Host genetic polymorphisms associated with beta human papillomavirus seropositivity

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Received: 8 February 2021 / Accepted: 25 April 2021 / Published online: 11 June 2021
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Abstract

Human papillomaviruses (HPVs) cause superficial epidermal infections and are only cleared if they trigger an immunological response. We analysed SNPs that had previously been investigated for association with HPV infection to determine whether they play a role in the serological response to cutaneous beta-HPVs in an Australian population. Serum samples from 1,142 participants were analysed for seropositivity against the L1 protein of 21 beta-HPV types. Associations between seropositivity to beta-HPV types and the SNPs rs9264942 (HLA-C; HPV-9, p = 0.022, HPV-15, p = 0.043 and HPV-17, p = 0.004), rs12449858 (EVER1; HPV-23, p = 0.029), and rs2981451 (FGFR2; HPV-22, p = 0.049) were identified. We found that certain SNPs could be involved in the serological response to beta-HPVs.

Papillomaviruses are a diverse group of viruses that infect skin and mucosa and are the causative agents of warts and mucosal squamous cell carcinomas (SCCs). Over 220 different human papillomavirus (HPV) types have been fully characterised to date [7]. HPVs belong to different genera in the family Papillomaviridae, with most papillomaviruses that infect the skin belonging to the genera Betapapillomavirus and Gammapapillomavirus. Asymptomatic skin infections with beta-HPV types are very common in the general population [2, 6] and have been extensively investigated as a possible causal factor for cutaneous SCC. While some studies have suggested that beta-HPV infection is a potential risk factor, others have found no associations [1, 17].

Patients with the rare hereditary disease epidermodysplasia verruciformis (EV) have mutations in the two genes, EVER1 and EVER2, which affect their ability to clear their skin of HPV infections [23]. Studies of mutations in these genes have found associations with cutaneous SCCs, cervical cancer, and cutaneous HPV seropositivity [8, 9, 20, 21, 25]. Another SNP (rs9264942) located 35 kb upstream of HLA-C (human leukocyte antigen C; a major histocompatibility complex) has been found to be important in serological responses to beta-HPV and hepatitis C virus (HCV) infections and to affect the viral load in human immunodeficiency virus (HIV) infections [12, 14, 19, 20]. rs9264942 has been shown to affect the expression of HLA-C and also to determine the viral load in HIV-positive individuals; i.e., the genotype CC leads to high expression of HLA-C and lower HIV viral load [14]. Here, we examined associations of SNP variants identified based on published evidence with seropositivity to 21 beta-HPV types that we published previously [3]. The findings reported here are of potential significance, given that HPV is only cleared if an immunological response is elicited.

The Nambour Skin Cancer Study is a prospective cohort study of 2,095 residents of Nambour, a township in Queensland, Australia, who were selected at random from the population-based electoral register and recruited in 1986 [13]. In 1992, all participants completed questionnaires about their personal details, including ancestry and medical and occupational histories. Blood samples were collected from...
693 randomly selected participants in 1992, again from 549 of these in 1993, and from 1,211 study participants in 1996 [13]. The Human Research Ethics Committee of the QIMR Berghofer Medical Research Institute approved this study. All participants gave written informed consent.

After collection, the serum samples were stored at -80°C. Sera were shipped to the German Cancer Research Center (DKFZ), Heidelberg, Germany, on dry ice. At the DKFZ, samples were analyzed for antibodies against the large capsid protein (L1) of 21 beta-HPV types: HPV-5, -8, -9, -14, -15, -17, -20, -21, -22, -23, -24, -36, -38, -47, -49, -75, -76, -80, -92, -93, and -96 [5]. The antibody detection method was based on glutathione S-transferase (GST) capture ELISA [18] in combination with fluorescent bead technology, as described previously [22]. The data on HPV serology used in this study were obtained from the Nambour Skin Cancer Study and were published previously [3].

Genotyping was carried out in two different batches, 863 samples in 2019 and 305 in 2020, both of which were analysed using Illumina Global Screening Array (GSA) chips (model GSAMD-24v1-0-20011747; manifest revision A4), run under contract by the Human Genomics Facility (HuGeF) at Erasmus Medical Centre, Rotterdam.

Genotypes were called using the Genotyping Module in Illumina GenomeStudio by standard procedures described in detail in Supplementary Material. Of the 1,168 genotyped samples, 1,142 samples were included in analysis after removing individuals as described in Supplementary Materials.

To select SNPs that had been investigated previously for association with risk of HPV infection or HPV serology, we performed an online search in PubMed. The following keywords were used, alone or in combination: “HPV”, “SNP”, and “association study”. This resulted in the identification of 25 SNPs from seven publications (SNPs listed together with references in Supplementary Material). Of those, four were already genotyped in our sample. Genotype information for SNPs rs12449858 [4], rs2981451 [25], rs9264942 [20], and rs9357152 was extracted for further analysis (Table 1).

To test the association of these SNPs with serological responses to beta-HPV types, we used a candidate gene case-control study design in study participants of European ancestry. To account for relatedness in our sample we used CERAMIC, an estimating equation approach that combines logistic regression and a linear mix-effects model (LMM) [24]. CERAMIC is a method for binary trait mapping that accounts for partially missing data and sample structure (relatedness) through the integration of a kinship matrix in the calculation of the algorithm. The output generated is a list of $p$-values per SNP. In this study, a $p$-value < 0.01 (using the Bonferroni correction) was considered significant.

