HLA-G Polymorphism Impacts the Outcome of Oral HPV Infections in Women

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Abstract

**Purpose:** Human leukocyte antigen (HLA)-G may have an important role in the natural history of human papillomavirus (HPV) infection. Our aim was to evaluate the role of HLA-G in the outcome of genital and oral HPV infections in women.

**Methods:** Analyses included 306 women from the Finnish Family HPV-study and were followed-up for six years. Genital and oral samples were tested for 24 different HPV types with multiplex HPV genotyping. HLA-G alleles were determined through direct DNA-sequencing. Unconditional logistic regression was used to determine the associations between HLA-G genotypes and HPV infection outcomes.

**Results:** Nine HLA-G alleles were identified. Most common HLA-G genotypes were the wild type G*01:01:01/01:01:01 (31.3%) followed by G*01:01:01/01:01:02 (26.8%). G*01:01:01/01:01:01 genotype was associated with increased risk of oral HPV infections by any HPV type or single-type with OR=1.86 (95% CI 1.14-3.04) and 2.22 (95% CI 1.14 - 3.71), respectively. G*04:01+ allele and the G*01:01:01/01:04:01 genotype both protected from any and single oral HPV infections; OR=0.46 (95% CI 0.23-0.89) and 0.53 (95% CI 0.23-0.97), respectively. G*01:01:02/01:04:01 genotype increased significantly the risk of infertility and its treatments, with respective OR= 5.06 (95% CI 1.22-21.02) and OR=9.07 (95% CI 1.22-39.50). Both HLA-G alleles and genotypes showed several significant associations with the outcomes of oral HPV infections, but none of them had any impact on the outcomes of genital HPV infections in these women.

**Conclusion:** The host HLA-G genotypes appear to impact the outcomes of oral HPV infections in women but have little if any effect on genital HPV status or infection outcomes.

Introduction

Human Papillomavirus (HPV) infections are very common and it is estimated that 80% of sexually active men and women contract a genital HPV-infection during their lifetime[1]. Persistent genital infection with high-risk (HR) HPV is shown to be involved in nearly all cervical cancers and its precursors[2]. Increasing evidence also implicates that HPV infections play a significant role in the etiology of head and neck carcinomas, oropharyngeal cancer in particular[3]. Globally, HPV infections are associated with nearly 10% of all human cancers[4].

Only a small percentage of HPV infections progress to cervical cancer (CC). Impaired reactivity of the cell-mediated immunity (CMI) and the human leukocyte antigen (HLA) system to viral antigens seem to increase the risk of CC[5]. The vast majority of the published literature has focused on the association between HLA class II alleles and CC[6], but data are emerging to implicate an association between HLA-G polymorphisms and cervical HPV infections as well as CC [7, 8]. Human leukocyte antigen (HLA)-G is a non-classical HLA class Ib molecule, first identified to be present in placental cells of fetal origin and playing a role in immune tolerance during pregnancy [6, 9, 10]. However, HLA-G can be expressed de novo at high levels in several pathological conditions, including some tumors as well as during microbial or viral infections, leading to the impairment of the immune response against tumor cells or infectious pathogens, respectively [11].

Various HLA-G alleles have been reported to be associated with HPV-infections, including their prevalence, persistence and progression to CC [12, 13]. Furthermore, HLA-G has been found to play an important role in several aspects of the female reproductive health [14]. In the present study, our aim was to evaluate the potential impact of HLA-G in the outcomes of genital and oral HPV infections in women. In addition, we evaluated whether different HLA-G genotypes and alleles are of predictive value in different characteristics of the female reproductive health.

Methods

**Finnish Family HPV-study**

The present study is part of the Finnish Family HPV (FFHPV) study, which was initiated in 1998 to investigate the dynamics of HPV transmission between the family members[15, 16]. At baseline, 329 pregnant mothers, 171 fathers and 331 their newborn babies were recruited. All the families are of Caucasian origin (native Finnish population). At the baseline visit, all women were pregnant (36 gestational weeks), with the mean age of 25.6 years (SD ± 0.2), range 18–46 years. Detailed data were collected by structured questionnaire at baseline, complemented with the information about the delivery as well as the child’s anthropometrics. The Research Ethics Committee of Turku University Hospital (#3/1998 and #2/2006) has approved the study protocol and its amendments. All the participants gave their written consent for the cohort study.

**Samples**

Oral and genital scrapings from all women were collected for HPV-testing with a cytobrush (MedScand, Malmö, Sweden) at baseline and during the follow-up visits at 2, 6, 12, 24, 36 and 72 months [15]. HPV was detected with PCR, using My09/My11 and GP05+/GP06+. 

