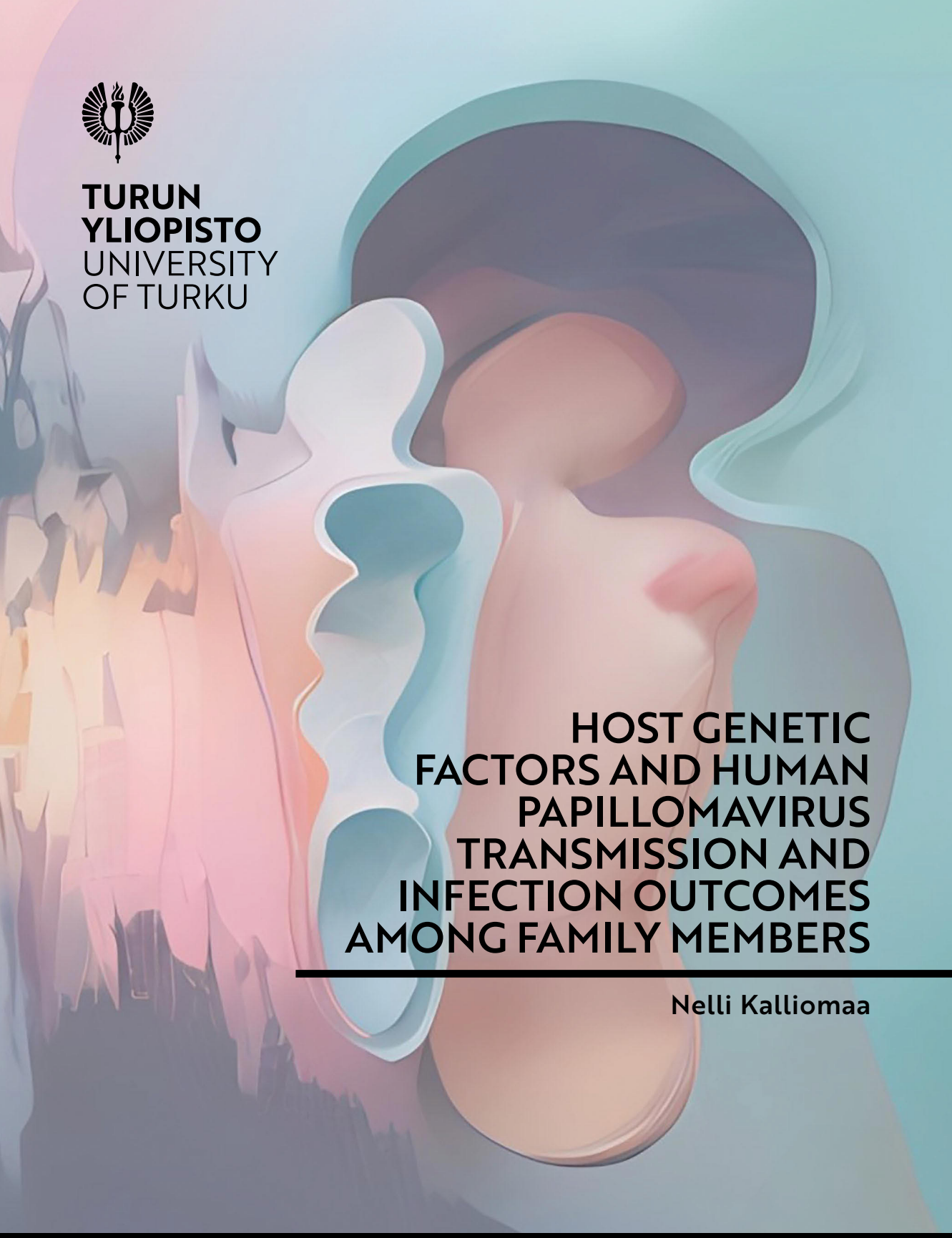

TURUN YLIOPISTO
UNIVERSITY OF TURKU

HOST GENETIC FACTORS AND HUMAN PAPILLOMAVIRUS TRANSMISSION AND INFECTION OUTCOMES AMONG FAMILY MEMBERS

Nelli Kalliomaa

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To my family

UNIVERSITY OF TURKU
Faculty of Medicine
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NELLI KALLIOMAA: Host genetic factors and human papillomavirus transmission and infection outcomes among family members
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ABSTRACT

The natural history of human papillomavirus (HPV) infections is complex, and more information is greatly needed to identify the host genetic and immunological factors that contribute to the infection susceptibility and its progression. HPV is the most common sexually transmitted infection. However, in the last few decades, more data on non-sexual HPV transmission routes have become available. The knowledge on vertical HPV transmission from the mother/father to the child remains insufficient.

This doctoral dissertation evaluated 319 mothers, 132 fathers and their 321 newborn offspring from the prospective Finnish Family HPV (FFHPV) Study cohort. Participants' HPV status and HLA-G genotypes were determined. The aim was to investigate the vertical HPV transmission from parents to newborn, and the role of HLA-G in the father-newborn HPV transmission and the fathers' HPV infection outcomes. In **Study I**, the associations between the fathers' human leukocyte antigen G (HLA-G) alleles/genotypes and the fathers' HPV infection outcomes were evaluated. The allele G*01:01:02 showed to be protective against oral HPV infections whereas the allele G*01:01:03 increased the risk of genital high-risk HPV infection. Fathers with the allele G*01:01:01 showed a reduced risk for incident and persistent oral infections. **Study II** evaluated the association between the father-newborn HLA-G allele/genotype concordance and the father-newborn HPV concordance at birth as well as at the postpartum period; the father-newborn HLA-G *allele* concordance, but not *genotype* concordance, was associated with the HPV concordance. In **Study III** the HPV genotype concordances between 321 mother-newborn pairs and 134 father-newborn pairs were evaluated. The HPV concordances were statistically significant with HPV6, HPV16, HPV18, HPV31 and HPV56 between the mother-newborn pairs, and with HPV6 and HPV31 between the father-newborn pairs, respectively.

While the results of the genotype-specific HPV concordance between parents and their newborns are suggestive of a vertical HPV transmission, the father-newborn transmission remains more uncertain. The father's HLA-G may potentially influence HPV infection outcomes and his child's risk of acquiring a perinatal HPV infection.

KEYWORDS: Human papillomavirus, HPV, vertical transmission, mother, father, child, perinatal, HLA-G, oral, genital

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Synnytys- ja naistentautioppi
NELLI KALLIOMAA: Geneettiset tekijät perheen sisäisessä ihmisen papilloomavirustartunnassa ja infektion ilmenemisessä
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TIIVISTELMÄ

Ihmisen papilloomavirus (HPV) -infektion taudinkulku on monimuotoinen. Geneettisistä ja immunologisista tekijöistä, jotka vaikuttavat HPV-infektioalttiuteen ja taudin etenemiseen, kaivataan lisää tietoa. HPV on yleisin seksivälitteisesti tarttuva infektio. Viime vuosikymmenten aikana on kuitenkin saatu lisää tietoa HPV:n ei-seksivälitteisistä tartuntareiteistä. HPV:n vertikaalisesta, äideistä tai isistä lapsiin tapahtuvasta, tarttumisesta tiedetään vielä vähän.

Tähän väitöskirjatutkimukseen sisällytettiin prospektiivisesta Finnish Family HPV (FFHPV) tutkimuksesta 319 äitiä, 132 isää ja heidän 321 vastasyntynyttä lastaan, joilta oli kerätty HPV-näytteet sekä määritetty HLA-G genotyypit. Tutkimuksen tavoitteena oli osoittaa vertikaalinen HPV-tartunta vanhempien ja lasten välillä sekä tutkia Ihmisen leukosyyttiantigeeni G:n (HLA-G) roolia isä-lapsi-HPV-tartunnassa ja isien HPV-infektion ilmenemisessä. **Osatyössä I** tutkittiin isien HLA-G alleelien/genotyyppien vaikutusta HPV-infektion ilmenemiseen. Tulokset osoittivat, että alleeli G*01:01:02 oli suojaava tekijä suun HPV-infektiolta. Alleeli G*01:01:03 oli riskitekijä genitaalialueen korkean riskin HPV-infektiolle. Isät, joilla oli alleeli G*01:01:01, olivat pienemmässä riskissä saada suun HPV-infektio ja pienemmässä riskissä infektion kroonistumiselle. **Osatyössä II** tutkittiin isä-lapsiparien HLA-G alleeli-/genotyyppikonkordanssin vaikutusta isä-lapsiparien HPV-konkordanssiin syntymähetkellä ja syntymän jälkeen. HLA-G *alleelien* konkordanssi oli yhteydessä HPV-konkordanssiin isä-lapsipareilla. Sen sijaan HLA-G *genotyyppien* konkordanssilla ei ollut vaikutusta HPV-konkordanssiin. **Osatyössä III** 321 äiti-lapsiparilta ja 134 isä-lapsiparilta määritettiin HPV-konkordanssit HPV-tyypeittäin. Äiti-lapsipareilla tilastollisesti merkitseviä olivat HPV6-, HPV16-, HPV18-, HPV31- ja HPV56-konkordanssit ja isä-lapsipareilla HPV6- ja HPV31-konkordanssit.