After quality control, 1,142 study participants of European ancestry were included in the analysis. The mean age of the participants was 49 years (range, 25-75 years), and 55% were females.

The SNP rs9264942 was associated with seropositivity to three beta-HPV types: HPV-9, -15 and -17 (for allele C; $p$-values 0.022, 0.043, and 0.004, respectively; Table 2). For rs12449858, allele A was associated with seropositivity to HPV-23 ($p = 0.029$), and rs2981451 (allele G) with seropositivity to HPV-22 ($p = 0.049$; Table 2). We found no associations with rs9357152 on chromosome 6 with seropositivity to any of the 21 beta-HPV types analysed.

The two beta-HPV types with the highest seroprevalence in our previously published study [3] from this cohort were HPV-8 and HPV-38 (both 33%).

### Table 1 Details of SNPs investigated in this study

| SNP     | Gene                | Chromosome | Position (GRCh38) | Alleles (MAF) | HPV association/publication | Clinical significance                  |
|---------|---------------------|------------|------------------|---------------|-----------------------------|----------------------------------------|
| rs9264942 | LOC112267902        | 6          | 31306603         | T/C (C = 0.363) | Beta-HPV seropositivity [20] | Susceptibility to HIV-1 viremia [12]   |
| rs9357152 | None                | 6          | 32697183         | A/G (G = 0.269) | HPV-8 seropositivity (a beta-HPV type) [3] | None reported                         |
| rs2981451 | FGFR2               | 10         | 121519400        | G/T (T = 0.335) | Cervical HPV-58 infection (an alpha-HPV type) [25] | None reported                         |
| rs12449858 | EVER1 (TMC6)        | 17         | 78125237         | G/A (A = 0.105) | HPV status in mucosal head and neck cancer (caused by alpha-HPV types) [4] | None reported                         |

### Table 2 SNPs significantly associated with serology for beta-HPV types

| HPV type | SNP      | A1 | A2 | MAF (full cohort) | $p$-value |
|----------|----------|----|----|-------------------|-----------|
| HPV-9    | rs9264942| C  | T  | 0.353             | 0.022     |
| HPV-15   | rs9264942| C  | T  | 0.353             | 0.043     |
| HPV-17   | rs9264942| C  | T  | 0.353             | 0.004     |
| HPV-22   | rs2981451| T  | G  | 0.483             | 0.049     |
| HPV-23   | rs12449858| A  | G  | 0.093             | 0.029     |
seroprevalence for the five beta-HPV types that were associated with the SNPs investigated here were HPV-9 (17%), HPV-15 (22%), HPV-17 (23%), HPV-22 (15%), and HPV-23 (12%).

We evaluated the association of four selected SNPs with seropositivity to 21 different beta-HPV types. We showed that the rs9264942-T allele increased susceptibility to seropositivity for species 2 beta-HPV types (HPV types 9, 15, and 17). The SNP rs9264942 has previously been implicated in immunological response to viral infections, including HIV viral load, and seropositivity to HCV and beta-HPV types [12, 14, 19, 20]. A previous case-control study of skin SCC identified an association with the CC genotype of this SNP and seronegativity to HPV types from the beta-2 species in controls, but not in cutaneous SCC cases [20]. An antibody response to HPV would be important in helping to clear HPV infection. In addition to viral control and serological responses to viral infections, this particular SNP in the HLA-C gene has also been associated with a predisposition to psoriasis (TT genotype), a relatively common inflammatory skin disease [11]. Interestingly, there have been studies suggesting that high viral load of certain beta-HPV types could act as autoantigens and illicit psoriasis through cytokine release [10, 11, 16].

The EVER genes have been identified as genes that play a role in susceptibility to infection with skin HPV types [15]. We investigated SNP rs12449858 located in the EVER gene and found genotype GG to be associated with seropositivity to HPV-23. We investigated this particular SNP in a previous study on HPV infection in mucosal cancers of the head and neck but found no significant association with mucosal (alpha) HPVs [4]. We also found that rs2981451 was associated with seropositivity to HPV-22, and this SNP has previously been associated with HPV-58 infection of cervical cells [25]. While a previous study found associations with rs9357152 and HPV-8 seropositivity, we found no associations between this SNP and serological responses to any of the HPV types investigated here. However, we acknowledge the limitation that the relatively small sample size of this study has lessened its statistical power.

In summary, rs9264942 has been investigated previously and seems to be important for immunological control of viral infections and serological responses by regulating HLA-C expression on cells. Our data support previous findings and show that this SNP (rs9264942) is strongly associated with serological response to beta-HPV types.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05137-4.

Acknowledgements We would like to thank all participants of the Nambour Skin Cancer Study. We thank Prof Lyn Griffiths, Ms Rebecca Grealy, and Griffith University for their invaluable contributions to the Nambour Skin Cancer Study DNA resource, including sharing sample processing and storage. We are also grateful to Profs Nick Martin and David Whiteman for their support.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Annika Antonsson, Astrid J. Rodriguez-Acevedo, Maria Celia B. Hughes, and Adele C. Green. The first draft of the manuscript was written by Annika Antonsson, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by a Program Grant (1073898) from the National Health and Medical Research Council of Australia (NHMRC). The funding body played no role in the design or conduct of the study. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding body.

Availability of data and material Available upon request from authors.

Code availability Available upon request from authors.

Declarations

Conflict of interest The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval QIMR Berghofer Medical Research Institute’s Human Research Ethics Committee (P327).

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