Whole-Transcriptome Sequencing–Based Profiling of the Cutaneous Virome in Patients with Secondary Immunodeficiency

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Most viral infections can be self-limited, with no requirement for medical intervention. However, the same viruses can cause severe diseases in patients with compromised immunity due to single-gene diseases, acquired immune deficiency syndrome, or hematologic malignancies or those receiving immunosuppressive drugs. Occasionally, these immunocompromised patients harbor >1 infectious agent, requiring several concomitant diagnostic tests. We have developed, to our knowledge, a previously unreported whole-transcriptome sequencing–based pipeline that allows virome profiling, quantitation, and expression pattern analysis of 926 distinct viruses by sequencing of RNA isolated from a single lesional skin biopsy. This pipeline can also explore host genetics if there is a Mendelian predisposition to infection. We applied this pipeline to 6 Iranian patients with viral-induced skin lesions associated with immune deficiency secondary to HIV, human T-lymphotropic virus 1, chronic lymphocytic leukemia, and post transplant immunosuppression. In 5 cases, definitive human papillomavirus infections were identified, some caused by multiple viral types. In addition to human papillomavirus, coinfection with other viruses (Merkle cell polyomavirus, cytomegalovirus, and human herpesvirus 4) was detected in some lesions. In 1 case, whole-transcriptome sequencing validated the clinical diagnosis of adult T-cell leukemia/lymphoma in a patient with an initial diagnosis of mycosis fungoides/Sézary syndrome. These findings attest to the power of whole-transcriptome sequencing in profiling the cutaneous virome in the context of compromised immunity.

Keywords: Cutaneous virome, Human papillomavirus, Immunodeficiency, RNA-Seq, Whole-transcriptome sequencing

JID Innovations (2024);**4:**100278 doi:10.1016/j.xjidi.2024.100278

INTRODUCTION

The human cutaneous virome, a compilation of up to 1000 genetically distinct viruses, can cause skin infections that may remain latent and asymptomatic or can cause cutaneous diseases that can be severe, particularly in patients with compromised immunity. As an example, human papillomaviruses (HPVs), which comprise a group of 441 different types belonging to 5 distinct subfamilies, can cause a spectrum of skin disorders with a phenotypic range from self-limited cutaneous warts, some of which can become recalcitrant to genetically determined conditions, such as epidermodysplasia verruciformis and the Tree man syndrome

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Abbreviations: CMV, cytomegalovirus; HPV, human papillomavirus; HTLV1, human T-lymphotropic virus 1; MAEC, maximum exon coverage; RNA-Seq, RNA-sequencing

Received 11 March 2022; revised 17 February 2024; accepted 22 February 2024; accepted manuscript published online XXX; corrected published online XXX

Cite this article as: JID Innovations 2024;4:100278

(McBride, 2022; Uitto et al, 2022). Many families have a well-defined immune deficiency due to compromised T-cell immunity or the keratinocyte-intrinsic immune system (Biglari et al, 2024). In addition to genetically determined susceptibility to viral infections in patients with primary immune deficiency, many cases have been documented where the cutaneous lesions are associated with acquired immune deficiency, for example, secondary to acquired immune deficiency syndrome or human T-lymphotropic virus 1 (HTLV1) infections (Akinboro et al, 2013; Cockerell, 1991; Valencia and Moreno, 2017).

Previously, some technologies based on antibody characterization or RNA and DNA analysis such as low-throughput methods have been applied for viral typing in cutaneous lesions. Some of them are strictly limited to specific subtypes of viruses, such as the presence of high- and low-risk oncogenic HPVs. Recently, high-throughput DNA-based next-generation sequencing technologies have been developed to detect virome. These DNA-based next-generation sequencing detect the presence of the viral genomes and cannot determine active viral infections (Béziat et al, 2021). Whole-transcriptome sequencing—based technologies have the advantage of detecting active viral agents in the tissues. We have recently developed a whole transcriptome—based pipeline that allows concomitant detection of the cutaneous virome and characterization of the underlying genetic alterations in the immune-associated genes in patients with primary immune deficiency due to inborn errors of immunity (Saeidian et al, 2023, 2022). In this study, we have tested the applicability of this pipeline to determine the viral profile in cutaneous lesions in patients with secondary immune deficiencies, often with multiple infectious agents, including patients with acquired immune deficiency syndrome, endemic HTLV1 infection, chronic lymphocytic leukemia, and immunosuppressive treatment after renal transplantation.