Tulokset vanhempien ja lasten välisestä HPV-konkordanssista voivat viitata HPV:n vertikaaliseen tarttumiseen, vaikkakin isä-lapsitartunta vaikuttaa epävarmemmalta. HLA-G saattaa vaikuttaa isän HPV-infektion ilmenemiseen ja hänen lapsensa riskiin saada perinataalinen HPV-infektio.

AVAINSANAT: Ihmisen papilloomavirus, HPV, vertikaalinen tartunta, äiti, isä, lapsi, perinataalinen, HLA-G, suu, genitaali

Table of Contents

Abbreviations

AF	Attributable fraction
AIS	Adenocarcinoma in situ
aOR	Adjusted odd ratio
AORRP	Adult-onset recurrent respiratory papillomatosis
APC	Antigen presenting cell
BV	Bacterial vaginosis
cDNA	Complementary DNA
CI	Confidence intervals
CIN	Cervical intraepithelial neoplasia
DB	Dot-blot hybridisation
DC	Dendritic cell
EDTA	Ethylenediaminetetra-acetic acid
FEH	Focal epithelial hyperplasia
FFHPV	The Finnish Family HPV Study
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLA-G	Human leukocyte antigen G
HNSCC	Head and neck squamous cell carcinomas
HPV	Human papillomavirus
HR	High-risk
HSIL	High-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
IMGT/HLA	International Immunogenetics Database
ISH	In situ hybridisation
ITIM	Immunoreceptor tyrosine-based inhibition motif
JORRP	Juvenile-onset recurrent respiratory papillomatosis
KIR2DL4	Killer cell immunoglobin-like receptor 2DL4
LBC	Liquid-based cytology
LC	Langerhans cell
LEEP	Loop electrosurgical excision procedure

LIRB1	Leukocyte immunoglobulin-like receptor B1
LIRB2	Leukocyte immunoglobulin-like receptor B2
LR	Low-risk
LSIL	Low-grade squamous intraepithelial lesion
MFI	Median fluorescence intensity
MHC	Major histocompatibility complex
miRNA	Micro ribonucleic acid
MPG	Multiplex HPV genotyping
mRNA	Messenger ribonucleic acid
MSM	Men having sex with men
MSW	Men having sex with women
NASBA	Nucleic acid sequence-based amplification
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
NHANES	National Health and Nutrition Examination Survey
NK cell	Natural killer cell
OC	Oral contraceptive
OPC	Oropharyngeal cancer
OR	Odds ratios
ORF	Open reading frame
Pap smear	Papanicolaou smear
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
RNA	Ribonucleic acid
RRP	Recurrent respiratory papillomatosis
RT-PCR	Reverse-transcriptase PCR
SCC	Squamous cell carcinoma
SNP	Single nucleotide polymorphism
Th2	T helper 2 cell
TM	Transmembrane region
Treg	Regulatory T cell
TZ	Transformation zone
URR	Upstream regulatory region
UTR	Untranslated region
VIA	Visual inspection with acetic acid
VILI	Visual inspection with Lugol’s iodine
WHO	World Health Organization
αHPV	Alphapapillomavirus
β2m	β2-microglobulin chain
κ	Cohen’s kappa

3′UTR	3′ untranslated region
5′UTR	5′ untranslated region

List of Original Publications

This doctoral dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I **Suominen N***, Jaakola A, Roger M, Faucher M-C, Syrjänen K, Grénman S, Syrjänen S and Louvanto K. The association of HLA-G polymorphism with oral and genital HPV infection in men. *European Journal of Clinical Microbiology & Infectious Diseases*, 2022; 41(2): 219–226.

II **Suominen N***, Roger M, Faucher M-C, Syrjänen K, Grénman S, Syrjänen S and Louvanto K. HLA-G alleles impact the perinatal father–child HPV transmission. *Current Issues in Molecular Biology*, 2023; 45(7): 5798–5810.

III **Suominen N***, Luukkaala T, Laprise C, Haataja M, Grénman S, Syrjänen S and Louvanto K. Human papillomavirus concordance between parents and their newborn offspring: Results from the Finnish Family Human Papillomavirus Study. *The Journal of Infectious Diseases*, 2023; jiad330, https://doi.org/10.1093/infdis/jiad330

*Kalliomaa Nelli, formerly Suominen Nelli

1 Introduction

Human papillomavirus (HPV) is a common pathogen that causes various types of lesions, ranging from common warts to cancer. To date, more than 200 HPV types have been recognised, and over 40 of those infect the anogenital and other mucosal areas of the body (Bouvard et al., 2009; *Papillomavirus Episteme*, 2023; Trottier & Franco, 2006). Although sexual intercourse is considered the main transmission route for mucosal infections, evidence has emerged in the last few decades, suggesting that HPV also has various non-sexual modes of transmission that affect both the adult and paediatric populations (Z. Liu et al., 2016). HPV DNA has been detected in perinatal-aged children, indicating that the infection can be transmitted vertically from the mother to the child during pregnancy or childbirth (Medeiros et al., 2005). However, the father's role in perinatal HPV transmission remains unresolved.

Although HPV infections are common, the vast majority of infections are asymptomatic and cleared spontaneously by the host's immune system (de Sanjosé et al., 2018). However, in the adult population, a persistent high-risk (HR-) HPV infection may lead to precancerous lesions and invasive cancer. In fact, HPV is responsible for nearly all cervical cancer cases and a variating fraction of other anogenital cancers (vulvar, vaginal, penile, and anal cancers), as well as oropharyngeal cancers (de Martel et al., 2017). In children, HPV infections are commonly manifested as benign oral, genital, or skin warts, and its association with cancer progression has not been shown. However, for newborns and young children, perinatally acquired HPV can cause potentially severe juvenile-onset recurrent respiratory papillomatosis (JORRP), which can be difficult to treat (Benedict & Derkay, 2021).

It is still partly unclear why HPV infections persist and progress to precancerous/cancerous lesions in some individuals, while in most cases, HPV infections clear spontaneously (de Sanjosé et al., 2018). By now, the natural course of the infection is supposed to be multifactorial; among sexual behavioural risk factors, viral and host genetic factors have an impact (Trottier & Franco, 2006). As the host's immunological mechanisms play an important role in acquiring and clearing HPV infections, it is important to recognise the host's genetic and

immunological co-factors that affect the natural course of HPV infections (Moscicki et al., 2019).

Human leukocyte antigen G (HLA-G) is one immunological co-factor that is suggested to facilitate the natural course of an HPV infection (H. H. Xu et al., 2020). HLA-G is a part of the human leukocyte antigen (HLA) system. The HLA genes that are involved in the immune response fall into two classes: HLA I (including classical and non-classical HLA-class I) and HLA II. The function of the classical HLA I molecules (HLA-A, -B, and -C) in immune recognition is well understood. These highly polymorphic molecules play a central role in activation of specific immune response by presenting viral or tumoral peptides to T-cells. In contrast, non-classical HLA-G molecule is a tolerogenic molecule regulating immune responses. HLA-G has an inhibitory effect on cells involved in innate and adaptive immunity favoring escape from immune control. HLA-G takes part in immune responses to infectious diseases (Amiot et al., 2014; Rizzo et al., 2014) and immune escapes in cancer progression (Carosella et al., 2015; Lin & Yan, 2019). Moreover, the expression of HLA-G molecule in foetal trophoblastic cells maintains a tolerance in maternal-foetal interface during pregnancy (Kovats et al., 1990).

Compared to classical HLA I genes, HLA-G gene has a low polymorphism and limited protein variability. While the role of HLA-G polymorphism in the natural history of HPV infections still remains uncertain, it has been proposed as a prognostic biomarker for estimating the cervical cancer risk. Moreover, with its immunosuppressive properties and preferential expression at the maternal-foetal interface, HLA-G has suggested to be important in a vertical mother-to-child transmission of viral infections (Aikhionbare et al., 2001; Luo et al., 2013; Martinetti et al., 2006; Segat et al., 2009).