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Discussion

addition, HLA-G*01:01:01/01:01:03 genotype showed a significant association with an abnormal placenta, OR = 5.51 (95% CI 1.03–29.37). G*01:01:01/01:01:02 and G*01:01:01/01:01:03, with OR = 4.85 (95% CI 1.13–20.85) and OR = 6.00 (95% CI 1.11–32.46), respectively. In likelihood of skin wart history during follow-up, OR = 0.18 (95%CI 0.04–0.87). Self-reported oral warts were associated with genotypes respective OR = 5.06 (95%CI 1.22–21.02) and OR = 9.07 (95% CI 1.22–39.50). Genotype G*01:01:02/01:04:01 was shown to decrease the genotype level (Table 4), HLA-G*01:01:02/01:04:01 was associated with an increased infertility and the recorded treatments, with OR = 0.07 (95%CI 0.01–0.72). This trend was also seen in the high resolution wild type alleles, but it did not reach statistical significance. At CI 1.28–32.77) (Table 3). In HLA-G allele groups, G*01:01- group was associated with decreased risk of having an early labor (< 37 week), risk of having atopic tendency, OR = 1.9 (95% CI 1.00-3.73) while allele *01:06 increased the risk having an inducted labour, OR = 6.48 (95% CI 1.06–3.30). Similar association with permanent HPV-negative status was also found for genotype G*01:01:02/01:04:01, with OR = 2.18 (95% CI 1.12–4.26). Genotype G*01:01:01/01:01:01 was related to increased clearance of any oral HPV infection but also decreased the probability of permanent HPV-negative status with OR = 1.18 (95% CI 1-28-3.71) and OR = 0.54 (95% CI 0.33–0.88), respectively (Table 2).

We also investigated the associations between HLA-G alleles and genotypes with risk factors of several reproductive health endpoints. An incident HPV-infection was recorded when a baseline HPV-negative woman tested HPV-positive at any of the follow-up visits. HPV clearance denotes all cases where a previously HPV-positive women tested HPV-negative at any follow-up visits and remained HPV-negative until the end of the follow-up. Persistent HPV infection was defined when HPV positivity was recorded in two or more consecutive visits during the follow-up.

Statistical analysis

This study includes 306 women of the FFHPV-Study. Stata 15.0 (Stata Corp., College Station, TX) was used for all statistical analyses, performed two-sided and declared significant at the P-value < 0.05 level. Women with no HLA-G genotyping result available (n = 23/329) were excluded from the analyses. Single and multiple HPV infections were recorded, separately. HPV positivity was defined as being positive at any follow-up visits for one or multiple HPV genotypes. In statistical analysis, we only considered HLA alleles and genotypes that were present in at least in 3% of the study group. Unconditional logistic regression analysis was used to determine the associations between HLA-G alleles or genotypes and the genital and oral HPV-infection outcomes as well as their role as risk factors of several reproductive health endpoints. An incident HPV-infection was recorded when a baseline HPV-negative woman tested HPV-positive at any of the follow-up visits. HPV clearance denotes all cases where a previously HPV-positive women tested HPV-negative at any follow-up visits and remained HPV-negative until the end of the follow-up. Persistent HPV infection was defined when HPV positivity was recorded in two or more consecutive visits during the follow-up.

Results

Ten different HLA-G alleles and 24 different genotype combinations were identified in these women. HLA-G alleles and genotypes with > 3% prevalence are shown in Fig. 1. The most common HLA-G allele was the wild-type *01:01:01: 32% (n = 98) of the women being homozygous, 52% (n = 159) heterozygous and only 16% (n = 49) women missing this allele. Of the HLA-G genotypes, *01:01:01/01:01:01 (31.3%) was the most prevalent, followed by G*01:01:01/01:01:02 (26.8%).

HLA-G alleles and genotypes as related to genital and oral HPV-prevalence (single and multiple-type) are depicted in Table 1. No significant associations were observed between HLA-G alleles/genotypes and genital HPV infections. As to oral HPV infections, allele G*01:01:01:03 and genotype G* 01:01:01/01:01:03 were associated with increased risk of multiple-type oral HPV infections during the follow up, OR = 3.32 (95 % CI 1.18–9.34) and OR = 4.44 (95 % CI 1.39–14.20), respectively. G*01:01:01/01:04:01 and the G*01:04 group both protected from any and single-type oral HPV infections; OR = 0.46 (95% CI 0.23–0.89) and OR = 0.53 (95% CI 0.23–0.97), respectively. G*01:01:01/01:01:01 genotype seemed to predispose the women to any HPV type or single-type infection with OR = 1.86 (95% CI 1.14–3.04) and OR = 2.22 (95% CI 1.14–3.71), respectively.