RESULTS

Biopsies were obtained from skin lesions and adjacent normal-appearing skin from 6 patients with secondary immune deficiencies. RNA was isolated, and the recently developed whole-transcriptome—based RNA-sequencing (RNA-Seq) pipeline was applied to determine the viral profile using bioinformatics analysis of distinct viruses in the pipeline reference database. The compiled viral genome reference consisted of 926 unique viral species, including over 441 different HPV types; the sequence information on these viruses is available from the National Center for Biotechnology Information. This pipeline provides virus-specific data on RNA obtained from skin biopsies, first by excluding human sequences on the basis of genome and transcriptome reference databases. The bioinformatics analysis then allows the identification of specific viruses by the variant caller FreeBayes, annotating the output with the variant call file SnpEff and quantifying the absolute count of viral features, including exons and transcripts. Combining these features allows for development of a consensus sequence for identifying the viral profile in cutaneous lesions. The relative quantity of the virus was expressed as the maximum exon coverage (MAEC), and the expression profiles of the viruses were visualized by Sashimi plots in the context of the corresponding viral genome. These approaches were applied to 6 patients with skin lesions associated with secondary immune deficiency.

Case reports

Patient 1. A male patient aged 46 years with HIV under treatment from 7 years ago presented with an 8-month history of extensive warts on the neck and the genital area. Histopathology of these lesions was consistent with the HPV infection (Figure 1a and b). He received several cryotherapy treatments for these warts, but there was no improvement. Oral acitretin treatment, 25 mg 3 times weekly, was initiated, and after 3 months of follow-up, there was a complete resolution of these lesions. The patient was also receiving medications for acquired immune deficiency syndrome. Virome analysis revealed the presence of HPV types 3 and 76 in cutaneous lesions, but HPV3 was the predominant type, with MAEC almost 100 times higher than that of HPV76 (57,218 vs 640). However, full-length transcripts of both viruses, including L1 and L2 genes, were detected, implying active infection (Figure 1c). These HPVs were absent from adjacent normal cutaneous tissue. HIV was not detectable from the cutaneous lesion.

Patient 2. A female aged 29 years with a history of chronic renal disease had undergone kidney transplantation with the subsequent immunosuppressive treatment consisting of 1 g/day mycophenolate mofetil, 3.5 mg/d tacrolimus, and 5 mg/d prednisolone. Subsequently, she developed multiple hyperkeratotic warts on her hands and feet that became increasingly severe and resistant to treatment by cryotherapy, topical salicylic acid, and carbon dioxide laser (Figure 1d). Histopathology of the skin lesions was consistent with a viral infection (Figure 1e). The whole-transcriptome analysis identified the presence of HPV2 in the patient's warts, which was absent in adjacent normal cutaneous tissue (Figure 1f).

Patient 3. A male aged 59 years with negative results on immunophenotyping and HIV test had a history of severe acute respiratory syndrome coronavirus 2 infection in October 2020 that did not require hospitalization. However, he presented with complaints of asymptomatic papules on his right hand since March 2021, and their number progressively increased over time. On physical examination, multiple erythematous and violaceous firm papules of varying sizes were noted, and the lesions extended to the right hand and forearm. The initial clinical diagnosis was Kaposi's sarcoma, and skin biopsies from the lesions revealed the proliferation of spindle cells with thin, slit-like vascular spaces, supportive of this diagnosis (Figure 1g and h). However, the immunohistochemistry of excisional tissue for human herpesvirus 8 was negative. Virome analysis identified low levels of HPV128 (MAEC = 84), and expressions of L1 and L2 genes was very low, suggesting the presence of the inactive virus (Figure 1j). According to the immunochemistry result, RNA-Seq did not detect the Kaposi sarcoma—associated herpesvirus, also known as human herpesvirus 8. Cryotherapy was used to treat minor skin lesions. The large lesions were treated by total excision.