The Finnish Family HPV (FFHPV) Study is a longitudinal cohort study, which was originally designed to evaluate the dynamics of HPV infections between family members. Between 1998 and 2002, 329 families with 329 mothers, 132 fathers, and their 331 newborn offspring were enrolled in the study. HPV samples were collected from different anatomic sites (oral, genital, semen, placenta, and umbilical cord blood) of the family members, and they were followed up for six years. In this thesis, part of the data from the original FFHPV Study was analysed. The vertical HPV transmission from the parents to their newborn were evaluated by determining genotype-specific HPV concordances between the mother-newborn pairs and father-newborn pairs. Moreover, this unique data gave us the opportunity to be the first to assess the role of HLA-G polymorphism in men's HPV infection outcomes and the father-to-newborn HPV transmission, both of which are unstudied subjects.

2 Review of the Literature

2.1 Human papillomavirus

2.1.1 Genomic structure

HPVs are small non-enveloped DNA viruses, approximately 55 nm in diameter (Williams et al., 1961). An icosahedral protein capsid, composed of 72 capsomers, encloses the double-stranded circular DNA genome (Doorbar et al., 2015). The genome of nearly 8000 base-pairs with eight or nine open reading frames (ORFs) can be divided into three regions:

1. the upstream regulatory region (URR) (non-coding regulatory region, including viral promoters, enhancers, and the replication origin);
2. the early coding region (early genes E1, E2, E4, E5, E6, E7) involved in viral replication, transcription, and cellular transformation; and
3. the late coding region (late genes L1, L2) that encodes the major and minor capsid proteins involved in virus assembly (Doorbar et al., 2015; McBride, 2022).

The genome organisation of HPV16, the most carcinogenic HPV type, is shown in **Figure 1.**

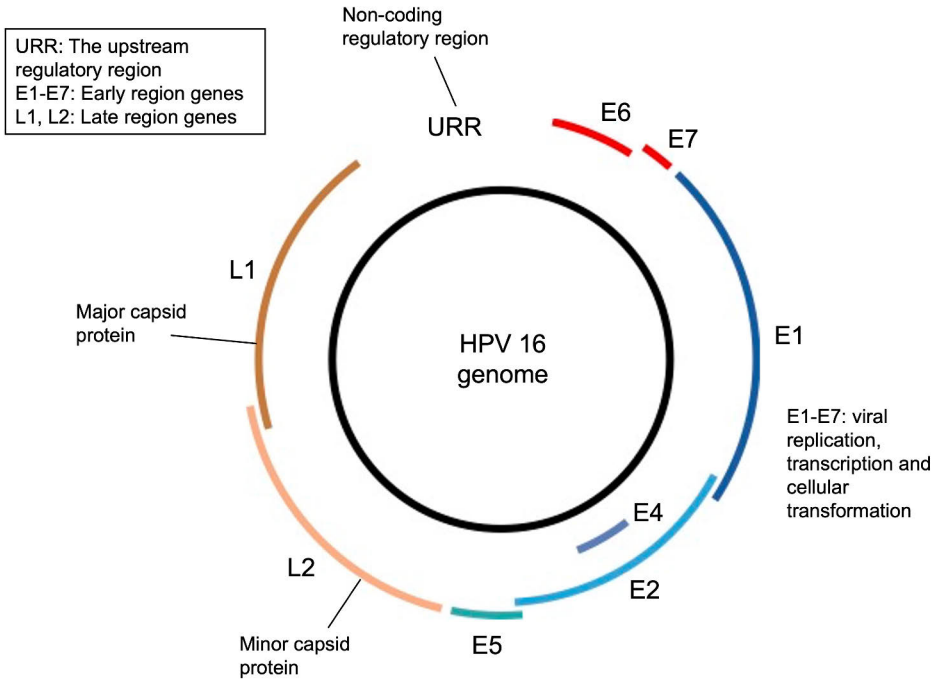

Figure 1. Genome organisation of HPV16. Modified from Rautava and Syrjänen, 2011 (Rautava & Syrjänen, 2011).

2.1.2 Classification

Taxonomy

HPVs, as well as animal papillomaviruses, belong to the taxonomic family of Papillomaviridae. HPVs are divided into genera, species, types, and variants (i.e., variant lineages) according to the shared nucleotide identity in L1 ORF, which is the most conserved ORF within the HPV genome (De Villiers et al., 2004). The phylogenetic tree of HPVs is shown in **Figure 2**. Five phylogenetic genera of HPV include: Alphapapillomaviruses, Betapapillomaviruses, Gammapapillomaviruses, Mupapillomaviruses and Nupapillomaviruses. The Alphapapillomavirus genus (αHPVs) is the most studied, as it includes all the mucosal HPV types of which subset are related to cancer (de Martel et al., 2017).

Each genus is further divided into species, which cover a various amount of HPV types. HPVs belonging to the same species share between 60% and 70% nucleotide sequence identity in the L1 ORF, and typically have similar pathological properties (De Villiers et al., 2004). To date, more that 200 HPV types have been identified (*Papillomavirus Episteme*, 2023). If there is over a 10% difference in the L1 ORF

compared to any other HPV type, it meets the criteria for recognising the new HPV type (De Villiers et al., 2004). The full genome sequencing has enabled HPV types to be further categorised as variant lineages and sublineages. Differences of 1.0–10.0% and 0.5–1.0% across the L1 ORF are used to identify variant lineages and sublineages, respectively. As the HPV co-evolution with humans has led to geographic dispersion, four commonly recognised HPV16 variant lineages A, B, C, and D are from different continents (Burk et al., 2013). These variant lineages include the following sublineages: A1-3 (European), A4 (Asian), B1-2 (African-1), D1 (North American), and D2-3 (Asian-American) (Burk et al., 2013). In general, variant lineages differ in the risk of persistence, but some HPV types – more importantly, HPV16 – differ in the risk of progression to pre-cancer and cancer (Mirabello et al., 2016; Schiffman et al., 2010).

Classification according to oncogenic potential

Based on genome sequencing and epidemiological data, HPV types are categorised by their oncogenic potential to the high-risk (HR) types and low-risk (LR) types (Burk et al., 2009; Muñoz et al., 2003). Based on the evidence of cervical cancer, the International Agency for Research on Cancer (IARC) has classified 12 αHPVs as oncogenic (i.e., HR) types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59; and 13 αHPVs as probably or possibly oncogenic types: 68, 26, 53, 66, 67, 70, 73, 82, 30, 34, 69, 85, 97 (Bouvard et al., 2009). In **Figure 2**, HR-HPV types are indicated according to Schiffman et al. (Schiffman et al., 2010). In fact, all established carcinogenic HPV types belong to five species (α5, α6, α7, α9, and α11), which all originate from the same clade (i.e., branch) of the alpha genus (Schiffman et al., 2010). Considered the most important species, α9 is largely comprised of HR-types, including the most carcinogenic type HPV16 (Schiffman et al., 2010).

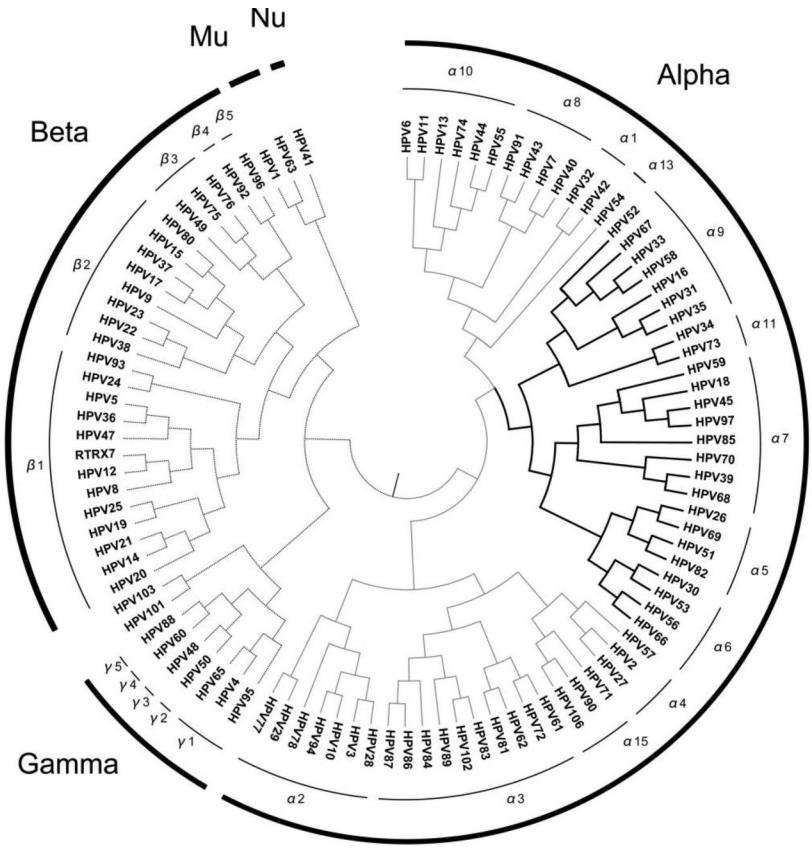

Figure 2. Phylogenetic tree of HPVs. HPVs are classified into five phylogenetic genera: Alpha-, Beta-, Gamma-, Mu- and Nupapillomaviruses. The distribution of HPV species and genotypes across the five genera is indicated. The cluster of high-risk mucosal HPV types is highlighted with bold lines. Reproduced with permission from Schiffman et al., 2010 (Schiffman et al., 2010), Copyright Clearance Center's RightsLink® service.

