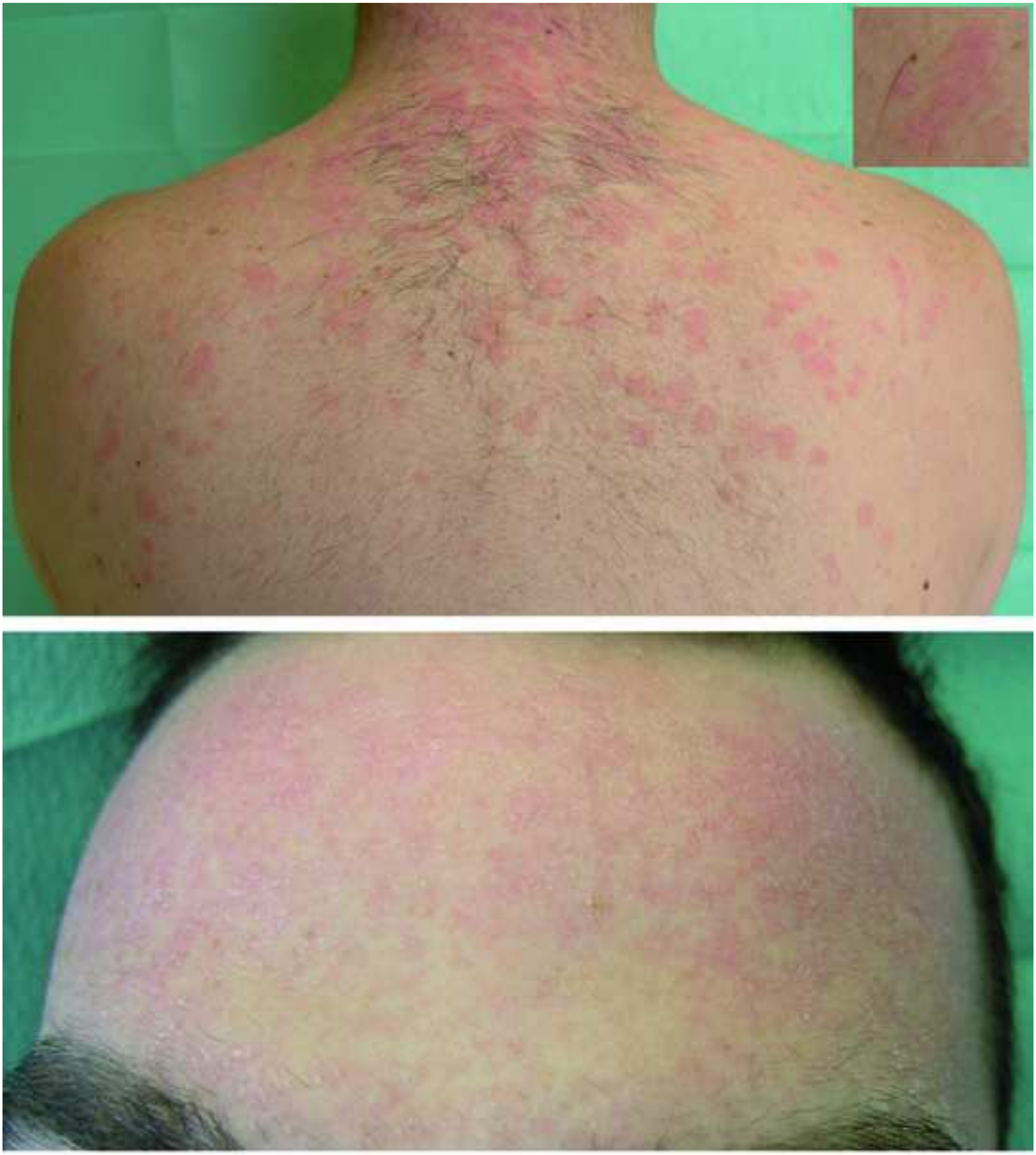
We gratefully acknowledge "Fondazione Banca Popolare di Novara per il territorio" for their contribution in purchasing the digital scanner Pannoramic MIDI. This work was supported by grants from Compagnia di San Paolo (CSP2012 to M.G.) and Associazione Italiana per la Ricerca sul Cancro - AIRC (IG 2012 to M.G.), ESCMID Research Grant 2013 (ESCMID 2013 to C.B.) and from the Ministry of Education, Universities and Research – MIUR (FIRB 2008 to M.D.A.). The PhD fellowship of A. P. is funded by "Fondazione Franca Capurro per Novara Onlus".