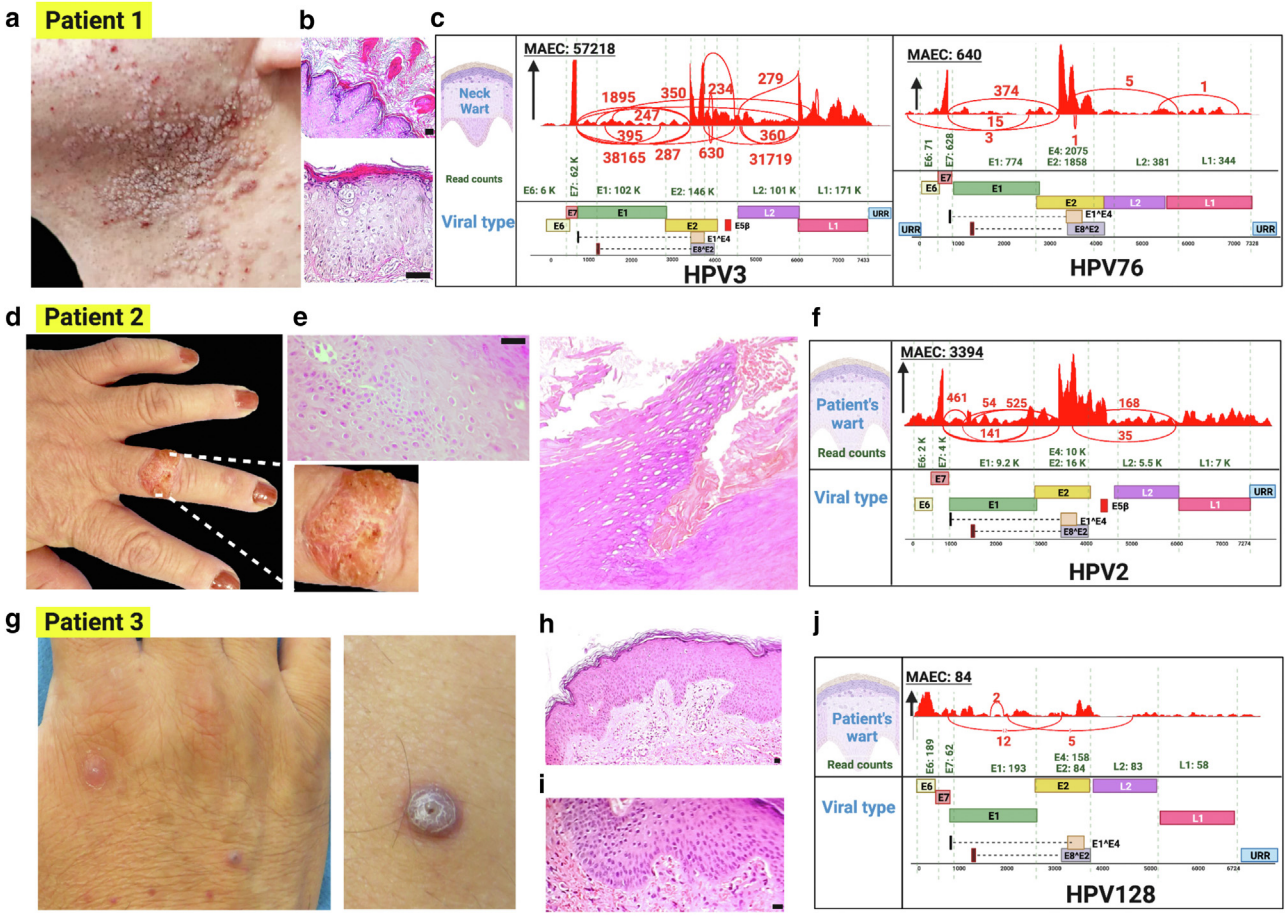

Figure 1. Virome detection in recalcitrant warts and cutaneous lesions in patients affected by SIDs. (**a**) A male aged 46 years (patient 1) with AIDS had extensive warts on the neck and genital area. (**b**) Histopathology showed acanthosis, orthokeratosis, and marked papillomatosis with hypergranulosis. (**c**) RNA-Seq analysis by VirPy detected HPV76 and HPV3 in neck warts. (**d**) A female aged 29 years (patient 2) with kidney transplantation developed recalcitrant warts on her hands and feet. (**e**) Histopathology revealed hyperkeratosis and koilocytes (H&E staining). (**f**) RNA-Seq analysis by VirPy detected HPV2 in the wart. (**g**) A male aged 59 years (patient 3) affected by COVID-19 developed asymptomatic erythematous papules on his right hand. (**h, i**) Skin histopathology revealed the proliferation of spindle cells with thin, slit-like vascular spaces and erythrocyte extravasation consistent with Kaposi's sarcoma. RNA-Seq compatible with negative HHV8 immunochemistry could not detect transcripts of this virus. (**j**) RNA-Seq of a skin lesion detected HPV128 (bar = 50 μm for all histopathology slides). AIDS, acquired immune deficiency syndrome; HHV8, human herpesvirus 8; HPV, human papillomavirus; MAEC, maximum exon coverage; RNA-Seq, RNA sequencing; SID, secondary immunodeficiency; URR, upstream regulatory region.

Patient 4. A female aged 25 years presented to the emergency department with weakness, blurred vision, and dizziness. HIV-positive status was identified during a previous admission 1 month prior when the CD4+ count was 10 (the normal range is between 500 and 1500 cells/mm^{-3}). Hepatitis C virus and hepatitis B virus coinfections were not present. Upon physical examination, cytomegalovirus (CMV) retinitis and a perianal skin ulcer were noted, and cerebrospinal fluid was positive for CMV. Skin biopsies were obtained from the perianal lesion and perilesional normal-appearing skin (Figure 2a). The histopathology and immunohistochemistry staining confirmed the CMV infection (Figure 2b and c). Transcriptome RNA-Seq analysis revealed the presence of HPV2 and HIV at low levels in the perianal lesion. In addition, human herpesvirus 5 and human herpesvirus 4 were detected in the lesion and not in the normal adjacent tissue (Figure 2d). The patient was treated with antiretroviral therapy as well as intravenous and intravitreal ganciclovir. The skin lesion healed with mild postinflammatory hyperpigmentation, the subsequent ophthalmologic examination was typical, and the CMV viral load in cerebrospinal fluid was undetectable.