2.1.3 Tissue tropism

HPVs are tissue-specific viruses with high tropism towards squamous epithelial cells, thus infecting the cutaneous and mucosal areas of the human body. More than 40 HPV types, mostly αHPVs, are known to infect the mucosal epithelia of anogenital and oropharyngeal regions. HPV manifestations by each anatomic site are discussed in Chapter *2.4.5 HPV manifestations*.

In the anogenital region, HPV infects the cervix, vulva, vagina, and anus in women, whereas in men, HPV is found in the anus, penis and urethra (Taylor et al., 2016). The cervix consists of three epithelial types: a conventional stratified epithelium of the ectocervix, a columnar epithelium of the endocervix, and a transformational zone (TZ) between those. The TZ is the site for squamous metaplasia, meaning a physiological replacement of the everted columnar epithelium

by the squamous epithelium. HPV can infect all these three epithelial types of the cervix, but the cervical TZ is especially susceptible to HPV infections and cancer progression. However, the reason for that is not yet completely resolved (Doorbar & Griffin, 2019). Interestingly, the TZ in the anorectal junction seems to be less prone to carcinogenesis than the cervical TZ (E. J. Yang et al., 2015).

In the head and neck region, oropharyngeal, oral and laryngeal cancers are related to HPV (de Martel et al., 2017). According to one meta-analysis that comprised over 12,000 head and neck squamous cell carcinoma (SCC) cases, the HPV DNA prevalence estimates were significantly higher for oropharyngeal SCC (45.8%) than oral cavity SCC (24.2%) or laryngeal SCC (22.1%) (Ndiaye et al., 2014). Anatomic borderlines separate the site of HPV infections, as well as cancers; oropharyngeal cancers arise from the base of the tongue, tonsils, posterior pharyngeal wall, or soft palate. In fact, oropharyngeal cancers are frequently found in the tonsils (Herrero et al., 2003). Tonsil crypts, which are lined with reticulated epithelium and immune cells, might serve as a good reservoir for HPV (Roberts et al., 2019). Laryngeal cancers are found in the supraglottic, glottic (true vocal cords, and anterior and posterior commissures), or subglottic larynx. Oral cancers arise from the oral cavity, including the anterior tongue, floor of the mouth, hard palate, and gums. The oral mucosa consists of stratified squamous epithelium. In fact, three different types of mucosa can be found in the oral cavity: 1) lining mucosa with non-keratinising squamous epithelium covering the mobile structures of the oral cavity, such as buccal mucosa, inner mucosa of the lips, and the floor of the mouth; 2) masticatory mucosa with keratinised epithelium covering the hard palate and the gingiva; 3) specialised mucosa with keratinised epithelium, including the lingual papillae and taste buds as specialised structures, as well as covering the dorsal part of the tongue as a part of the oropharynx (Groeger & Meyle, 2019)

Although HPV primarily infects epithelial cells, HPV DNA has been isolated from seminal fluid and spermatozoa as well (Laprise et al., 2014; Lyu et al., 2017; Pakendorf et al., 1998). Interestingly, some studies have also shown that HPV can be isolated from the trophoblastic cells of a placenta (Ambühl et al., 2016). Trophoblast cells have shown to be broadly permissive for HPV (You et al., 2003), and HPV16 is able to undergo a complete life cycle in trophoblast cell lines (Y. Liu et al., 2001).

2.1.4 Viral life cycle

The life cycle of αHPVs occurs in the squamous epithelia of the genital and oropharyngeal regions. Squamous epithelia are stratified epithelia consisting of basal, parabasal, intermediate, and superficial layers. **Figure 3** shows the structure of squamous epithelia in relation to the HPV life cycle. The function of the cells in

the basal layer (basal cells) is to renew the squamous epithelia by dividing into two daughter cells; one maintains the basal layer, while the other cell differentiates to form the upper layers of the epithelium. Finally, fully differentiated keratinocytes exfoliate from the superficial layer of the epithelium. HPV abuses this differentiation process of the host epithelial cell by establishing basal cells as a reservoir for persistent infections, whereas an active infection, including viral production, occurs in terminally differentiated cells (McBride, 2022). By taking advantage of the keratinocyte's cell cycle, HPV can evade immune defence. The viral cycle takes theoretically at least three weeks, during which the keratinocytes differentiate from basal cells to fully differentiated keratinocytes. However, the appearance of HPV-induced lesions may vary from weeks to months (Stanley et al., 2007). In the following section, the four phases of the HPV life cycles are explained briefly:

Phase 1: Viral entry

Initially, HPV must gain access to the deepest layer of the epithelium, the basal layer. This first event in HPV life cycle is thought to result from a wound or microabrasion in the epithelium. Especially the TZ, the junction between squamous and columnar epithelial cells in the cervix or anus, is known to be a susceptible target for the infection. Microabrasion exposes heparin sulfate proteoglycans on the basement membrane (lamina propria). The virus binds to these glycoproteins, leading to conformational changes of the viral capsid and receptors on the basal cell surface. The virus is taken into the basal cell via endocytosis; L2 protein covers the virus in a membrane vesicle to avoid detection by the immune system (McBride, 2022).

Phase 2: Low activity of replication and gene expression

The initial amplification of the viral genome begins in undifferentiated basal cells. In these cells, a low copy number of the viral episome, 50-200/cell, is maintained with the replication proteins E1 and E2 (Doorbar et al., 2015). The HPV genome is divided into daughter cells with the interaction of host mitotic chromosomes in the basal cell division. This low-level viral activity with low gene expression allows for viral persistence as well.

Phase 3: High activity of replication and gene expression

When the infected keratinocyte has differentiated, migrated to the upper epithelia and exited the cell cycle, there is a massive up-regulation of the viral gene expression (especially early genes E6 and E7 and late genes) and viral DNA replication (Stanley et al., 2007). At this stage, the amplificated viral copy number is at least 1000

copies/cell (Stanley et al., 2007). HPV DNA replication is dependent on DNA polymerases and replication factors of the host mitotic cells.

The E5, E6, and E7 proteins affect the cell proliferation and differentiation to enable the low-activity infection in basal cells and active infection in differentiated cells to occur. The low-activity infection is strengthened by driving cells that are leaving the basal layer to re-enter instead of exiting the cell cycle. To support activity in the upper layers of epithelia, the cell differentiation is delayed.

Phase 4: Assembly and release

The completion of the HPV life cycle happens only in terminally differentiated keratinocytes, which is the reason for viral interest only in delaying the cell differentiation process and not destroying it. Finally, the L1 and L2 capsid protein expression and viral particle production occur. After viral assembly, the virions are released from the exfoliated cells to the environment.

