

# Do beta-human papilloma viruses cause cutaneous squamous cell carcinoma in immunocompetent individuals?

## Abstract

**Background:** Existing epidemiological evidence is controversial regarding the possible association between Beta Human papillomavirus (B-HPV) and cutaneous squamous cell carcinoma (cSCC) in immunocompetent individuals.

**Objectives:** Perform a systematic review of cohort and case-control studies that assessed B-HPV and the risk of cSCC in immunocompetent individuals to assess the current data.

**Materials and methods:** We performed a systematic literature search for studies in humans through March 17, 2014, with no specified start date or language restrictions and employed predefined search criteria. The included studies complied with the predefined inclusion and exclusion criteria and were agreed upon by both authors after review of abstracts and full text. Data extraction included general study information comprising latitude of study location, study design, the number of cases and controls, method of HPV detection and the number of types of HPV detected. Furthermore, study results were designated as the general and/or type specific B-HPV adjusted Odds Ratio or Relative Risk.

**Results:** This is the largest review to be conducted on this topic in healthy individuals. We evaluated 4,056 cSCC cases and 7,067 controls that were collected from 16 case-control and 4 cohort studies included in this review. Studies were subdivided into six categories depending on the method of HPV detection used. Among the 20 studies, 18 (90%) showed a significant association between B-HPV and cSCC. More specifically, HPV8 and HPV38 were most commonly reported to have a significant association with cSCC.

**Conclusion:** This systematic review provides further evidence supporting the role of B-HPV in the development of cSCC in healthy individuals and supports a possible type-specific HPV 8, 38 involvements in cSCC. Our findings highlight the need for large multi-center prospective research to validate these associations.

**Keywords:** squamous cell carcinoma, Non melanoma skin cancer, skin cancer, human papillomavirus (HPV), beta-HPV

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**Abbreviations:** HPV, human papillomavirus; cSCC, cutaneous squamous cell carcinoma; IARC, international agency for research of cancer; HHV-8, human herpes virus-8

## Introduction

Skin cancer represents the most prevalent malignancy worldwide, with non-melanoma skin cancer accounting for one third of all oncological cases in the USA. More specifically, cutaneous squamous cell carcinoma (cSCC) accounts for approximately 20% of all non-melanoma skin cancer cases with an estimate of over 700,000 new cases diagnosed yearly in the USA.<sup>1,2</sup> The most important risk factors implicated in cSCC development are ultraviolet radiation exposure, immunosuppression and fair skin. While Human Papillomavirus (HPV) is the most incriminated virus, sero-epidemiological studies remain controversial. According to the International Agency for Research of Cancer (IARC) working group, data remains insufficient to establish a causal relationship.<sup>3</sup> The theory of viral oncogenesis first emerged in the 1950s, when Gross et al described a plausible connection. Today, viruses are associated with more than 15% of the oncological burden.<sup>4-5</sup>

Two compelling pieces of evidence in cSCC epidemiology render

HPV a thought-provoking virus for causality assessment. First, the increased occurrence of cSCC in organ transplant recipients associated with significantly higher rates of B-HPV which is similar to the human herpes virus-8 (HHV-8) induced Kaposi sarcoma.<sup>6-9</sup> Second, the proven causality between specific HPV types and the development of cSCC in individuals diagnosed with epidermodysplasia verruciformis.<sup>10-11</sup> This mechanism was established as a rare inherited disorder with mutations in the transmembrane channel genes TMC6 or TMC8 acting as an immuno-modifier and supporting the amplification of specific types of HPV; most commonly HPV5 or HPV8.

The detailed examination of molecular pathways explaining the biological plausibility of B-HPV's implication in the cancerogenesis of cSCC is beyond the scope of our review. We will limit our discussion to the following three novel molecular mechanisms:

- The increased susceptibility to UV induced oncogenesis of transgenic mice's skin keratinocyte expressing HPV type 38 E6 and E7 oncoproteins.<sup>12-13</sup> This represents an *in-vivo* proof of HPV38 E6 and E7 oncoproteins involvement in cSCC tumor initiation.
- Another biological explanation lies in HPV type 8 E6 oncoprotein capacities to inhibit the PDZ domain protein syntenin-2. This

is possibly a crucial element in the control of viral oncogenic potential as this deregulation caused by HPV 8 may contribute to cSCC initiation. In the future further understanding of the cellular pathway downstream from syntenin-2 will help better understand the HPV pathogenesis.<sup>14-15</sup>