Patient 5. A male aged 68 years presented with extensive warts. The patient had a medical history of chronic lymphocytic leukemia, which was being treated with chemotherapy. A few months after chemotherapy, he started developing multiple warts that extended to the neck, axilla, and anogenital area (Figure 3a). Histopathology of these lesions was consistent with a viral infection (Figure 3b). Virome analysis of warts revealed a high level of expression of HPV6 in genital warts (MAEC = 35,454). In addition, the expression of HPV14 was noted in the genital and nongenital warts. In addition, warts from the genital area expressed the Merkel cell polyomavirus (Figure 3c). Merkle cell polyomavirus was detected in about 25% of these chronic lymphocytic leukemia cases. The role of Merkle cell polyomavirus in oncogenesis is still unknown (Pantulu et al, 2010)

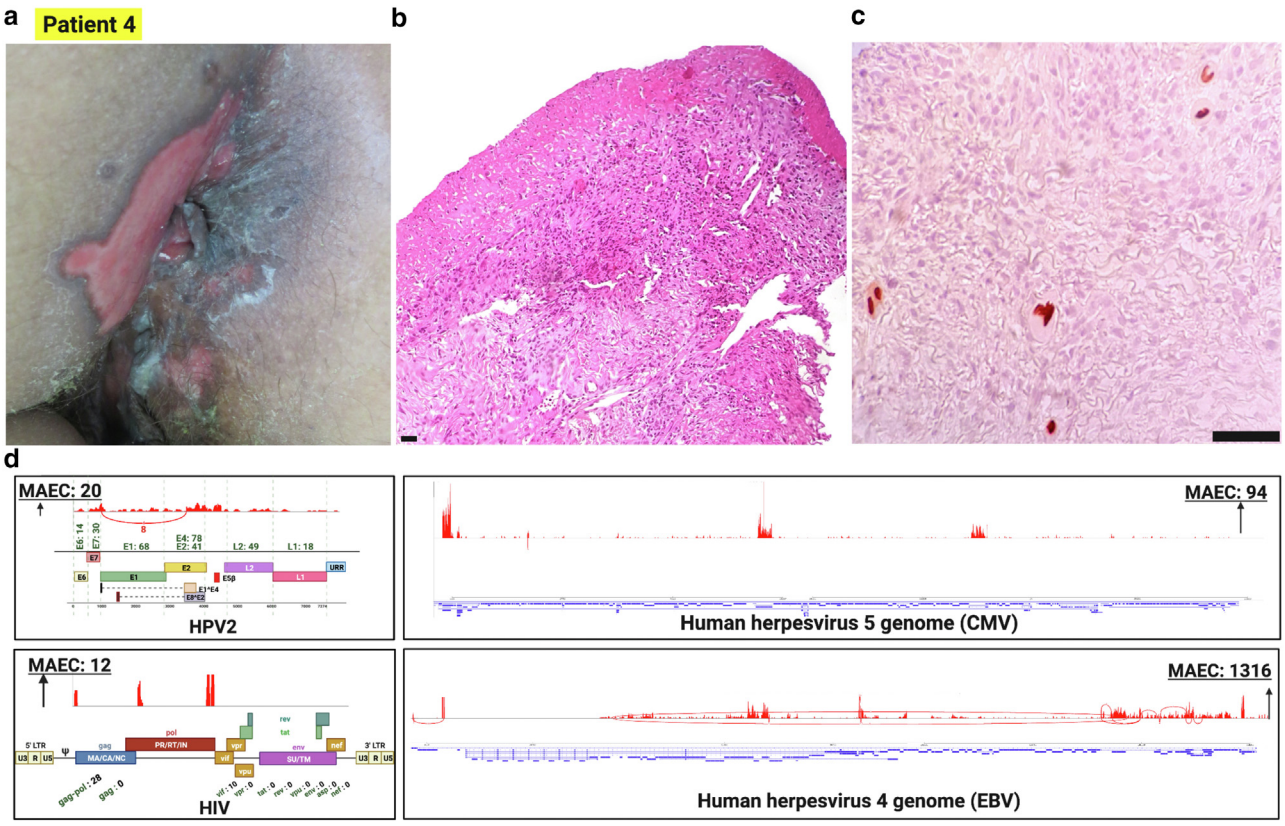

Figure 2. Viral determinants of a cutaneous lesion in a patient affected by secondary immunodeficiency. (**a**–**c**) Female aged 25 years (patient 4) with AIDS due to HIV infection presented with CMV retinitis and anal cutaneous ulcer that was confirmed by histopathology and immunohistochemistry using mAbs against early and late CMV antigens. (**d**) RNA-Seq analysis was performed on a skin biopsy obtained from an anal skin lesion. It detected viruses, including HPV2, CMV, HIV, and EBV (bars = 50 μm and 100 μm for the middle and right histopathology slides, respectively). CMV, cytomegalovirus; EBV, human herpesvirus 4; HPV, human papillomavirus; MAEC, maximum exon coverage; RNA-Seq, RNA sequencing.