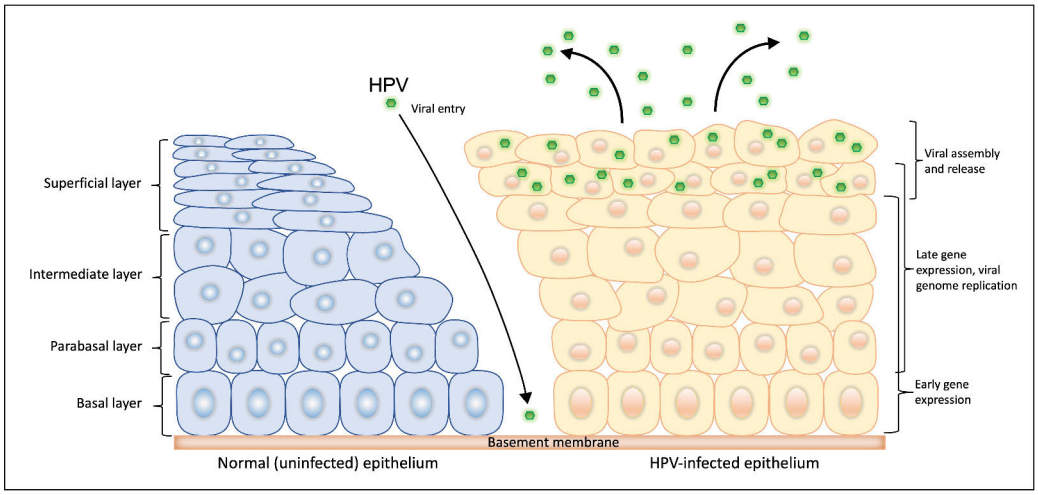

Figure 3. HPV life cycle in epithelial cell. Modified from Moody and Laimins, 2010 (Moody & Laimins, 2010).

2.1.5 Deregulated viral life cycle and carcinogenesis

HPV may induce carcinogenesis in the cell, although it is not the main goal for the virus. The main interest of HPV is to replicate, and from that point of view, carcinogenesis is an unproductive event. A persistent HPV infection is the most crucial event leading to carcinogenesis. Other co-factors, e.g., hormones and smoking, may contribute to carcinogenesis (Luhn et al., 2013). In addition, epigenetic changes, such as viral and host DNA methylation, have been associated

with the progression of cancer (Clarke et al., 2012; Giuliano et al., 2020; Louvanto et al., 2020; Mirabello et al., 2013). Co-factors in HPV infections and carcinogenesis are discussed in detail in Chapter *2.4.6 Risk factors for HPV infection and HPV-related pathology*.

HR-HPV's early genes E6 and E7 are known as oncogenes, as their continued expression is a key factor in HPV-related cancer progression. The early genes E6 and E7 inhibit the host cell's tumour suppressor proteins, and more importantly, p53 and pRB (de Sanjosé et al., 2018). In addition, they serve a function in telomerase activation, immune evasion, and the deregulation of the keratinocyte cell cycle and proliferation, as well as in survival-signalling, leading to the host's genome instability (de Sanjosé et al., 2018; Katzenellenbogen, 2017). These are main events leading to carcinogenesis. Moreover, viral DNA can be integrated into the host's genome, leading to carcinogenesis in several alternative ways. In fact, HR-HPV differs from LR-HPV by the ability to integrate its genome as a part of the host's genome. In the earliest model, HPV integration was suggested to lead an expression of E6 and E7 via the inactivation of E2, which is the main inhibitor of E6 and E7 (McBride & Warburton, 2017). Now it is known that other epigenetic events without the direct inactivation of E2 can modulate the expression of E6 and E7 (McBride & Warburton, 2017).

2.2 Host immune response and human leukocyte antigen (HLA) system

2.2.1 Host immune response and HPV immune evasion

The host immune system is divided into innate immunity and adaptive immunity. Innate immunity generally provides the initial defence against infections through the epithelial barriers (skin and mucosa), phagocytes (i.e., macrophages), innate lymphoid cells (i.e., natural killer cells (NK cells)), and complement system. Antigen presenting cells (APC) act as a bridge between innate immunity and adaptive immunity. The HLA system, further discussed in detail in Chapter *2.2.2 HLA system*, plays a crucial role in the adaptive immune responses. Antigens (i.e., peptide fragments originated from the host cell itself or microbes) are presented by HLA molecules on the surface of APC. Antigen presentation to the naïve T-cell will initiate the adaptive immune responses, which are mediated by T- and B-lymphocytes and their products, such as cytokines and antibodies produced by B-cells.

HPV is incorporated in the host's dendritic cell (DC), a type of APC. If presented in the parabasal or lower suprabasal layers of the epithelium, DCs are also called Langerhans cells (LCs). As other APCs, LCs take up HPV antigens and become

activated via the induction of signalling cascades, costimulatory molecules, and proinflammatory cytokines. The activated APCs migrate to the lymph node where they present the HPV antigen to naïve T-cells, which induce T-cell activation followed by autocrine cytokine secretion and T-cell proliferation. Clonally expanded T-cells differentiate into either effector T-cells or long-lived pre-activated T-cells, i.e., memory T-cells, which are involved in immunologic memory. These differentiated T-cells, together with immune cells of the innate immune system, are responsible for the complex pathways of immune responses against pathogens.

HPV is a successful pathogen, as it can achieve persistent infections by evading the host's immune surveillance in multiple ways. At its best, HPV may practically be invisible to the host's immune defences, allowing a long period of persistence. HPV does not cause viremia, and only minimal amounts of the virus are exposed to the host's immune surveillance. The infection cycle itself is an immune evasion mechanism, as HPV inhibits and delays the host's immune responses as discussed above. High-level viral gene expression with viral production occurs only in the differentiated upper layers of the epithelium, which are ready to desquamate. Thus, the virus can hide far from the sites of immune activity. Moreover, HPV does not induce cell death nor inflammation, which would call for immune cells. Overall, during the infection cycle, a small amount of pro-inflammatory cytokines, which would be crucial for APC activation, is released (Stanley & Sterling, 2014). However, not only does HPV hide from the immune system, but it also downregulates many immune responses in several ways, such as downregulating cytokine production (Doorbar et al., 2015; Stanley & Sterling, 2014).

2.2.2 HLA system

The HLA gene family is the human version of the major histocompatibility complex (MHC) found in many animals. HLA genes encode for proteins that are key mediators for immune responses against pathogens and the development of self-tolerance. This gene family is clustered in a region on the short arm of chromosome 6 (**Figure 4**), containing more than 200 highly polymorphic genes; of those, over 40 encode leukocyte antigens. An individual inherits and expresses only two alleles of each HLA gene (one from each parent) representing very few of the many variants in the population.

HLA genes, as well as the molecules they code, can be structurally and functionally divided into three classes: HLA-class I includes classical HLA-class I (HLA- A, -B, -C) and non-classical HLA-class I (HLA-E, -F, -G); HLA-class II (HLA-DR, -DQ, -DP); and HLA-class III. Of those, HLA-G, as a part of the non-classical class I (Ib), is discussed in detail in Chapter *2.2.3 HLA-G*. Class I molecules are expressed in most somatic cells, whereas class II molecules are expressed in

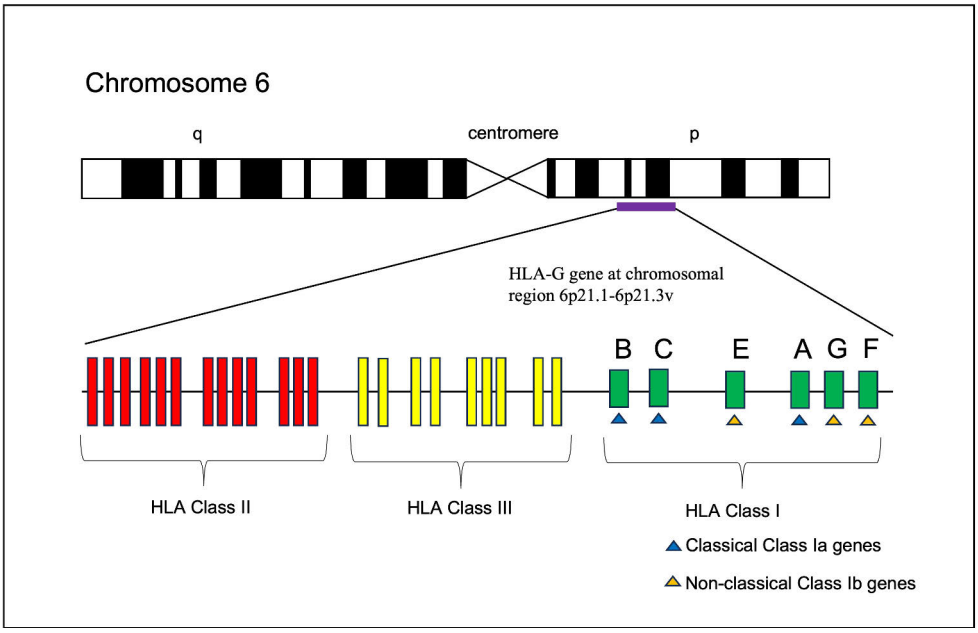

Figure 4. HLA-region on the short arm of chromosome 6. Modified from Arnaiz-Villena, 2021 (Arnaiz-Villena et al., 2021).

immune cells, such as dendritic cells, macrophages, activated T-cells, B-cells and thymic epithelial cells, but can be also induced in other cell types by the cytokine interferon-γ. Class I HLA and class II HLA are primarily known to be involved in the adaptive immune response, whereas class III HLA molecules are involved as an example in complement activation and inflammation, and contain numerous functions that are not directly implicated with the immune system (Hauptmann & Bahram, 2004).