Table
Click here to download Table: Landini Table 1.doc

Table 1. Human Papillomavirus DNA genotyping in swabs and hair bulbs from different body sites

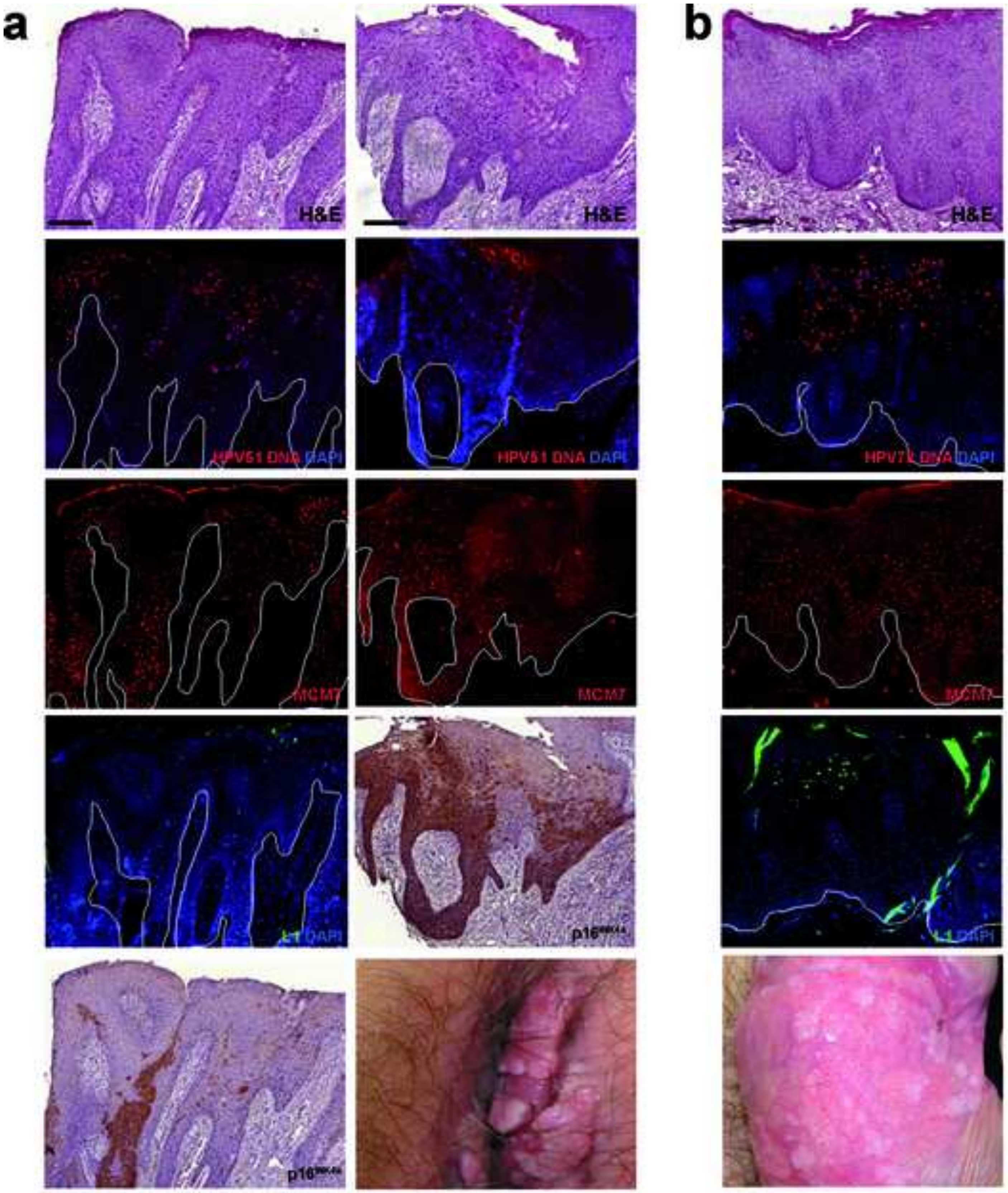
Samples	**alpha HPV types (copies/cell)**	**beta HPV types (copies/cell)**
Hair bulbs		
Eyebrows	**18** (**<**0.1), **51** (<0.1), **61** (<0.1)	**8** (16), **24** (273)
inguinal hair	**16** (<0.1), **51** (<0.1), **61** (<0.1)	**8** (0.2), **24** (19)
Swabs		
forehead (macular lesion)	**51** (<0.1)	**8** ($1x10^3$), **24** ($6x10^3$)
arm (normal skin)	**51** (1)	**8** (<0.1), **24** ($1x10^5$)
anal region (condyloma)	**51** (228), **61** (2), **72** (60)	**8** (<0.1), **24** (80)
buttock (normal skin)	**51** (<0.1), **61** (<0.1)	**8** (<0.1), **24** ($2x10^3$)
penis (condyloma)	**51** (<0.1), **72** (4)	**8** (<0.1), **24** (8)
genital region (normal skin)	**51** (<0.1)	**8** (<0.1), **24** ($2x10^3$)