Patient 6. A female aged 50 years presented to the dermatology clinic with a chief complaint of generalized pruritic skin lesions all over the body, except palms, soles, and face, which manifested with generalized hyperpigmented and reticulated patches and plaques along with areas of erythema, atrophy, and poikiloderma (Figure 4a). These lesions had been present over the past 8 years, and associated moderate pruritus was managed with symptomatic treatments. However, the lesions' extent and pruritus' severity increased progressively. Histopathology of the skin lesions revealed the presence of dermal lymphocytic infiltrates with mild cellular atypia and epidermotropism (Figure 4c). Our initial diagnosis was mycosis fungoides with Sézary syndrome. Whole-transcriptome sequencing of a skin biopsy disclosed the presence of the HTLV1 virus, and serological examination confirmed HTLV1 lymphoma (Figure 4e). Histochemical staining of her bone marrow and a skin biopsy revealed markers positive for CD2, CD3, CD4, and CD25, confirming the diagnosis of adult T-cell leukemia/lymphoma. A peripheral blood smear revealed many medium-to-large-sized lymphocytes, some with nuclear irregularity and lobulation (Figure 4b). Further investigations included computed tomography that revealed axillary, mesenteric, and pelvic lymphadenopathy; splenomegaly; and left lung consolidation (Figure 4d). The patient was treated with a combination of simultaneous chemotherapy and antiviral therapy (Bazarbachi et al, 2011). Chemotherapy included 4 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone. Antiviral therapy included lower doses of zidovudine at 500 mg/day and pegylated IFN-α at 1.5 mcg/Kg/weekly. Thus, whole-transcriptome–based sequencing detected HTLV1 in patient 6 and validated the clinical diagnosis of adult T-cell leukemia/lymphoma.

DISCUSSION

In this study, we have tested the applicability of a previously unreported whole-transcriptome sequencing–based pipeline for profiling the cutaneous virome in skin diseases. This approach is based on the analysis of RNA isolated from lesional skin biopsies compared with that from adjacent normal-appearing skin biopsies, coupled with a bioinformatics pipeline that interrogates 926 genetically distinct viruses with sequence information from the National Center for Biotechnology Information. We have previously developed the RNA-Seq–based pipeline to identify genetic variants in the human genome in a process that combines the human genome and transcriptome reference databases for optimal identification of sequence variations and their consequences at the RNA level (Youssefian et al, 2021). We extended this next-generation sequencing approach to the analysis of viral sequences by examining those reads that do not align or only partially align with the human sequences, assuming that the