The structures of HLA class I and II molecules are shown in **Figure 5.** Both class I and II HLA molecules are responsible for presenting pathogen-derived peptides to T-cells, leading to T-cell activation and the initiation of the adaptive immune response. Class I molecules present *endogenous peptides* (peptides originated from the cytosol of an infected cell or a tumour cell, or entered in the cytosol by phagosomes) to CD8+ T-cells, whereas class II molecules present mainly *extracellular peptides* to CD4+ T-cells. As a result, endogenous/exogenous peptide processing APCs are adorned with 100,000–300,000 peptide-laden HLA molecules; each HLA-molecule has one peptide, self-peptide or non-self-peptide, bound to it (Klein & Sato, 2000). Each cell presents a heterogenous collection of peptides; some peptides are just a few copies and others are thousands of copies. Thus, a single T-cell may need to see a peptide displayed by only a few of hundreds of thousands HLA molecules.

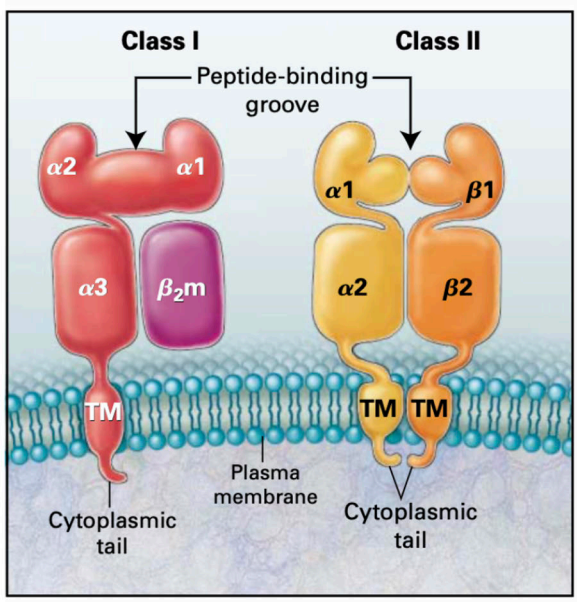

Figure 5. The structure of HLA class I and II molecules. Reproduced with the permission from Klein & Sato, 2000, ©Massachusetts Medical Society (Klein & Sato, 2000). A HLA class I molecule consists of α chain that includes two peptide-binding domains (α1 and α2), the immunoglobulin-like domain α3, a transmembrane region (TM), and a cytoplasmic tail. The α chain is noncovalently associated with a β2-microglobulin chain (β2m). The α chains, TM, and cytoplasmic tail are encoded by the class I gene on chromosome 6, whereas the β2-microglobulin chain is coded outside the HLA genes on chromosome 15. A HLA class II molecule consists of α and β chains, each having four domains: peptide-binding domain (α1 or β1), immunoglobulin-like domain (α2 or β2), TM, and cytoplasmic tail.

2.2.3 HLA-G

Discovered by Geraghty et al. in 1987 (Geraghty et al., 1987), HLA-G belongs to the family of the non-classical HLA class Ib genes. It differs from classical HLA-class Ia genes by the following features: 1) low degree of polymorphism, 2) limited protein variability, 3) presence of multiple isoforms (membrane-bound and soluble isoforms), 4) modulation of the immune response (suppression), 5) short cytoplasmic tail, and 6) expression restricted to certain tissues (Donadi et al., 2011). The alternative splicing of the primary messenger ribonucleic acid (mRNA) leads to seven isoforms of HLA-G: four membrane-bound (HLA-G1, G2, G3 and G4) isoforms and three soluble (G5, G6 and G7) isoforms (**Figure 6**).

Even if HLA-G presents a class I classical molecule structure, its main function is in immune response regulation, not in antigen presentation. Instead of an immune response stimulation, HLA-G is a tolerogenic molecule that holds inhibitory functions against many immune cells: NK cells, T-cells, and APCs through interaction with multiple inhibitory receptors of certain immune cells (Castelli et al., 2014).

HLA-G expression was initially observed on cytotrophoblasts at the maternal-foetal interface (Kovats et al., 1990), and the immune tolerance properties were recognised for the first time; HLA-G modulates the maternal immune responses to maintain its tolerance to the foetus. In addition to the foetal trophoblast cells, HLA-G has shown to be expressed in the cornea (Le Discorde et al., 2003), thymus (Mallet et al., 1999), nail matrix (Ito et al., 2005), pancreatic islets (Cirulli et al., 2006), and erythroblasts (Menier et al., 2004). Moreover, HLA-G has been found to be expressed under pathological conditions, such as pregnancy complications, cancer, autoimmune disease, tissue transplantation, and viral infections (Donadi et al., 2011; González et al., 2012; Hviid, 2006; Morandi et al., 2016; Nilsson et al., 2014). HLA-G is suggested to be involved in immune responses by promoting immune escape in various types of cancers (Lin & Yan, 2019). The HLA-G molecule has even been proposed as a novel biomarker and potential therapeutic target in various types of cancers.

There are a few alternative methods to investigate the role of HLA-G in pathological states. A commonly-used method is to determine the HLA-G gene polymorphism in the coding region, but also in the non-coding region, as the non-coding region affects HLA-G gene expression with functional significance. Moreover, the determination of the soluble HLA-G molecule level, as well as the mRNA level and stability, may be used to investigate the impact of HLA-G molecules in certain circumstances (Craenmehr et al., 2019; Hviid, 2006; Rebmann et al., 2001).

2.2.4 HLA-G gene structure and protein isoforms

The HLA-G gene at the chromosomal region 6p21.3 consists of three regions: 1) promoter region, i.e., 5′ untranslated region (5′UTR), 2) coding region, and 3) 3′ untranslated region (3′UTR) (Castelli et al., 2014). Between the National Center for Biotechnology Information (NCBI) (*National Center for Biotechnology Information*, 2023) and the International Immunogenetics Database (IMGT/HLA) (*IPD-IMGT/HLA Database*, 2023), there is no consensus regarding the HLA-G exon and intron nomenclature, and the exact location of the HLA-G transcription start point. In this thesis, the HLA-G gene structure and transcripts are presented according to NCBI. The HLA-G molecule consists of a heavy chain associated with the β2-microglobulin (**Figure 5**). Like classical class I genes, the HLA-G coding region encodes for the heavy chain, whereas the β2-microglobulin chain is coded outside the HLA genes on chromosome 15.

The HLA-G gene structure and transcripts by NCBI, as well as the protein isoform structures, are shown in **Figure 6.** The HLA-G gene includes eight exons and seven introns (Castelli et al., 2014). Exon 1 is located at 5′UTR. Exon 2 encodes the final portion of the 5′UTR and the HLA-G signal peptide containing the main

translation start point. Exon 3, 4, and 5 encode the extracellular α1, α2, and α3 domains, respectively. Exon 6 encodes the transmembrane domain. Exon 7 encodes the cytoplasmic tail; in fact, a premature stop codon at exon 7 leads to a shorter cytoplasmic tail, distinguishing HLA-G from other class I molecules. Exon 8 is a 3'UTR and is never translated.

The HLA-G DNA segment encodes a full-length mRNA and alternative smaller ones. The alternative splicing of the primary mRNA leads to seven transcript variants, which are translated to seven protein isoforms of HLA-G; G1–G4 isoforms are membranous (having transmembrane and cytoplasmic domains), whereas G5–G7 are soluble isoforms (Castelli et al., 2014). HLA-G1 is the complete membrane-bound isoform that includes all heavy domains (α1, α2, and α3 domains) associated with the β2 light chain; its structure resembles classical class I molecules (**Figure 6**). HLA-G2 lacks the α2 domain, HLA-G3 lacks the α2 and α3 domains, and HLA-G4 lacks the α3 domain. Three isoforms are soluble due to the lack of the transmembrane domain. The soluble HLA-G5 and HLA-G6 isoforms present the same extracellular domains of HLA-G1 and HLA-G2, respectively, but their transcript variants retain intron 5. A premature stop codon at intron 5 prevents the translation of the transmembrane and cytoplasmic tail implicating in their solubility. Similarly, the HLA-G7 transcript variant retains intron 3, leading to a premature stop codon and only the α1 domain is presented at the HLA-G7 isoform (Paul et al., 2000).