- c. A Third experimental evidence showed that B-HPV types 5,8,20 and 38 through E2, 6 and 7 oncoprotein increase the quantity of stem cell-like cells available during early carcinogenesis, thus enabling the persistence and accumulation of DNA damage necessary to generate malignant stem cells. This portrays the possible molecular mechanisms of B-HPV's involvement in the progression and persistence of the oncogenic process.<sup>16</sup>

Establishing a strong causal relationship between B-HPV and cSCC requires both a concomitant molecular plausibility and a well-established epidemiological proof. The detailed molecular pathways implicating B-HPV is beyond the scope of this manuscript and will be limited to the above discussion. As for the epidemiological case-control and cohort studies conducted in the general immunocompetent population, their results are debatable, rendering the association controversial till this date. The objective of our review is to examine whether B-genus HPV is associated with an increased risk of skin squamous cell carcinoma in the general population. To this purpose, we performed a systematic review of all case-control and cohort studies that analyzed this association in immunocompetent adult individuals. In addition, we assessed the limitations in today's epidemiological literature, to open the window on future research questions.

## Materials and methods

### Search strategy and Inclusion/Exclusion Criteria

We performed a systematic literature search for studies in humans through March 17, 2014, with no specified start date or language restrictions. We used PubMed to search for all trials lists and databases with the following search terms: (Human Papillomavirus or HPV or B-HPV) and (cutaneous Squamous Cell Carcinoma or Skin Squamous Cell Carcinoma or cSCC or non-melanoma skin neoplasms). Additionally, in order to ensure comprehensiveness, we examined the reference lists from retrieved manuscripts for supplementary relevant studies. We did not identify or obtain data from unpublished manuscripts. We conducted our systematic review in harmony with PRISMA guidelines. Furthermore, a medical reference librarian verified our search strategy, and independently repeated the search.

The two authors (J.C. and A.S.) conducted the eligibility assessment of the studies independently, adopting an unblinded standardized approach. All disagreements between J.C. and A.S. were resolved by consensus through discussion between authors and in-depth review of the articles. The inclusion criteria were related to study design (case-control or cohort), population (immunocompetent), exposure (HPV), outcome (cSCC) and detection method. Details about the inclusion and exclusion criteria are presented in Table 1. Based on these criteria, our search retrieved 20 studies with a total of 4,056 cSCC cases and 7,067 controls. Figure 1 illustrates the flowchart for the identification and selection of these articles.

**Table 1** Adopted Inclusion and Exclusion Criteria for Article Selection

Criteria	Inclusion	Exclusion
<b>Study Design</b>	Cohort or Case-Control studies examining the association between HPV and cSCC	Abstracts, letters to the editor or review articles without original data, case reports, case-control studies with less than 20 cases and 20 controls or cohort studies of less than 40 subjects
<b>Outcome</b>	The outcome of interest was the development of cSCC as primary episode	The outcome of interest is not cSCC The outcome of interest is recurrent cSCC (rather than first episode of cSCC)
<b>Exposure</b>	The exposure of interest was HPV prevalence	The exposure of interest was not HPV prevalence
<b>Population</b>	Humans Immunocompetent population solely	Non-humans Immunocompromised individuals
<b>Reported findings</b>	Odds Ratio (OR) or Relative Risk (RR) with 95% confidence intervals (CI)	No ORs or RRs, or CIs different than 95%
<b>HPV Detection Method</b>	Sero-prevalence alone or, Eyebrow Hair (EBH) DNA sampling alone or, Skin biopsy alone using Polymerase Chain Reaction (PCR), or sero-prevalence and EBH DNA, or sero-prevalence and skin biopsy using PCR	No defined method of HPV quantification

cSCC, cutaneous squamous cell carcinoma; HPV, human papilloma virus; OR, odds ratio; RR, relative Risk; ELISA: Enzyme-Linked Immunosorbent Assay; EBH DNA: Eyebrow Hair DNA; PCR: Polymerase Chain Reaction