HLA-G alleles, groups and genotypes as related to genital and oral HPV infection outcomes are shown in Table 2. No significant association was detected between the genital HPV-infection outcomes and HLA-G alleles or genotypes. As to oral HPV infections, allele G*01:01:01:02 was associated with decreased clearance of oral HPV infection OR = 0.50 (95% CI 0.29–0.79) and the genotype G*01:01:02/01:04:01 increased the risk of oral persistence, with OR = 4.01 (95% CI 1.19–13.53). HLA Group G04:01 + decreased the risk of incident oral HPV by 2-fold; OR = 0.51 (95% CI 0.27–0.98) and increased the probability of women remaining HPV-negative throughout the follow-up; OR = 1.87 (95% CI 1.06–3.30). Similar association with permanent HPV-negative status was also found for genotype G*01:01:02/01:04:01, with OR = 2.18 (95% CI 1.12–4.26). Genotype G*01:01:01/01:01:01 was related to increased clearance of any oral HPV infection but also decreased the probability of permanent HPV-negative status with OR = 1.18 (95% CI 1-28-3.71) and OR = 0.54 (95% CI 0.33–0.88), respectively (Table 2).

We also investigated the associations between HLA-G alleles and genotypes with risk factors of HPV infection recorded in these women at baseline and during the follow-up (Table 3 and Table 4). At the HLA-G allele level only allele G*01:01:02 showed associated with increased risk of having atopic tendency, OR = 1.9 (95% CI 1.00-3.73) while allele *01:06 increased the risk having an inducted labour, OR = 6.48 (95% CI 1.28–32.77) (Table 3). In HLA-G allele groups, G*01:01- group was associated with decreased risk of having an early labor (< 37 week), OR = 0.07 (95%CI 0.01–0.72). This trend was also seen in the high resolution wild type alleles, but it did not reach statistical significance. At the genotype level (Table 4), HLA-G*01:01:02/01:04:01 was associated with an increased infertility and the recorded treatments, with respective OR = 5.06 (95%CI 1.22–21.02) and OR = 9.07 (95% CI 1.22–39.50). Genotype G*01:01:02/01:04:01 was shown to decrease the likelihood of skin wart history during follow-up, OR = 0.18 (95%CI 0.04–0.87). Self-reported oral warts were associated with genotypes G*01:01:01/01:01:02 and G*01:01:01/01:01:03, with OR = 4.85 (95% CI 1.13–20.85) and OR = 6.00 (95% CI 1.11–32.46), respectively. In addition, HLA-G*01:01:01/01:01:03 genotype showed a significant association with an abnormal placenta, OR = 5.51 (95% CI 1.03–29.37).
In the present study, we identified ten different HLA-G alleles and 24 different genotype combinations among the 306 women of the FFHPV-Study. We found an association with certain HLA-G alleles and genotypes with the outcomes of oral HPV-infections, but interesting enough no such associations with the outcomes of genital HPV-infections. Additionally, some HLA-G alleles and genotypes seemed to be linked with some characteristics of the women's reproductive health. There are only a few studies available investigating the impact of HLA-G polymorphism in the natural history of HPV-infections, most of these previous studies being published on cervical HPV-induced premalignant and malignant lesions. The data on HLA-G polymorphism in oral HPV-infections are, to our knowledge, lacking, with anecdotal data on malignant lesions of the oropharynx where HLA-G polymorphism have been studied, but only using immunohistochemistry in the biopsy samples with mono-/or polyclonal antibodies for HLA-G or assessing the polymorphic sites at 5'URR (upstream regulatory region) and 3'UTR (untranslated region) by polyacrylamide gel electrophoresis. In another study, HLA-G gene alleles *0106, *010106, *01010106 and *0105N were significantly higher in patients with embryonic implantation failure on infertility treatments. The data on HLA-G polymorphism in oral HPV-infections are, to our knowledge, lacking, with anecdotal data on malignant lesions of the oropharynx where HLA-G polymorphism have been studied, but only using immunohistochemistry in the biopsy samples with mono-/or polyclonal antibodies for HLA-G or assessing the polymorphic sites at 5'URR (upstream regulatory region) and 3'UTR (untranslated region) by polyacrylamide gel electrophoresis.

This is the first study to report that HLA-G has a potentially an important role in the natural history of oral HPV infections in women. The findings of the present study are in agreement with our recent observations showing that HLA-G molecules are associated with the newborn's likelihood of oral HPV infection at birth. Accordingly, HLA-G*01:01:02/01:01:02 genotype concordance between mother and her child was shown to increase the risk of oral infection of the child by any HPV genotype and/or HR-HPV genotypes (OR 2.45). This raised the possibility that oral HPV infections detected in young women might be determined by their mother's HLA-G status.