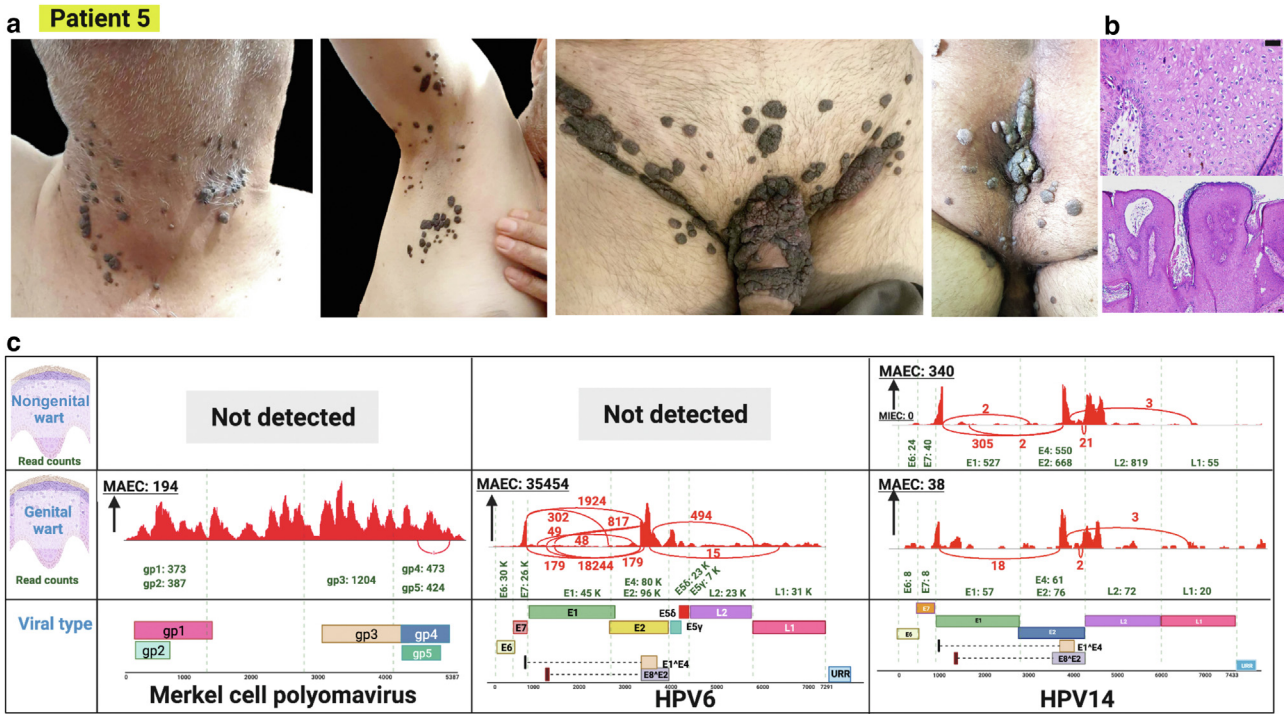

Figure 3. Viral repertoire of cutaneous lesions in a patient with chronic lymphocytic leukemia. (**a**) A male aged 68 years (patient 5) with chronic lymphocytic leukemia developed recalcitrant warts after chemotherapy. (**b**) Histopathology results showed the presence of koilocytes indicating viral infection (upper panel) (H&E staining) and verrucous proliferation of the epidermis with hyperkeratosis (lower panel). (**c**) RNA-Seq analysis by VirPy detected Merkel cell polyomavirus, HPV6, and HPV14 in genital and HPV14 nongenital warts (bar = 50 μm for all histopathology slides). HPV, human papillomavirus; MAEC, maximum exon coverage; RNA-Seq, RNA sequencing; URR, upstream regulatory region.

remaining sequences represent microbiota in human skin (Saeidian et al, 2023, 2022). We now applied this pipeline to patients with viral-induced skin manifestations associated with underlying secondary immunodeficiencies. The results allowed us to identify the presence of specific viruses in these patients' cutaneous lesions. The paramount innovation of using our newly developed pipeline is the ability to detect human sequence variants and viruses concomitantly through a single-run pipeline. This means that we use the methods as a first-tier approach for detecting pathogenic human sequence variants in about 500 predisposing genes to infections in the context of patients with heritable inborn errors of immunity (Tangye et al, 2022). This robust tool is currently limited to the research setting but is anticipated to appear as a clinical test later. Besides, RNA-Seq provides information about the pathogenicity and consequences of sequence variants (Saeidian et al, 2020). In addition, our laboratory used unmapped reads to host (human) genome/transcriptome and aligned them to the viral genomes. The unmapped readspace of RNA-Seq data tends to be significant but is often overlooked. We hypothesized that unmapped readspace of RNA-Seq data might possess valuable signals for nonhuman infection agents, and all viral and other microbial determinants of lesion biopsies obtained from patients with inborn errors of immunity can be probed. Host genetic study is critical because there is increasing evidence that even in unsuspicious clinical scenarios, predisposing fully penetrant sequence variants can be detected in patients with infections (Casanova, 2015).

Five of the 6 cases studied had evidence of HPV infection, manifesting clinically as recalcitrant cutaneous warts, resistant to multiple treatment modalities (cases 1–4) and a sizable perianal erosion (case 4). In 3 cases (case numbers 1, 2, and 3), HPVs were the only viruses identified. Case 1 had HPV3 and 76, the predominant virus being HPV3, which has been associated with cutaneous warts of the alpha family of HPV. This patient also demonstrated the presence of HPV76, a member of the beta family suggested to be associated with epidermodysplasia verruciformis. Case 2 was found to have the presence of the HPV2 virus, another member of the alpha genus. Case 3 showed exclusively the presence of HPV128, a member of the gamma family of this virus, previously associated with cutaneous warts. We previously showed the association of HPV128 with aggressive and metastatic cutaneous squamous cell carcinoma (Saeidian et al, 2023). Patient 4 with the HIV infection also demonstrated the presence of HIV at a low level in the perianal skin lesion.

Interestingly, the human herpesvirus 5 and the human herpesvirus 4 genomes were also present in this same lesion. In patient 5, warts on the genital area demonstrated the presence of HPV6 and 14, representatives of the alpha and beta families, associated with anogenital warts and laryngeal papillomas and epidermodysplasia verruciformis and immunosuppression, respectively. The nongenital cutaneous warts in this patient did not contain HPV6 but instead had HPV14 as the predominant type. In addition to HPV, patient 5 demonstrated the presence of Merkel cell polyomavirus in genital warts, which was previously shown to be associated