### Data extraction

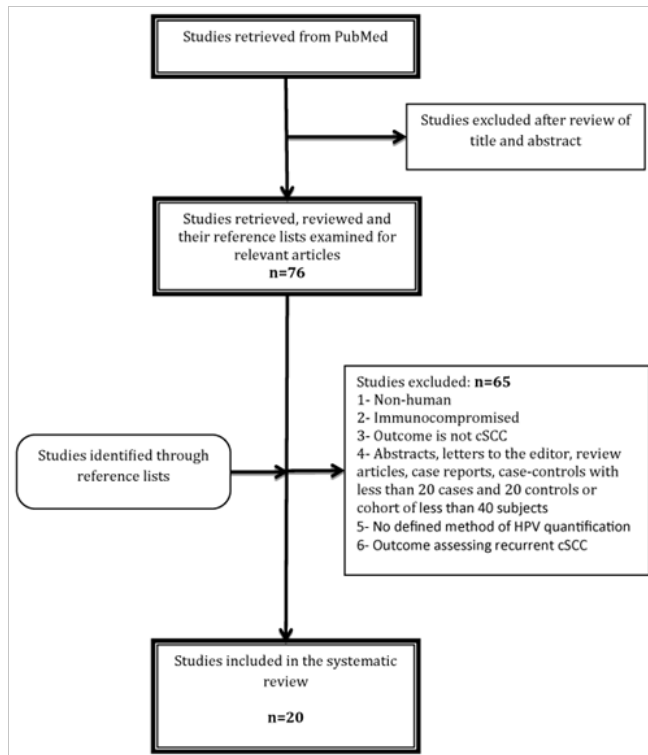
Each study was thoroughly reviewed to extract information of interest. One Author retrieved the data from selected studies and the second author verified its accuracy and comprehensiveness. Discrepancies were resolved by discussion between authors and in-depth review of the data. We monitored studies for duplicate data from overlapping publications using a simple algorithm entailing the comparison by authors' names, study geographical location and sample size. We excluded duplicate data from the systematic review after discussion and we included the most updated data between the two sources.

The retrieved data falls under the following three categories:

**Global study information:** last name of first author, year of publication, and location (Continent or country where the study population was enrolled; for studies with samples from multiple countries, data were extracted separately by country when possible), we also identified the study location by Latitude an average estimate of latitude was estimated since exact latitude was not provided in most of the studies;

**Study design and population:** Case-Control or Cohort, number of cases and cohort size (or alternatively number of controls), HPV detection method, and number of specific HPV type detected;

**Study results:** odds ratio (OR) or relative risk (RR) with the corresponding 95% confidence intervals (CI) (we extracted the OR or RR that reflected the greatest degree of adjustment for possible confounding factors) and type of HPV detected (if applicable) and the odds ratio (OR) or relative risk (RR) with the corresponding 95% confidence intervals (CI) for different type of HPV detected (if applicable).



**Figure 1** Strategic search of studies.

## Results

All the studies that tackled the association between B-HPV and cSCC were published after the year 2003 and the number of publications increased in the last five years. This highlights the relatively recent interest in validating this association in the immunocompetent population, in view of more robust novel evidence confirming the role of B-HPV in immunosuppressed and transplant population.<sup>17</sup> Each study included in our review, performed calculations of odds ratio (OR) or relative risk (RR) with a confidence interval of 95% and adjusted for sex and age. All the included studies reported either the overall or type-specific B-HPV OR or RR to measure the association of interest. Some of studies adjusted their OR further than age and sex, accounting for skin sensitivity, cigarette smoking, and number of lifetime painful sunburns and level of education.

## Location

The geographical distribution of the pool of studies was mainly restricted to the European continent and the USA. A total of 12 studies (60%) were conducted in Europe; the Netherlands and Sweden leading the way with 4 studies each. Six studies (30%) were published in the USA mostly out of the 2 states of New Hampshire and Florida. The two remaining studies were conducted in Australia. The exclusion of the African continent seems understandable, since it is established that people with dark skin complexity are at significantly lower risk for developing cSCC. On the other hand the lack of any reported data

from the Asian continent, excludes a large population with specific genetic buildup, sun exposure, and viral carriage.

The geographical distribution is important, knowing that each population had different sun exposure which is a known risk factor in the development of keratinocyte cancer and a possible key element in the pathogenesis of B-HPV and cSCC. Thus, we collected the latitude of each study center included in our systematic review, excluding only 2 multicenter studies due to large differences between the centers' location.

Since the quantity of ultraviolet (UV) in sunlight differs significantly with latitude, we grouped studies into three categories:

- Conducted at latitudes greater or equal to 45° north classified as 'high latitude', this category was the largest with 12 out of the 18 studies;
- Included only 2 out of 18 studies that were conducted at latitudes between 44° and 35° north and classified as 'middle latitude'; and
- Included 6 studies, conducted between 34° north/south and 20° north/south classified as the 'low latitude'.

## Design

The study design most adopted by investigators in was the case-control design with a total of 16 studies and the remaining 4 were cohort studies. The latter consisted of small collected samples which rendered questionable any further interpretation of the cohort results. The case-control studies were further subdivided as follows: single institution (10 studies), population based (3 studies) and multicenter (3 studies).

## HPV Detection Method

We identified a significant variability between studies in terms of the adopted HPV detection method. Thus, we grouped the collected articles into the following 6 categories:

### Category A: Sero-prevalence alone

The group of studies that "only collected sero-prevalence of B-HPV" was the largest, with nine studies accounting for 1,707 cSCC cases and 3,837 controls/cohort<sup>18-26</sup> This category included 6 case-control studies and the highest number of cohort studies (3 out of a total of 4 cohort studies) which highlights the importance of serology as a cost-effective, noninvasive and easily reproducible procedure on large population.