Figure 1
Click here to download high resolution image



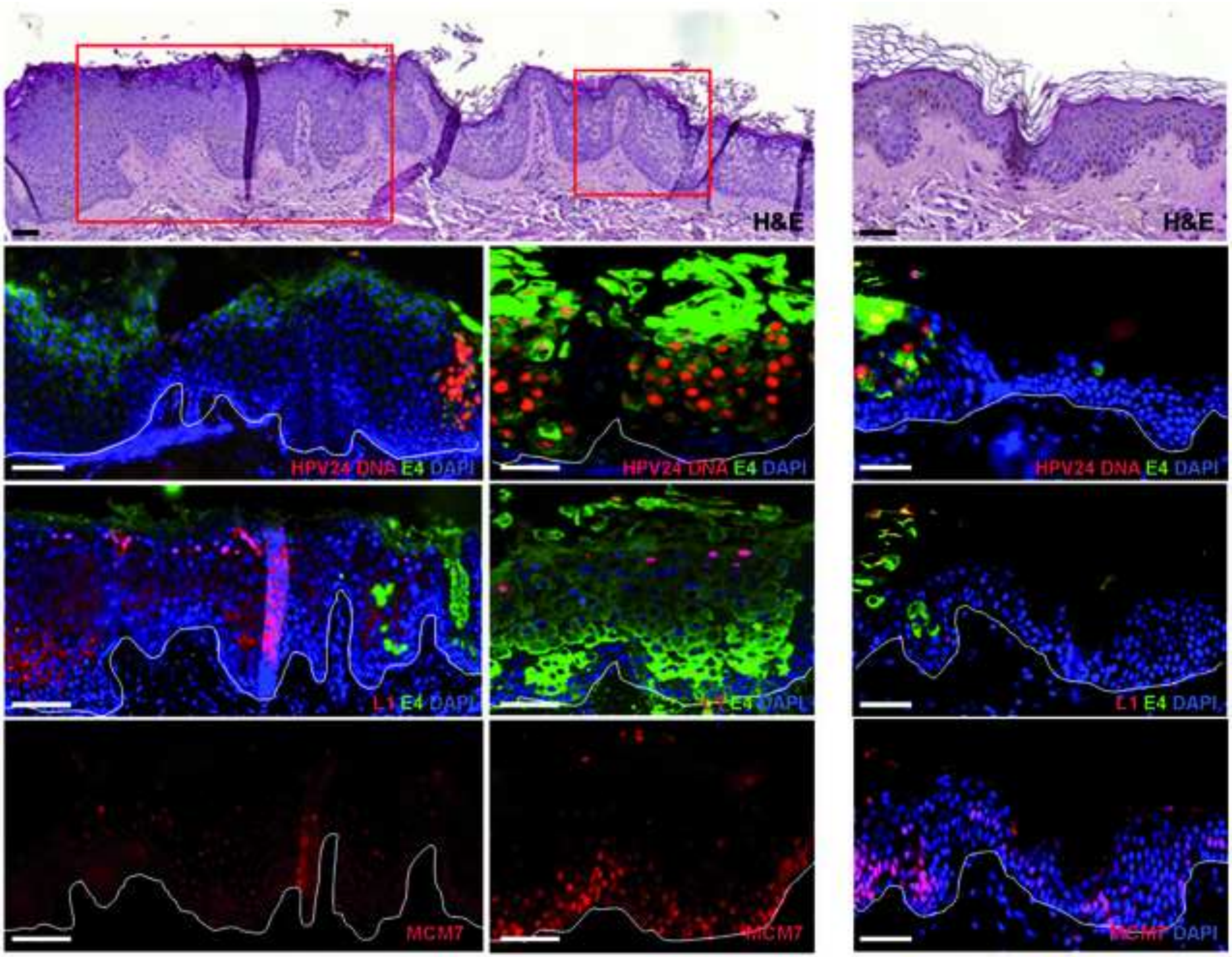
Landini et al., Figure 1

Figure 2
Click here to download high resolution image



Landini et al,. Figure 2

Figure 3
Click here to download high resolution image



Landini et al., Figure 3