In the present female cohort, homozygous genotype G*01:01:01/01:01:01 was shown to increase both the clearance of any oral HPV infection (OR = 1.18) and having any oral HPV-infections during the follow-up (OR = 1.86). However, this wild-type genotype did not impact on the HPV persistence that would be needed for the progression to a premalignant lesion. A similar protective effect for oral HPV infection was seen with G*04:01+ (OR = 1.87) as well as genotype G*01:01:01/01:01:02 (OR = 2.18). In the genital mucosa, Metcalfe and coworkers showed that allele G*01:01:01 (OR = 2.23) and heterozygous genotype with G*01:01:01 (OR = 2.14) were increasing both the risks of genital LR-HPV infection and genital multiple-type infections versus single-type infections. Ferguson et al. found significant associations between persistent genital HPV-16 and LR-HPV-infection with allele G*01:01:02, G*01:01:03, G*01:01:05, G*01:01:08 and G*01:03 (OR's 1.90, 2.07, 2.52, 2.17, 2.99 respectively). Later, they also reported that the homozygous G*01:01:02 genotype increased the risk of developing invasive cervical squamous cell carcinoma (OR = 3.52). Contradictory to the latter, Metcalfe et al. observed that allele G*01:01:02 was significantly associated with decreased risk of any genital HPV infection (OR = 0.64) and had a protective effect against multiple type infections (OR = 0.45)

In the present study, we found allele G*01:01:02 to reduce the probability of clearance of oral HPV-infection (OR = 0.50) and its combined genotype G*01:01:02/01:01:01 to increase oral HPV persistence (OR = 4.01). Protective effect against multiple-type infections was also noted with genotype G*01:01:01/01:01:02 (OR = 0.49) but not with the allele G*01:01:02. With regard allele G*01:01:03, Ferguson et al. found it to increase the risk of genital HPV infections (OR = 2.07). This finding was also observed in our study for multiple-type oral infections (OR = 3.32) but not for genital infections (Table 1). In previous studies, there seems to be some parallel trends recorded in both genital and oral infections, but interestingly, the present cohort failed to show any association between HLA-G polymorphism and genital HPV infections. The potential reasons might be that the previous studies have been somewhat larger (N = 539–636) and importantly, focused on populations of different origin (Hispanics, Inuites)

Only a few studies have assessed the associations between HLA-G polymorphism and female reproductive health. Craenmehr et al. reported HLA-G over-expression in the full-term placenta of the women with a history of recurrent miscarriages. In another study, HLA-G gene alleles *0106, *010106, *01010106 and *0105N were significantly higher in patients with embryonic implantation failure on infertility treatments. In the present study, we could not confirm these results on HLA-G alleles, but we showed an association with HLA-G G*01:01:02/01:04:01 genotype and risk of both infertility and undergoing treatments for infertility (OR = 5.06 and OR = 9.07, respectively). In addition, genotype G*01:01:01/01:01:03 was significantly associated with an abnormal placenta (OR = 5.51). Of note, all women in our cohort were pregnant at study baseline. Thus, our cohort is not a representative series of infertility clinic patients and all the data on their reproductive health are based on the women's self-reported statements.

This study has some potential limitations. The FFHPV cohort consists only of Caucasian Finnish women, and accordingly, the generalization to other populations is limited because Finland has its limited gene pool. Cohort size of women was quite small, only 306 women, thus impeding the power to evaluate any associations of the rarest HLA-G genotypes. The main strength of this study is the vast database from a long-term prospective study that included study subjects with similar lifestyle and biological background, followed-up for a long period of time with detailed HPV status of both the genital and oral sites.
In conclusion, we identified six clinically significant HLA-G genotypes affecting the oral HPV status and infection outcomes, and being related to some characteristics of the reproductive health of the women in the FFHPV study. The host HLA-G genotypes and certain alleles alone appear to have a potentially important impact in the outcome of oral HPV infection in women, but only a minor or no role in the natural history of genital HPV infections. Further studies on HLA-G polymorphism are warranted to confirm their impact as predictors of the natural history of HPV infections at different anatomic sites.

**Declarations**

**Funding**

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**Conflict of interest**

Nothing to declare.

**Availability of data and material**

Data is available upon reasonable request from the corresponding author.

**Code availability**

Data is available upon reasonable request from the corresponding author.

**Authors’ contributions**

All authors have contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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Tables

**Table 1.** HLA-G alleles, low-resolution groups and genotypes, as related to genital and oral HPV status of the women
| Genital | Oral |
|---------|------|
| HLA-G   | Any HPV | Single | Multiple (2+) | Any HPV | Single | Multiple (2+) |
| (n=250) |        | (n=119) | (n=131)       | (n=154) | (n=120) | (n=34)       |