As for the specific serological method of detection used in the above studies, only two published in 2003 used ELISA, before the availability of multiplex serology testing.<sup>18-19</sup> The remaining 7 studies in this category used multiplex serology. Only one study did not perform HPV type-specific analysis, 26 with the other 8 studies testing for different types of HPV with lowest number of types tested being 4 HPV types<sup>18</sup> and the highest number being 38.<sup>21</sup>

Among the 9 studies included in this first category, 7 (77.7 %) showed a significant association between overall or type-specific B-HPV antigenicity and cSCC. Interestingly, B-HPV type 38 was the most cited as having a significant adjusted OR for this association, with 4 out of the 6 studies (66%) that calculated HPV 38 specific OR reporting significant adjusted ORs ranging between lowest 1.3 and highest 3.0 with the respective 95% confidence intervals of 1.3 (1.0-1.8)<sup>25</sup> and 3.0 (1.2-7.9)<sup>22</sup>, 3.0 (1.1-8.4)<sup>19</sup>. Additionally, the largest

cohort study performed by Andersson et al. that included 353 cSCC cases highlighted further the implication of HPV 38 in cSCC, since this specific HPV type had the strongest association with cSCC in comparison to others.<sup>25</sup>

On the other hand, HPV 8 was had the second strongest association with cSCC with OR ranging between lowest 1.45 and highest 14.7 with respective 95% confidence interval of 1.45 (1.08-1.97)<sup>23</sup> and 14.7 (1.6-135).<sup>19</sup> Nevertheless, two studies did not show any significant association between B-HPV antigenicity and cSCC. Interestingly, they were both cohort studies with small sample sizes. The first was a pilot study and only included 39 cSCC cases and thus was not powered to allow any conclusions.<sup>21</sup> While the second study that followed 150 newly diagnosed cases of cSCC between 1992 and 1996, allowed more in-depth analysis. Although no significant sero-conversion was observed, the authors concluded that the limited number of followed cases hindered the power of association. The same study demonstrated a significant association with a twofold increased risk of cSCC in the population aged below 50 years old with any type of B-HPV.<sup>27</sup>

### Category B: Sero-prevalence and EBH DNA

This second category “collected both sero-prevalence and EBH DNA of B-HPV”, and included 2 studies with a total of 753 cSCC cases and 902 controls. Both studies adopted a case-control design

with one consisting of a multi-center effort that collected data from two European countries (Netherlands and Italy) and from Australia. This study showed a significant increase in the risk of developing cSCC in B-HPV seropositive Australian population. However, the population from Netherlands and Italy showed a significant increased risk associated with EBH DNA carriage.<sup>28</sup> This study did not perform any type-specific analysis of data, which renders questionable the interpretation of its results. In this same category the second study that assessed type-specific HPV also showed similar results that to those in category A highlighting the importance of HPV type 8.<sup>29</sup>

### Category C: Sero-prevalence and Skin Biopsy DNA

The third category “collected Sero-prevalence and Skin Biopsy DNA of B-HPV” included 4 studies with a total of 964 cSCC cases and 1,149 controls/cohort.<sup>30-33</sup> This category is the second largest in this systematic review, consisting of 3 single institution case-control studies with relatively small sample sizes and one large cohort study. Only one of those studies performed type-specific analysis while another performed none B-HPV type-specific analysis (HPV types 16,18) and the remaining two studies did not perform any HPV type-specific analysis.<sup>33</sup> All the studies showed a significant association between the involvement of B-HPV skin DNA and cSCC. In the following paragraphs we will present the most prominent findings of each of the four studies that were not included in (Table 2).