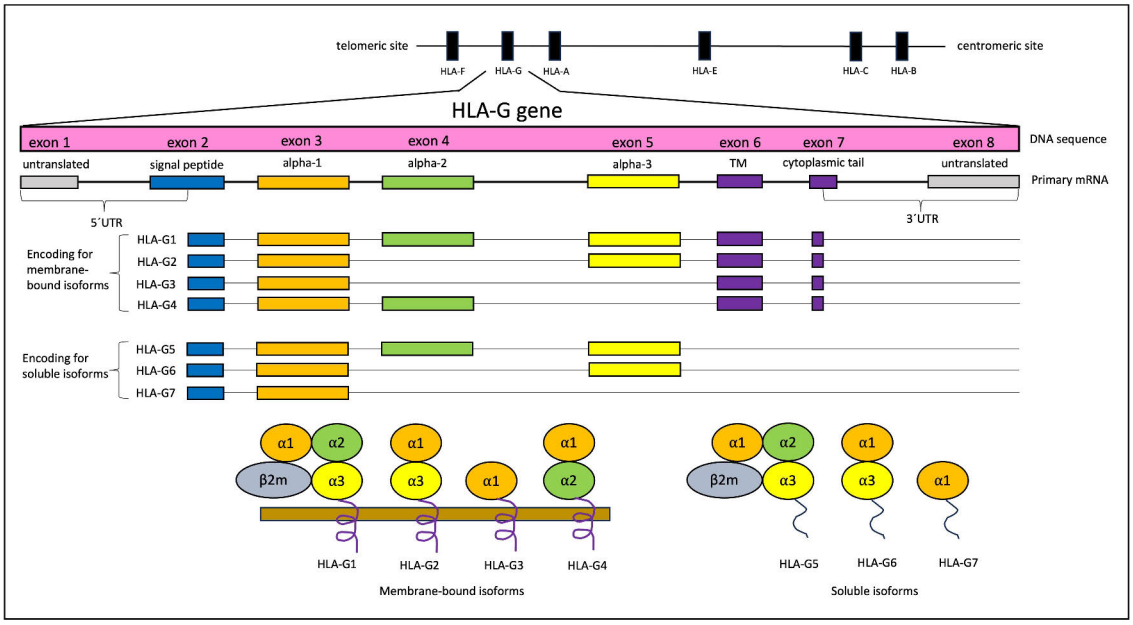

Figure 6. The HLA-G gene structure and transcripts by NCBI, and protein isoform structures. Modified from Castelli et al 2014 and Donadi et al 2011. (Castelli et al., 2014; Donadi et al., 2011).

2.2.5 HLA-G coding region polymorphism

To date, 157 different HLA-G alleles with 48 coded proteins have been identified (IPD-IMGT/HLA Database, November 2023). The nomenclature of the HLA-G allele is explained in **Figure 7**. As an example, HLA-G coding alleles G*01:01:01:01, G*01:01:01:04, G*01:01:01:05, G*01:01:02:01, G*01:01:03:01, G*01:01:05, and G*01:01:07 all carry intronic or synonymous mutations and encode for the same full-length HLA-G molecule known as G*01:01. Allele HLA-G*01:01:01:01 was the first one described and usually the most common allele in the populations studied so far, although the diversity of different HLA-G alleles varies between populations. The limited polymorphism in the HLA-G coding region is distributed among α1, α2, and α3 domains. Amino acid substitutions may account for differences in the HLA-G biological function, such as the production of protein isoforms (selection) and peptide-binding, which may modulate the suppression of immune cells and explain the HLA-G disease association (Donadi et al., 2011).

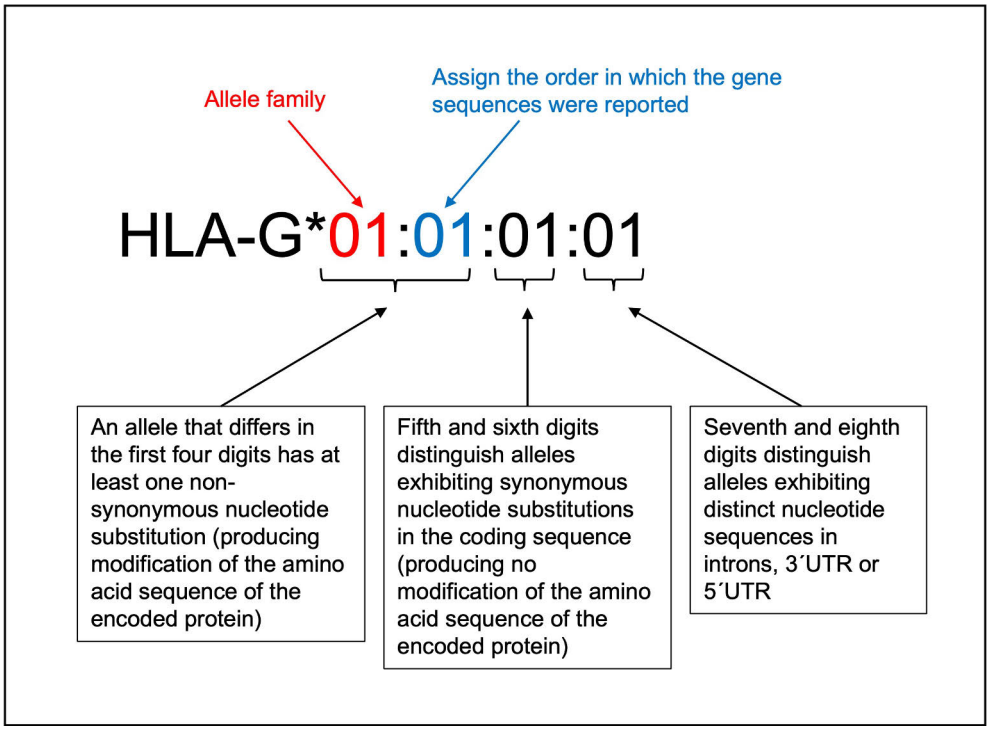

Figure 7. The nomenclature of the HLA-G allele.

HLA-G polymorphism and protein isoform production

Theoretically, nearly all of the described HLA-G alleles could encode all protein isoforms through the alternative splicing process. However, null alleles can lead to an incomplete isoform formation or the prevention of protein isoform production. For instance, the G*01:05N null allele presents a single base deletion, preventing the translation of isoforms possessing the α2 domain (HLA-G1, -G4, -G5) and allowing the production of other isoforms (HLA-G2, -G3, G6, G7) (Le Discorde et al., 2005). Another null allele G*01:13N presents a single nucleotide change, leading to an early stop codon at the α1 domain, and presumably preventing the formation of all protein isoforms or leading to non-functional proteins (Lajoie et al., 2008).

HLA-G polymorphism and peptide binding

The peptide repertoire of HLA-G is limited, as antigen presentation is not the main function of HLA-G. The conformation of the HLA-G and bound peptide complex is similar to that observed in classical HLA I molecules; however, the peptide is buried deeper in the HLA-G cleft (Clements et al., 2007). By now, it seems that the polymorphic sites around the peptide groove may not affect the HLA-G function. However, the molecular structure of the distinct HLA-G proteins and their bindings to different peptides have not been fully investigated. Thus, a broader repertoire of different peptides may exist.

HLA-G modulating immune responses

HLA-G acts as a tolerogenic molecule of the adaptive immune system by suppressing immune reactions. Compared to other HLA Class I molecules, HLA-G has a shorter cytoplasmic tail and a longer half-time of the molecule, enabling multiple interactions with the cells of the immune system. HLA-G polymorphism in the coding region may influence these interactions and potentially modulate immune responses.

The interaction between HLA-G and leukocyte receptors, including leukocyte immunoglobulin-like receptors B1 and B2 (LILRB1 and LILRB2), the killer cell immunoglobin-like receptor 2DL4 (KIR2DL4), and CD8, are supposed to play an important role in the HLA-G derived modulation of the immune response (Gao et al., 2000; Shiroishi et al., 2006). NK cells and macrophages express activator and inhibitor receptors (KIR); the balance between the incoming activating/inhibiting signals determines the effector function (Raulet et al., 2001). In that point of view, the interaction between KIR2DL4 and HLA-G may be significant in immune response regulation. The binding site of KIR2DL4 to HLA-G has not been

recognised. However, the α1 domain is known to be a KIR recognition site in HLA class I molecules.