**Alleles**

- **01:01:01**: 1.01 (0.46-2.21) 0.84 (0.36-1.98) 1.20 (0.50-2.87) 0.88 (0.48-1.62) 1.01 (0.52-1.97) 0.58 (0.23-1.44)
- **01:01:02**: 0.94 (0.52-1.70) 0.90 (0.47-1.73) 0.97 (0.52-1.84) 0.83 (0.53-1.31) 0.83 (0.51-1.35) 0.85 (0.40-1.83)
- **01:01:03**: 0.88 (0.34-2.28) 0.76 (0.26-2.22) 1.00 (0.36-2.74) 1.80 (0.83-3.93) 1.42 (0.61-3.35) **3.32 (1.18-9.34)**
- **01:03:01**: 0.89 (0.18-4.32) 0.94 (0.17-5.29) 0.85 (0.15-4.78) 0.24 (0.05-1.13) 0.15 (0.02-1.23) 0.55 (0.07-4.51)
- **01:04:01**: 1.02 (0.49-2.12) 1.03 (0.47-2.29) 1.01 (0.46-2.22) 0.57 (0.30-1.02) 0.55 (0.29-1.02) 0.67 (0.26-1.73)
- **01:06**: 2.29 (0.29-18.28) 3.44 (0.41-28.64) 1.29 (0.13-12.67) 1.76 (0.51-6.15) 1.28 (0.31-5.21) 3.58 (0.76-16.81)

**Groups**

1. **G01:01**: 1.12 (0.12-10.20) 1.06 (0.09-11.98) 1.17 (0.10-13.20) 0.67 (0.11-4.07) 0.79 (0.11-5.67) 0.44 (0.04-5.00)
2. **G04:01**: 1.07 (0.52-2.22) 1.09 (0.49-2.40) 1.06 (0.49-2.32) **0.53 (0.30-0.94)** 0.51 (0.28-0.95) 0.62 (0.24-1.61)

**Genotypes**

- **010101:010101**: 0.80 (0.44-1.47) 0.84 (0.43-1.65) 0.76 (0.39-1.48) **1.86 (1.14-3.04)** 2.22 (1.32-3.71) 0.92 (0.39-2.21)
- **010101:010102**: 0.82 (0.44-1.55) 0.71 (0.35-1.44) 0.94 (0.47-1.86) 0.73 (0.44-1.21) 0.80 (0.47-1.37) 0.49 (0.19-1.27)
- **010101:010103**: 1.97 (0.44-8.78) 1.95 (0.40-9.48) 1.99 (0.42-9.53) 1.75 (0.67-4.57) 1.09 (0.36-3.33) **4.44 (1.39-14.20)**
- **010101:010401**: 1.01 (0.44-2.31) 0.93 (0.37-1.33) 1.08 (0.45-2.62) **0.46 (0.23-0.89)** 0.47 (0.23-0.97) 0.41 (0.12-1.44)
- **010102:010102**: 2.05 (0.25-16.55) 2.92 (0.43-24.86) 1.29 (0.13-12.67) 1.50 (0.41-5.42) 1.28 (0.31-5.21) 2.31 (0.41-13.17)
- **010102:010401**: 2.29 (0.29-18.28) 2.92 (0.34-24.86) 1.73 (0.19-15.85) 1.19 (0.36-3.99) 1.01 (0.27-3.86) 1.84 (0.34-9.90)

1. HPV positivity taken as positive for any HPV type during the 72months follow-up (part of single or multiple infection). Single infection: always only one HPV type present at the follow-up visits and multiple: two or more HPV types presented during the follow up visits.
2. Groups as low resolution for the following alleles G01:01: *010101, *010102, *010103, *010114 and G04:01: *01040 and *010404. Only those HLA-G alleles and genotypes that where >3% prevalent among the women where included to the analyses. The significant associations are bolded.