**Table 2** Description of individual case-control and cohort studies examining the association between B-HPV and cSCC

Author,Year	Design and Population	Location	Exclusion/Inclusion Criteria and Detection Methods	Results
<b>Category A - Sero-prevalence alone: cSCC cases: 1,707 and controls/cohort: 3,837</b>				
<b>Masini et al.<sup>18</sup></b>	Single institution based case-control study Cases: n=46 cSCC Controls: n=84	In a referral center in Italy (Rome) Latitude 41, 54 N	Serology ELISA 4 HPV types (8,15,23,36)	Adjusted for sex, age, history of lifetime exposure to sunlight and eye color only significant for <b>HPV 8: OR 3.2 (1.3-7.9)</b>
<b>Feltkamp et al.<sup>19</sup></b>	Single institution based case-control study Cases n=161 cSCC; Controls: n=386	Netherlands Latitude 52.16 N	Serology ELISA; 7 HPV types (5, 8, 15, 20, 24, 38, 16 a)	Adjusted OR for age and sex only significant for <b>HPV 8: OR 14.7 (1.6-135)</b> <b>HPV 38: OR 3.0 (1.1-8.4)</b>
<b>Karagas et al.<sup>20</sup></b>	Population-based case-control Cases: 252 cSCC; 525 BCC Control: 461 subjects	New Hampshire Latitude 45 N	Multiplex serology 16 HPV types	Adjusted for age, sex, and skin sensitivity <b>All B-HPV: OR 1.6 (1.2-2.3)</b> <b>HPV 5: OR 1.8 (1.0-3.1)</b>
<b>Casabone et al.<sup>21</sup></b>	Prospective pilot cohort study Cases: 39 Controls: 80	United kingdom Latitude 51.7 N	Multiplex serology 38 HPV types	Adjusted for age, sex and region of residence The numbers of cases examined are small and differences were not statistically significant
<b>Waterboer et al.<sup>22</sup></b>	Single institution based case-control study Cases: 43 cSCC Controls: 77	Rome Italy Latitude 41.54 N	Multiplex serology 31 HPV types	Adjusted for age, sex, eye color and history of lifetime sun exposure Significantly increased cSCC risks observed for the beta HPV types HPV 15: OR 1.8 (1.1-7.1), HPV 17: OR 2.6 (1.01-6.5) and HPV 38: OR 3.0 (1.2-7.9) and Any type of the beta 2 species: 3.3 (1.2-8.7)



Table Continued...

Author,Year	Design and Population	Location	Exclusion/Inclusion Criteria and Detection Methods	Results
Karagas et al. <sup>23</sup>	Population based case-control study Cases: n=663 cSCC Controls: n=805	New Hampshire, USA Latitude 45 N	Multiplex serology: 16 HPV $\beta$ types 5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96, and 107	Adjusted for age, sex, level of education, cigarette smoking, skin sensitivity, and number of lifetime painful sunburns Any B-HPV type: OR 1.6 (1.2-2.3). HPV 38 OR 1.74 (1.27-2.4) HPV 8 OR 1.45 (1.08-1.97) All tested HPV types provided significant increased risk for cSCC except of HPV type 5 and 36
Plasmeijer et al. <sup>24</sup>	Cohort from 1992 until 2007. Cohort: 1,311 cSCC newly diagnosed: 150 people	Australia Latitude 26_S	Multiplex serology: 21 different $\beta$ HPV types: 5, 8, 9, 14, 15, 17, 20, 21, 22, 23, 24, 36, 38, 47, 49, 75, 76, 80, 92, 93, and 96	Adjusted for age and sex and used Cox proportional hazards Did not observe any association with overall antibody positivity, antibodies to multiple $\beta$ HPV types or to specific types previously shown to be associated with cSCC.
Andersson et al. <sup>25</sup>	Cohort 850,000 subjects Cases: 353 cSCC Controls: 633	Norway and Sweden Latitude 60 N	Multiplex serology 33 HPV types	Adjusted sex and age and country Any B2 type OR = 1.3 (1.1-1.7) HPV 38 = OR 1.3 (1.0-1.8)
Farzan et al. <sup>26</sup>	Population based case-control Cases: 663 cSCC Controls: 805	New Hampshire, USA Latitude 45 N	Multiplex serology: 25 HPV types 2-5, 7-10, 15, 17, 20, 23, 24, 27b, 36, 38, 48-50, 57, 65, 75-77, 88, 92, 95, 96, 101, 103 and 107 -NO HPV TYPE-SPECIFIC ANALYSIS performed	Adjusted for age and sex B-HPV: OR of 1.41 (CI 5 1.13-1.75)
<b>Category B - Sero-prevalence + EBH DNA prevalence: cSCC cases: 753 and cSCC controls: 902</b>				
Struijk et al. <sup>29</sup>	Single institution based case-control Cases: 64 cSCC. Controls: 57 tumor-free	Brisbane, Australia Latitude 26 S	-Serology ELISA -EBH DNA analysis by HPV type-specific PCR, for 7 types 5, 8, 15, 16, 20, 24, and 38.	Adjusted for age and sex -B-HPV serology method: Any B-HPV had OR = 3.9 (1.4-10.7) HPV 8= OR 9.3 (1.9-45.6) HPV 38: not reported EBH DNA method: Any $\beta$ HPV OR = 0.93 (0.43-2.0)
Bouwes Bavinck et al. <sup>28</sup>	Multicenter case-control Total cases: 689 Total controls: 845 From Netherlands: Cases: 155; Controls: 278 Italy: Cases: 228; Controls: 257 Australia: Cases: 306; Controls: 310	Netherlands, Italy, and Australia Latitude NA	- Highly sensitive HPV DNA genotyping assay in eyebrow hair follicles: 1-25 $\beta$ HPV types - Serology multiplex luminex $\beta$ HPV types HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, and 93) -NO HPV TYPE-SPECIFIC ANALYSIS	Adjusted to sex and age -B-HPV serology: Australia any B-HPV OR 1.8 (1.3-2.6). Netherlands any B-HPV OR 1.4 (0.93-2.1) Italy any B-HPV OR 1.2 (0.81-1.8) -EBH B-HPV DNA: Netherlands any B-HPV OR 2.8(1.3-5.8) Italy any B-HPV OR 1.7 (0.79-3) Australia OR 0.91 (0.5-1.3)
<b>Category C - Sero-prevalence + Skin Biopsy DNA: cSCC cases: 963 and cSCC controls/cohort: 1,149</b>				
Andersson et al. <sup>30</sup>	Single institution based case-control Case: 72 cSCC Control: 121	Sweden and Austria Latitude 59 N	- Biopsies analyzed for HPV DNA by 4 different PCR in 3 different labs - Serology multiplex 13 types of HPV1, 5, 6, 8, 9, 10, 15, 16, 20, 24, 32, 36, 38 -NO HPV TYPE-SPECIFIC ANALYSIS	Adjusted to sex and age I-Any skin B- HPV DNA OR:2.08 (1.034.22)