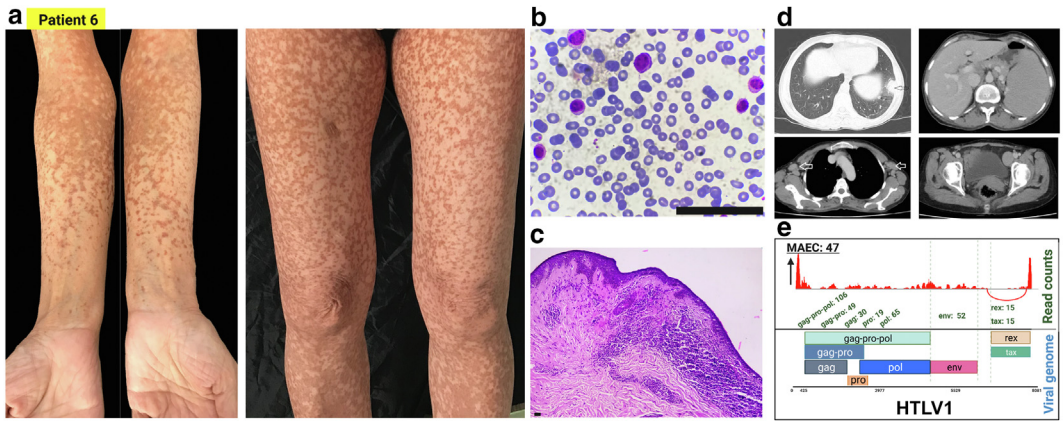

Figure 4. RNA-Seq confirmed the clinical diagnosis of ATLL in a patient with a suspected diagnosis of mycosis fungoides/Sézary syndrome. (**a**) A female aged 55 years (patient 6) presented with generalized pruritic skin lesions, hyperpigmented reticulated patches, and plaques, along with areas of erythema, atrophy, and poikiloderma. (**b**) A peripheral blood smear showing the presence of medium-to-large-sized lymphocytes confirmed the diagnosis of ATLL. (**c**) Histopathology showed dermal lymphocytic infiltrates with cellular atypia and epidermotropism. (**d**) A thorax, abdomen, and pelvis computed tomography scan displayed left lung consolidation; splenomegaly; and mesenteric, axillary, and pelvic lymphadenopathy. (**e**) RNA-Seq of the skin biopsy identified the presence of the HTLV1 (bar = 50 μm for histopathology slides). ATLL, acute T cell leukemia-lymphoma; HTLV1, human T-lymphotropic virus 1; MAEC, maximum exon coverage; RNA-Seq, RNA sequencing.

with chronic lymphocytic leukemia and has an unknown role in carcinogenesis (Pantulu et al, 2010).

Our results suggest that certain types of warts are specific to HPV subtypes, but multiple viral species can exist in 1 skin lesion. It should be noted that the demonstration of the presence of various HPV viruses in the same skin biopsy cannot be explained by nonspecific cross-identification of different HPV subtypes by the sequencing technique because sequence reads that do not align concordantly or have additional secondary alignments are filtered out in the bioinformatics process to avoid incorrectly attributing genome segments that are common between viruses. Thus, our pipeline demonstrates high fidelity for viral detection, and it has recently been validated with few, if any, false-positive and few false-negative findings (Saeidian et al, 2023).

Patient 6 had an interesting, rare, and unusual presentation with erythrodermic poikiloderma–like lesions, primarily on the arms and legs. Without HTLV1, clinical, pathology, and imaging data suggest the clinical diagnosis of mycosis fungoides with Sézary syndrome (AbdullGaffar and Abdulrahman, 2021). RNA-Seq identified HTLV1 in her skin, leading to suspicion of adult T-cell leukemia/lymphoma, a rare T-cell leukemia/lymphoma affecting adults caused by the HTLV1 virus. The patient resided in the northeastern region of Iran, where HTLV1 infection is endemic (Hedayati-Moghaddam et al, 2011). This diagnosis was also confirmed by hematological examination, bone marrow pathology, and computed tomography imaging analysis demonstrating lymphadenopathy and the presence of tumor infiltrates in the lungs. Thus, analysis of the skin biopsy for the presence of viruses was diagnostic in this case, with strikingly unusual cutaneous findings.

Previously, HPV typing has been based on DNA or RNA; some HPV tests will detect the DNA, and other HPV tests identify E6/E7 mRNA (Biglari et al, 2024). Routinely, these approaches test only for the presence of a subset of this group of viruses, particularly those with oncogenic potential (Youssefian et al, 2019). Our whole-transcriptome–based analysis currently allows for identification of 926 genetically distinct genes, including 441 different HPV types, for which the sequence information is presently available from National Center for Biotechnology Information. The virus library dataset in our pipeline can be expanded as new viral sequences become available. Thus, the virus detection pipeline presented in this study can be modified to be up to date for direct evaluation of the presence of the entire cutaneous virome. In addition to identifying specific viruses, this RNA-Seq–based pipeline allows for relative quantitation by MAEC count. Sashimi plots can determine the expression profile and splicing pattern of individual viruses in the context of their genomic organization. For example, HPV viruses consist of early (*E1–8*) and late (*L1*, *L2*) genes and the upstream regulatory region. The expressions of *L1* and *L2* genes encoding capsid proteins indicate an active virus and thus allow for assessment of the lesions in terms of the presence of an active virus. This feature distinguishes RNA-based sequencing from DNA-based analysis, which only detects the presence of gene fragments without functional assessment of their expression.