**Table 2.** HLA-G alleles, low-resolution groups and genotypes as related to outcomes of the genital and oral HPV infections during the six-year follow-up.
| HLA-G | Always negative | Incidence | Clearance | Persistence | Always negative | Incidence | Clearance | Persistence |
|-------|----------------|-----------|-----------|-------------|----------------|-----------|-----------|-------------|
| **Genital HPV infection outcomes** | | | | | **Oral HPV infection outcomes** | | | | |
| *01:01:01* | 0.99 (0.45-2.19) | 0.72 (0.37-1.40) | 1.14 (0.61-2.12) | 0.81 (0.44-1.50) | 1.14 (0.62-2.10) | 1.14 (0.59-2.21) | 1.92 (0.86-4.29) | 0.68 (0.34-1.32) |
| *01:01:02* | 1.06 (0.59-1.92) | 1.08 (0.67-1.75) | 0.88 (0.55-1.40) | 1.06 (0.67-1.67) | 1.20 (0.76-1.90) | 0.87 (0.53-1.42) | 0.50 (0.29-0.79) | 0.96 (0.56-1.65) |
| *01:01:03* | 1.13 (0.44-2.91) | 1.26 (0.56-2.87) | 1.18 (0.54-2.64) | 1.34 (0.63-2.85) | 0.55 (0.25-1.21) | 1.90 (0.89-4.07) | 1.07 (0.46-2.51) | 1.16 (0.49-2.72) |
| *01:03:01* | 1.12 (0.23-5.42) | 0.79 (0.22-2.86) | 1.14 (0.32-4.13) | 2.75 (0.70-10.82) | 4.22 (0.88-20.22) | 0.50 (0.10-2.39) | NC | 0.34 (0.04-2.72) |
| *01:04:01* | 0.98 (0.47-2.03) | 0.78 (0.44-1.38) | 1.08 (0.61-1.91) | 0.81 (0.46-1.43) | 1.74 (0.98-3.09) | 0.54 (0.28-1.04) | 0.66 (0.33-1.33) | 0.92 (0.47-1.78) |
| *01:06* | 0.44 (0.05-3.48) | 2.45 (0.52-11.55) | 1.34 (0.38-4.68) | 0.64 (0.18-2.24) | 0.57 (0.16-1.98) | 1.17 (0.33-4.08) | 1.10 (0.28-4.25) | 1.84 (0.52-6.46) |
| **Groups** | | | | | | | | |
| **G01:01** | 0.89 (0.10-8.16) | 1.26 (0.21-7.68) | 0.32 (0.04-2.94) | 0.58 (0.95-3.52) | 1.49 (0.25-9.05) | 0.74 (0.12-4.47) | 1.38 (0.15-12.49) | 0.47 (0.77-2.88) |
| **G04:01** | 0.93 (0.45-1.92) | 8.83 (0.47-1.47) | 1.06 (0.60-1.87) | 0.89 (0.51-1.55) | 1.87 (1.06-3.30) | 0.51 (0.27-0.98) | 0.63 (0.32-1.26) | 0.87 (0.45-1.69) |
| **Genotypes** | | | | | | | | |
| 010101:010101 | 1.25 (0.68-2.29) | 1.19 (0.71-1.99) | 1.01 (0.61-1.65) | 0.87 (0.54-1.41) | 0.54 (0.33-0.88) | 1.49 (0.90-2.47) | 1.18 (1.28-3.71) | 1.44 (0.83-2.49) |
| 010101:010102 | 1.22 (0.64-2.29) | 0.79 (0.47-1.33) | 1.09 (0.66-1.84) | 0.89 (0.54-1.47) | 1.37 (0.83-2.28) | 0.90 (0.52-1.55) | 0.62 (0.33-1.51) | 0.75 (0.41-1.38) |
| 010101:010103 | 0.51 (0.11-2.26) | 2.99 (0.85-10.49) | 1.09 (0.40-2.94) | 1.61 (0.63-4.13) | 0.57 (0.22-1.49) | 1.91 (0.75-4.85) | 1.05 (0.36-3.01) | 1.49 (0.54-4.06) |
| 010101:010401 | 0.99 (0.43-2.27) | 0.59 (0.31-1.12) | 0.95 (0.50-1.82) | 0.68 (0.35-1.31) | 2.18 (1.12-4.26) | 0.64 (0.31-1.32) | 0.97 (0.46-2.03) | 0.45 (0.18-1.11) |
| 010102:010102 | 0.49 (0.06-3.92) | 1.25 (0.32-4.92) | 0.60 (0.16-2.27) | 1.14 (0.32-4.04) | 0.67 (0.18-2.41) | 0.87 (0.22-3.42) | 1.26 (0.32-5.01) | 1.36 (0.34-5.39) |
| 010102:010401 | 0.44 (0.05-3.48) | 5.53 (0.70-43.77) | 0.91 (0.27-3.04) | 1.38 (0.41-4.63) | 0.84 (0.25-2.81) | 0.20 (0.02-1.54) | NC | 4.01 (1.19-13.53) |

1. HPV outcomes definitions: incidence: baseline negative and acquired a HPV infection during follow-up, clearance: HPV positive and cleared the HPV (staying HPV negative to the end of the follow-up); persistence: recorded two or more consequence visits as HPV positive during the follow-up. 2. Groups as low resolution for the following alleles: G01:01: *01:0101, *01:0102, *01:0103, *01:0114, and G04:01: *01:0401 and *01:0404. Only those HLA-G alleles and genotypes that were >3% prevalent among the women were included to the analyses. NC: Not Computable. The significant associations are bolded.