Table Continued...

Author,Year	Design and Population	Location	Exclusion/Inclusion Criteria and Detection Methods	Results
<b>Asgari et al.<sup>31</sup></b>	Single institution based case-control Cases: 85 Control: 95	University of Washington Medical Center. (Seattle,WA) Latitude 47.6 N	- Biopsy: highly sensitive PCR-detection assays -NO HPV TYPE-SPECIFIC ANALYSIS	-Adjusted to age, sex, propensity to sunburn, as the other potential confounders, such as smoking status, skin sensitivity, freckling, tanning bed use, burn scars, and exposure to radiation. -HPV DNA from ANY $\beta$ -papillomavirus species 2 was more likely to be identified in tumors than in adjacent healthy tissue among cases OR 4.0(1.3–12.0).
<b>Iannacone et al.<sup>32</sup></b>	Single institution based case-control Cases: 173 cSCC Controls: 300	University of South Florida, Tampa Florida latitude 28 N	1- Multiplex serology 33 HPV type within genera alpha (2, 3, 7, 10, 27, 57, and 77); beta (5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96, and 107); gamma (4, 48, 50, 65, 88, 95, 101, and 103); mu (1); and nu (41). 2- HPV DNA was measured using an assay that detects 25 genus-beta HPV type with multiplex PCR and array primer extension (APEX) 3- Sero/biopsy concordance was assessed in 13 B-HPV types	-Adjusted for age and sex 1-Serology:Any genus-beta HPV type: OR 1.93 (1.23-3.02) HPV 8: OR 1.80(1.14-2.84) 2-Seropositivity to any B-HPV types tested was associated with overall DNA positivity of genus-B-HPV types in cSCC (OR, 2.81; 95% CI, 1.53–5.16) HPV 8: OR= 1.80 (1.14-2.84) 3-SERO/ DNA biopsy concordance HPV 5 OR 3.48 (1.27-9.59) HPV 24 OR 3.79 (1.24-11.5) HPV 17 OR 3.36 (1.29-8.72)
<b>Andersson et al.<sup>33</sup></b>	Cohort study: 850,000 subjects Cases: 633 cSCC Controls: 633 79 cSCC cases tested for DNA on skin biopsy blocks	Norway and Sweden Latitude 60 N	-Multiplex serology 9 for antibodies against 2 types of HPV: HPV16 and 18 L1 proteins. -Biopsy HPV 16-18 PCR	Adjusted for sex and age -cSCC was significantly increased among subjects seropositive for HPV16 and HPV18 -Only a very small proportion of cSCC tissues(4=79) were positive for HPV16 DNA
<b>Category D - EBH DNA alone: cSCC cases: 311 and cSCC controls: 691</b>				
<b>Strujik et al.<sup>34</sup></b>	Single institution based case-control Case: 155 Control: 371	Leiden Netherlands Latitude 52.16 N	-EBH DNA: HPV type-specific PCR -8 types of HPV: 2, 5, 8, 15, 16, 20, 24, and 38.	-Adjusted for age and sex Any B- HPV type: OR 1.7 (1.1-2.7) HPV 8: OR 2.5(1.3-4.8) HPV 15: OR= 2.5 (1.4-3.8) HPV 20: OR= 1.7 ( 1.0-2.9)
<b>Termorshuizen et al.<sup>35</sup></b>	Single institution based case-control study Cases: 156 cSCC Controls: 320	Netherlands Latitude 52.16 N	-EBH DNA type-specific PCR -6 HPV types 5, 8, 15, 20, 24, and 38	Adjusted to age and sex A significant association with the presence of cSCC was found for HPV EB DNA
<b>Category E - EBH DNA + skin biopsy HPV DNA: cSCC cases: 168 and cSCC controls: 290</b>				
<b>Iannacone et al.<sup>36</sup></b>	Single institution case-control study Cases: 168 cSCC Controls: 290 142 cases with biopsy	University of South Florida (USF) Latitude 28 N	-EBH DNA and skin tissue DNA - 8 types of HPV: HPV5, 8, 15, 20, 23, 24, 36 and 38	Adjusted for age, sex, education, hair color sunlight exposure, tanning ability, and ever-smoking status -EBH DNA:Any B HPV OR: 1.75 (1.00-3.5) EBH DNA HPV 38: OR 1.84(1.04-3.24) -EBH DNA /Skin DNA correspondence with cSCC:Any B HPV: OR: 2.13 (0.99-4.50) Type-specific concordance: HPV 5: OR 28.35 (4.77-168.47) HPV8: OR5.52 (1.81-16.8) HPV24: OR 35.60 (7.45-170.0) HPV 38: OR 6.31 (2.39-16.6)
<b>Category F - Skin Biopsy HPV DNA alone: cSCC cases: 154 and cSCC controls: 198</b>				
<b>Iftner<sup>37</sup></b>	Multicenter Case-control study Cases: 72 cSCC Controls: 106	Germany/ California Latitude NA	-Skin biopsy PCR; -NO HPV TYPE-SPECIFIC ANALYSIS	Adjusted for Age, sex and sun exposure Any B-HPV: OR 32 (10–100)