In conclusion, we have demonstrated that the recently developed whole-transcriptome–based next-generation sequencing analysis applies to viral profiling with quantitation and functional expression analysis. This approach can further characterize the cutaneous virome in the context of cutaneous lesions in patients with immunodeficiency.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board of the Pasteur Institute of Iran, and all subjects and parents of minor individuals gave written informed consent to participate in the research and to publish their clinical images. The eligibility criteria included patients with secondary immune deficiency who displayed viral-induced skin lesions in the absence of any single-gene inborn errors of immunity. The exclusion criterion was achieved by family history examination, and a negative result on testing with the first

part of the Virpy deals with mapping RNA-Seq reads to the human genome/transcriptome reference sequence. Six patients met the inclusion criterion, and thus, they were included in this study, and their lesion and normal skin biopsies were subjected to RNA-Seq (Saeidian et al, 2023, 2022).

RNA-Seq

RNA-Seq was performed on the RNA extracted from a 4-mm whole-skin biopsy of lesional tissue and the adjacent area of normal-looking skin. Sequencing was performed using 100 ng of total RNA with the TruSeq Stranded Total RNA Kit (Illumina), with the suggested manufacturer's instructions. The total mRNA capture and library preparation at 4-nmol/l concentration, including initial barcoding and sequencing on an Illumina NextSeq 500 machine, were performed according to routine procedures. With 150-bp paired-end chemistry, a sequencing depth of 50–100 million paired reads per sample was achieved.

VirPy

We recently developed a pipeline for comprehensive analysis of the human and virome profiles of the patients. This in-house pipeline, VirPy (accessible at VirPy.org), performs many computational procedures to generate a complete and comprehensive virome analysis. This Python-based pipeline employs third-party tools, including STAR (Dobin et al, 2013), HISAT2 (Kim et al, 2019), Samtools (Li et al, 2009), eXpress (Roberts and Pachter, 2013), Subread featureCounts (Liao et al, 2014), FreeBayes (http://arxiv.org/abs/1207.3907), Salmon (Patro et al, 2017), and SnpEff (Cingolani et al, 2012) packages. As an input, VirPy needs a Fastq paired-end read and a file of RNA-Seq on RNA to assess the quality by FastQC. Trimming is performed if required, and alignment steps occur with the STAR aligner. Unaligned reads or those only partially aligned to the human genome are assumed to be nonhuman in origin and represent genetic material from microorganism species. The nonhuman paired mates are extracted and realigned using the HISAT2 aligner to a compiled viral genome reference containing 926 unique viral species obtained from the National Center for Biotechnology Information, including 441 genetic types of HPV (Caldeira et al, 2003; Van Doorslaer et al, 2017). The virus-containing SAM (Sequence Alignment Map) file is filtered to contain only concordant pairs, and reads aligned to the viral genome reference are formatted into the aligned, sorted, and indexed BAM (Binary Alignment Map) files, which can be visualized in Integrated Genome Viewer (Thorvaldsdóttir et al, 2013) for bulk RNA-Seq (Saeidian et al, 2023, 2022).

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of the Pasteur Institute of Iran, and all subjects and parents of minor individuals gave written informed consent to participate in the research and to publish their clinical images.

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1085116.

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CONFLICT OF INTEREST

J-LC serves on the Scientific Advisory Boards of ADMA Biologics, Kymera Therapeutics, and Elixiron Immunotherapeutics. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank patients for their participation in this study. Carol Kelly assisted in manuscript preparation. The United States National Institutes of Health (grant R01AI143810) and the LEO Foundation (grant LF-OC-22-000965) supported the authors' original studies, reported in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: LY, JU, HV; Data Curation: LY, AHS, ZS, MA, FA, SM, MS-D, RD, FV, SZ, VB, EJ, J-LC, JU; Formal Analysis: LY, AHS, ZS, MA, FA, SM, MS-D, RD, FV, SZ, VB, EJ, J-LC, JU; Funding Acquisition: J-LC, JU, EJ; Supervision: J-LC, JU, HV; Writing – Original Draft Preparation: LY, AHS, JU, HV; Writing - Review and Editing: LY, AHS, ZS, MA, FA, SM, MS-D, RD, FV, SZ, VB, EJ, J-LC, JU, HV

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMs)

The authors did not use any generative artificial intelligence (AI) or large language models (LLMs) for the writing process of this manuscript.

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