Table 3. Association between HLA-G alleles and their low-resolution groups and risk factors for HPV infection recorded by the baseline questionnaire.
| Risk factors                                      | HLA-G alleles | OR (95%CI) | HLA-G groups | OR (95%CI) |
|--------------------------------------------------|---------------|------------|--------------|------------|
|                                                  |               | *01:01:01  | *01:01:02    | *01:01:03  | *01:03:01  | *01:04:01  | *01:06:01  | G01:01     | G04:01     |
| Miscarriages (≥1)                                | 0.56          | (0.25-1.29)| 1.67         | (0.83-3.35)| 1.51       | (0.53-4.28)| 2.87       | (0.71-11.65)| 0.59       | (0.22-1.60)| 0.63       | (0.08-5.03)| 0.62       | (0.07-5.74)| 0.56       | (0.21-1.52)|
| Infertility                                      | 0.44          | (0.17-1.13)| 2.28         | (0.97-5.34)| 0.38       | (0.05-2.95)| 1.19       | (0.14-9.80)| 0.78       | (0.26-2.38)| NC         | NC         | 0.74       | (0.24-2.27)|
| Infertility treatments                           | 0.37          | (0.12-1.15)| 2.34         | (0.81-6.77)| 0.65       | (0.08-5.11)| NC         | 2.11       | (0.69-6.45)| NC         | 0.22       | (0.02-0.88)| 2.01       | (0.66-6.13)|
| Sexually transmitted diseases (HSV, Chlamydia)   | 1.12          | (0.51-2.47)| 0.62         | (0.34-1.13)| 1.00       | (0.39-2.58)| 0.44       | (0.54-3.52)| 0.75       | (0.35-1.57)| 0.89       | (0.19-4.22)| 0.37       | (0.06-2.24)| 0.82       | (0.40-1.68)|
|Self reported warts                              |               |            |              |            |            |            |            |            |            |            |            |            |            |            |            |
| Genital                                          | 0.92          | (0.45-1.87)| 1.08         | (0.63-1.84)| 0.67       | (0.26-1.72)| 1.10       | (0.28-4.35)| 1.85       | (0.99-3.46)| 0.56       | (0.12-2.63)| 0.39       | (0.05-2.79)| 1.91       | (1.03-3.53)|
| Oral                                             | 1.35          | (0.16-11.25)| 4.82        | (0.96-24.36)| 3.21       | (0.62-16.77)| 4.07       | (0.45-36.75)| NC         | NC         | NC         | NC         | NC         |            |            |            |
| Skin                                             | 1.35          | (0.73-2.50)| 0.90         | (0.57-1.42)| 1.86       | (0.84-4.12)| 0.57       | (0.16-2.08)| 0.59       | (0.33-1.03)| 4.12       | (0.88-19.39)| 0.76       | (0.12-4.59)| 0.64       | (0.37-1.12)|
| Allergies                                        | 0.98          | (0.51-1.87)| 0.94         | (0.58-1.52)| 0.74       | (0.33-1.70)| 0.29       | (0.06-1.41)| 1.35       | (0.75-2.48)| 2.21       | (0.63-7.73)| 1.23       | (0.20-7.48)| 1.35       | (0.75-2.42)|
| Atopia                                           | 0.51          | (0.23-1.11)| 1.9 (1.00-3.73)| 1.23  | (0.44-3.45)| 0.58       | (0.07-4.69)| 0.93       | (0.40-2.14)| 1.19       | (0.25-5.69)| NC         | 1.23       | (0.57-2.69)| NC         |            |            |
| Vulvovaginitis                                   | 0.56          | (0.11-2.87)| 0.94         | (0.22-4.02)| NC         | 4.10       | (0.45-36.89)| NC         | NC         | NC         | NC         | NC         | NC         | NC         |            |            |
| Menarche                                         |               |            |              |            |            |            |            |            |            |            |            |            |            |            |            |            |
| Late (≥14years)                                  | 1.12          | (0.31-3.99)| 0.81         | (0.31-2.10)| 1.69       | (0.46-6.20)| 1.55       | (0.18-13.02)| 0.97       | (0.31-3.01)| NC         | NC         | 1.25       | (0.44-3.62)|
| Early (≤11years)                                 | 1.18          | (0.14-10.04)| 0.60        | (0.11-3.17)| NC         | NC         | 1.57       | (0.30-8.32)| 4.1 (0.45-37.35)| NC     | 1.50       | (0.28-7.93)|
| Start of labour                                  |               |            |              |            |            |            |            |            |            |            |            |            |            |            |            |
| Own contractions                                 | 1.33          | (0.69-2.59)| 0.83         | (0.51-1.36)| 0.52       | (0.23-1.19)| 1.19       | (0.31-4.54)| 1.07       | (0.59-1.95)| 2.93       | (0.58-14.79)| 0.35       | (0.04-3.37)| 1.02       | (0.57-1.85)|
| Rupture of the membranes                         | 1.15          | (0.48-2.76)| 1.22         | (0.66-2.29)| 0.33       | (0.75-1.43)| 0.55       | (0.07-4.48)| 0.59       | (0.25-1.40)| NC         | 0.67       | (0.07-6.58)| 0.57       | (0.24-1.36)|
| Induction                                        | 1.28          | (0.62-2.63)| 0.70         | (0.42-1.19)| 0.84       | (0.35-2.01)| 1.66       | (0.43-6.33)| 1.47       | (0.80-2.72)| 6.48       | (1.28-32.77)| 0.49       | (0.07-3.51)| 1.42       | (0.77-2.62)|
| Labour weeks                                     |               |            |              |            |            |            |            |            |            |            |            |            |            |            |            |
| Early (≤37weeks)                                 | 0.95          | (0.11-8.33)| 0.75         | (0.14-4.16)| NC         | NC         | 0.8 (0.09-6.98)| 5.8 (0.62-54.35)| 0.07       | (0.01-0.72)| 0.77       | (0.09-6.69)|

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The significant associations are bolded.