Table Continued...

Author,Year	Design and Population	Location	Exclusion/Inclusion Criteria and Detection Methods	Results
Forslund <sup>38</sup>	Multicenter case-control study Cases:82 cSCC Controls: 92	Sweden or Austria Latitude 59 N	Biopsy HPV DNA by PCR -NO HPV TYPE-SPECIFIC ANALYSIS	Adjusted to age, sex, skin type, sunburns, and eye color -Any B-HPVOR, 4.40 (1.92–10.06)

cSCC, cutaneous Squamous Cell Carcinoma; HPV, human papillomavirus; OR, odds ratio; RR, relative risk; ELISA, enzyme-linked immunosorbent assay; EBH DNA, eyebrow hair DNA; PCR, polymerase chain reaction

The study published in 2008 by Anderson et al.<sup>30</sup> identified that sero-positivity to any B-HPV type was almost two times more common among patients positive for HPV DNA of any HPV type. The second study performed by Asgari et al.<sup>31</sup> compared HPV DNA detection rates within cases, examining cSCCs skin lesions in comparison with the clinically uninvolved adjacent skin to determine the role of HPV in tumor progression. Their findings showed that cSCC lesion had B-HPV DNA 4 times more commonly than the normal adjacent skin. Another interesting finding in this same study was a six-fold increased likelihood of having three or more HPV types in cSCC lesions compared to the sun-protected controls (OR = 6.1, 95% CI = 1.4–26.1).

The third study conducted by Iannacone et al.<sup>32</sup> went beyond the general B-HPV antigenicity highlighting the trend of type-specific significantly elevated associations between HPV 8 and cSCC. Furthermore, the significant concordance of HPV types 5,17 and 24 in both serology analysis and skin biopsy DNA of cSCC lesions, mark the possible importance of these types in cSCC formation and progression. The last study in this category conducted by Andersson et al.<sup>33</sup> is the only prospective evidence of HPV 16 sero-positivity involvement in cSCC. This study did not analyze B-HPV types for cSCC association which represents a missed opportunity for more in-depth findings.

#### Category D: EBH DNA alone

The fourth category “collected only EBH DNA of B-HPV”, included two studies with a total of 311 cSCC cases and 691 controls. Both were case-control studies conducted in a single institution.<sup>34–35</sup> A significant association between any B-HPV type and cSCC was reported in both studies. Moreover, the type-specific analysis conducted by the two studies showed that HPV 8 EBH DNA was significantly more common in patients with cSCC with a twofold increased risk.

#### Category E: EBH DNA and Skin Biopsy DNA

The fifth category “collected EBH DNA and skin biopsy DNA of B-HPV”, included only one single-institution case-control study with 168 cSCC cases and 290 controls.<sup>36</sup> The findings showed a significant increased risk of developing cSCC in healthy individual that had any B-HPV EBH DNA. More specifically, HPV type 38 was associated with almost a twofold increased risk of cSCC. The same HPV 38 was the only type in this study that demonstrated a significant association between EBH DNA and cSCC, furthermore it showed a significant concordance between the two detection methods of EBH DNA and skin biopsy DNA.

#### Category F: Skin Biopsy DNA alone

This final category “collected only skin biopsy DNA of B-HPV”, included two multicenter case-control studies with 154 cSCC cases and 198 controls/cohort<sup>37–38</sup> Both studies had small sample sizes due to the invasiveness of the detection method, and none of these studies

calculated type-specific ORs for the associated risk of developing cSCC. Interestingly, both studies validated the association between skin biopsy B-HPV DNA and cSCC, demonstrating a significant increased risk of cSCC associated with the presence of B-HPV DNA in skin.

### Discussion

This systematic review included a total of 4,056 cSCC cases and 7,067 controls and, to our knowledge, is the largest review that assessed the association between B-HPV and cSCC in healthy individuals. We implemented strict inclusion and exclusion criteria for every case-control and cohort study included in the review. Our systematic review addressed the problem of heterogeneity between different studies, one of the biggest limitations to previous reviews on this topic. We achieved this by first strictly including data that assessed patients with confirmed cSCC, while all other skin lesions, being premalignant lesions or different types of skin cancer other than cSCC, were excluded from our review. Second, we divided these studies into six categories based on the utilized detection method of B-HPV. We adopted this approach in order to facilitate inter- and intra-categories comparisons. Third, we divided studies based on their status of multicenter case-control, single-institution case-control and cohort, in an effort to highlight the heterogeneity of the different designs present in the literature. In the case-control design, many authors have repeatedly raised the issue of causality between B-HPV and cSCC: “Is the presence of B-HPV causing the development of cSCC or is the simple inflammation caused by the presence of cSCC activating B-HPV?” In the latter scenario, the higher rates of B-HPV associated with cSCC would be merely a consequence of the cSCC and not the cause. The findings of two large prospective studies<sup>25,33</sup> included in our review succeed in answering this question and strengthen the position of those defending the opinion that B-HPV is implicated in either the initiation or the progression of cSCC. However, the utilization of a prospective cohort design in future studies remains the most powerful method to validate any significance between HPV and cSCC.

Our review collected B-HPV type-specific data from every included study when available. According to the above detailed results we can clearly see that this review is another proof that an association between B-HPV and cSCC exists in healthy individuals as evidenced by most of the conducted research. There remain a few exceptions consisting of 2 studies that according to their authors had a limited sample size to power a definitive answer regarding this association.<sup>21,24</sup>

The review of the majority of the analyzed data highlighted mainly two specific B-HPV types, 8 and 38, that presented a significant increased risk of cSCC development in most of the studied populations using most of the different methods of B-HPV detection. These findings may be strengthened further by conducting a quantitative meta-analysis. In addition, research in the future needs to be more oriented towards targeted B-HPV types in the hopes of finding the specific type to be targeted for cSCC prevention.

Based on the above discussion we suggest tailoring the best research model to answer our question regarding the association between B-HPV and cSCC. We propose a large multicenter prospective cohort study, with specific age stratification (younger than 50 years old and older than 50 years old) and long patient follow-up allowing evaluation for sero-conversion. As for the selection of the specific type of B-HPV to be tested, it appears from our review that types 8 and 38 are essential. The lack of meta-analysis assessing the type-specific data that we collected is one of the limitations of our review. Our team is currently in the process of conducting a pooled type-specific meta-OR that will provide a more certain answer regarding the B-HPV type to be included in future studies. As for the method of detection, we recommend a combination of type-specific multiplex PCR evaluation of B-HPV for the whole cohort, followed by the combination of multiplex serology and skin biopsy B-HPV DNA for individuals that would be newly diagnosed with cSCC for the duration of the cohort.

## Conclusion

This systematic review provides further evidence supporting the role of B-HPV as an important causative agent in cSCC in healthy individuals and supports a possible type-specific HPV<sup>8,38</sup> involvement in cSCC. However, this systematic review is not sufficient to confirm any of the aforementioned association. The main purpose of this paper is to serve as reminder of the need for prospective multi-center cohort studies examining the association between specific types of B-HPV and cSCC in immunocompetent individual.

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## Conflicts of interest

Authors declare there are no conflicts of interest.

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