**Table 4.** Association between HLA-G genotypes and risk factors for HPV infection recorded by the baseline questionnaire.
| Risk factors                          | HLA-G genotype OR (95% CI) | *010101:010101 | *010101:010102 | *010101:010103 | *010101:010401 | *010102:010102 | *010102:010401 |
|-------------------------------------|----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Miscarriages (≥1)                   |                            | 0.81 (0.37-1.75) | 1.13 (0.53-2.42) | 1.39 (0.38-5.10) | 0.50 (0.15-1.73) | 2.18 (0.42-11.24) | 0.70 (0.09-5.67) |
| Infertility                         |                            | 1.09 (0.45-2.66) | 1.13 (0.45-2.85) | 0.70 (0.09-5.51) | 0.24 (0.03-1.84) | 1.54 (0.18-13.07) | 5.06 (1.22-21.02) |
| Infertility treatments              |                            | 0.54 (0.15-1.95) | 1.36 (0.45-4.13) | 1.17 (0.14-9.52) | 0.42 (0.05-3.28) | NC              | 9.07 (2.08-39.50) |
| Sexually transmitted diseases       |                            | 1.40 (0.78-2.51) | 0.68 (0.35-1.33) | 1.08 (0.34-3.37) | 0.59 (0.24-1.48) | 0.44 (0.05-3.52) | 0.89 (0.19-4.22) |
| Warts                              |                            | 0.62 (0.34-1.12) | 1.18 (0.66-2.11) | 0.53 (0.15-1.89) | 1.88 (0.94-3.77) | 0.85 (0.17-4.29) | 0.72 (0.15-3.55) |
| Genital                            |                            | NC             | 4.85 (1.13-20.85) | 6.00 (1.11-32.46) | NC              | NC              | NC              |
| Oral                               |                            | 0.96 (0.59-1.56) | 1.29 (0.77-2.14) | 1.98 (0.73-5.35) | 0.69 (0.37-1.32) | 0.87 (0.25-3.08) | 0.18 (0.04-0.87) |
| Skin                               |                            | 1.07 (0.64-1.77) | 0.88 (0.52-1.50) | 0.95 (0.34-2.63) | 1.13 (0.58-2.20) | 2.08 (0.49-8.89) | 2.52 (0.62-10.29) |
| Allergies                          |                            | 0.62 (0.29-1.32) | 1.38 (0.68-2.79) | 0.34 (0.04-2.63) | 0.59 (0.20-1.74) | 1.80 (0.35-9.25) | 2.78 (0.67-11.55) |
| Vulvovaginitis                     |                            | 1.31 (0.31-5.60) | 0.39 (0.05-3.20) | NC              | NC              | NC              | NC              |
| Menarche                           |                            | 0.96 (0.36-2.59) | 0.67 (0.22-2.08) | 3.12 (0.81-12.03) | 2.37 (0.44-12.66) | NC              | 1.37 (0.16-11.38) |
| Late (≥14years)                    |                            | 1.71 (0.37-7.83) | 0.45 (0.05-3.82) | NC              | 1.01 (0.28-3.64) | NC              | NC              |
| Early (≤11years)                   |                            | 1.11 (0.66-1.87) | 0.96 (0.57-1.65) | 0.65 (0.24-1.75) | 1.12 (0.57-2.18) | 0.75 (0.20-2.86) | 0.75 (0.20-2.86) |
| Own contractions                   |                            | 1.43 (0.75-2.74) | 1.31 (0.67-2.55) | 0.26 (0.03-2.03) | 0.43 (0.15-1.28) | 1.28 (0.26-6.37) | 1.28 (0.26-6.37) |
| Rupture of the membranes           |                            | 0.87 (0.50-1.53) | 0.79 (0.44-1.41) | 1.12 (0.40-3.13) | 1.74 (0.89-3.44) | 0.57 (0.12-2.81) | 0.72 (0.81-4.07) |
| Induction                          |                            | 1.08 (0.19-5.99) | 2.75 (0.54-13.90) | NC              | NC              | NC              | NC              |
| Labour weeks                       |                            | 0.82 (0.51-1.33) | 0.97 (0.59-1.62) | 2.32 (0.81-6.61) | 1.30 (0.68-2.50) | 0.51 (0.14-1.86) | 0.95 (0.28-3.17) |
| Abnormal placenta                  |                            | 1.30 (0.30-5.56) | 0.89 (0.18-4.51) | 5.51 (1.03-29.37) | 0.85 (0.10-7.06) | NC              | NC              |

The significant associations are bolded.