LILRB1s are expressed on the surface of several leukocytes, including NK and lymphomononuclear cells (Brown et al., 2004). The expression of LILRB2 is restricted to certain cell types, such as monocytes and dendritic cells. The cytoplasmic tail of LILRB1 and LILRB2 include several immunoreceptor tyrosine-based inhibition motif (ITIM) receptors, which inhibit signalling events induced by stimulatory receptors (Dietrich et al., 2001). LILRB1 and LILRB2 bind to α3 and β2-microglobulin domains of HLA-G, and they are considered the main HLA-G receptors. Thus, polymorphic sites in the α3 domain might influence the inhibitory intracellular signalling of LILRB. As an example, alleles G*01:06, G*01:08, G*01:14 and G*01:16, all presenting non-synonymous polymorphic sites at the α3 coding region, may potentially influence LILRB interactions (Donadi et al., 2011).

The CD8 molecule interacts with the α3 domain of HLA class I molecules with a variating affinity between different HLA molecules; for instance, HLA-G has a higher affinity for CD8 than HLA-E. HLA-G polymorphism considering the α3 domain may potentially influence the affinity to CD8; however, no one has conducted affinity studies for different HLA-G molecule variants (Donadi et al., 2011). Moreover, soluble HLA-G (and HLA-A, -B and -C) molecules can induce apoptosis in CD8+ T-cells and NK cells, although the exact mechanism of interaction remains unclear (Contini et al., 2003).

2.2.6 HLA-G non-coding region polymorphism

Several factors potentially impact the transcriptional and post-transcriptional mechanisms regulating HLA-G expression. The 5′UTR and 3′UTR exhibit numerous nucleotide variations that may affect HLA-G expression and further tissue distribution under healthy and pathological conditions. Thus, besides the HLA-G coding region, the role of the non-coding region polymorphism in disease association has been intensively studied.

The functional level of mRNA is regulated by the rate of RNA synthesis, mainly driven by the promoter region (5′UTR). Numerous polymorphic single nucleotide variations in 5′UTR have been described (de Oliveira et al., 2018; Kuersten & Goodwin, 2003); they might affect the regulation of HLA-G expression. Moreover, nucleotide variations in 5′UTR have shown to coincide with other regulatory elements possibly influencing the binding of the corresponding regulatory factors (Castelli et al., 2014).

3′UTR includes several regulatory elements. Before moving to the cytoplasm, the primary transcript is bound by proteins (Aguilera, 2005). Proteins binding to mRNA may affect its translation, localisation, and degradation; 3′UTR

polymorphism is thought to influence the binding properties in this process (Donadi et al., 2011). Moreover, 3´UTR may be a target for microRNA (miRNA), small non-coding RNAs, which may downregulate gene expression (Kuersten & Goodwin, 2003). Several variation sites in the short segment of 3´UTR have been described; the subsequent sites represent true polymorphism (Castelli et al., 2014). It is worth mentioning three important polymorphic sites involving HLA-G expression. Firstly, 14-bp insertion/deletion (presence or absence of the nucleotide) in exon 8 has been related to mRNA stability (Donadi et al., 2011). In addition, single nucleotide polymorphism (SNP) at +3142 position is thought to be a target for certain miRNA, and SNP at the +3187 position has been associated with mRNA stability and degradation (Donadi et al., 2011). These polymorphic sites may hold importance in the regulation of HLA-G expression.

2.2.7 HLA-G and disease association

Viral infections

The immunosuppressive properties of HLA-G might contribute to the susceptibility for viral infections and their persistence. Generally, virus-infected cells should be susceptible to NK-cell mediated cytolysis as they downregulate HLA class 1 molecules. Viruses have created various methods to hide from NK-cell recognition; one of those is the HLA-G expression in a virus-infected cell, which may suppress the immune reactions and contribute to the infection outcome (Onno et al., 2000). The expression of HLA-G is influenced by several viral and host factors, including HLA-G polymorphism. However, the roles of the HLA-G molecule and HLA-G polymorphism have not been fully investigated. It has been reported that various HLA-G polymorphic sites at the coding and noncoding regions are associated with certain viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and HPV (Catamo et al., 2017; Cordero et al., 2009; Eskandari et al., 2017; Ferguson et al., 2011; Laaribi et al., 2015; Lajoie et al., 2006; Matte et al., 2004; Metcalfe et al., 2013; H. H. Xu et al., 2020). For example, the HLA-G 3´UTR 14-bp insertion/deletion has shown to influence the risk of a chronic HBV infection (Eskandari et al., 2017; Laaribi et al., 2015). The association between 5´UTR and 3´URT polymorphism and HCV infection has been reported (Catamo et al., 2017; Cordero et al., 2009). Moreover, among Zimbabwean women, the HLA-G*01:05N allele has shown to be associated with a decreased risk of HIV-1, and HLA-G*01:01:08 with an increased risk of an HIV-1 infection (Lajoie et al., 2006; Matte et al., 2004). HLA-G polymorphism in HPV susceptibility is discussed further in Chapter *2.4.6. Risk factors for HPV and HPV-related pathology.*

The HLA-G expression at the maternal-foetal interface has inspired researchers to establish the importance of HLA-G in the vertical mother-to-child HIV transmission. HLA-G polymorphism has shown to influence the risk of mother-to-child HIV transmission (Aikhionbare et al., 2001; Luo et al., 2013; Segat et al., 2009). One small prospective cohort study (N=34) run in New York City suggested that the mother-child discordance in exon 2 is associated with a reduced risk of perinatal HIV infection (Aikhionbare et al., 2001). In contrary, two larger studies did not find an association between the HLA-G allele concordance and vertical HIV transmission (H. A. Hong et al., 2015; Luo et al., 2013). Moreover, HLA-G has been shown to associate with the vertical mother-to-child HCV transmission (Martinetti et al., 2006). The role of HLA-G in vertical HPV transmission remains unresolved. To date, only one study by Louvanto et al. has elucidated the role of HLA-G in vertical HPV transmission; in that study, the role of HLA-G in the mother-to-child vertical transmission was explored in the FFHPV Study cohort (Louvanto et al., 2018). To our knowledge, there are no studies regarding HLA-G in the father-to-child vertical transmission of HPV nor other viruses.

Autoimmune disease

The roles of the HLA-G non-coding region polymorphism and HLA-G expression in autoimmune diseases have been studied. For instance, HLA-G has been related to asthma, rheumatoid arthritis, systemic sclerosis, and multiple sclerosis among others (Arnaiz-Villena et al., 2021).

Tissue transplantation

As HLA-G suppresses the host immune responses and maintains a tolerance in the maternal-foetal interface during pregnancy, there is great interest in the role of HLA-G in tissue transplantation. In particular, soluble HLA-G levels and UTR polymorphism have been the targets of transplantation research. The presence of HLA-G in the allograft and high soluble HLA-G plasma/serum levels have been associated with better graft acceptance and survival (Donadi et al., 2011). In the light of the current evidence, 3´UTR 14-bp insertion homozygosity is generally related to a higher risk of rejection, whereas deletion homozygosity is related to a decreased risk of rejection (Donadi et al., 2011). This may be due to the fact that 14-bp insertion is associated with lower HLA-G expression, and deletion with higher HLA-G expression.

Cancer

In tumour cells, tumour-associated antigens are expressed by HLA class I molecules, and these tumour cells are ideally recognised and destroyed by cytotoxic T-cells and NK cells. However, by tumour immunoediting, those tumour cells that do not respond to the immune system are evolved and proliferated, enabling uncontrolled tumour cell growth. This process is mediated by the tumour cell itself and the expression of many immunosuppressive factors, such as programmed cell death protein 1 (PD-1) and HLA-G as well (Carosella & Horuzsko, 2007; Dermani et al., 2019). HLA-G may be involved in cancer immunoediting by downregulating tumour cell elimination through inhibiting the cytotoxic function of T-cells and NK cells. For instance, trogocytosis, i.e., the process in which HLA-G is transferred from other cells to T-cells, reverses the T-cell function from effectors to regulatory cells (LeMaoult et al., 2007).

HLA-G expression has been detected in various cancers, such as cervical cancer, melanoma, colorectal cancer, ovarian carcinoma, and breast cancer (Arnaiz-Villena et al., 2021). Moreover, the polymorphic sites of HLA-G coding and non-coding regions have been associated with cancers (Arnaiz-Villena et al., 2021). As an example, HLA-G*01:04 and HLA-G*01:03 alleles have been associated with bladder carcinoma progression in a Brazilian population (Castelli et al., 2008). The HLA-G polymorphism in relation to cervical cancer is discussed in Chapter *2.4.6. Risk factors for HPV and HPV-related pathology.*

Pregnancy complications

HLA-G holds an important physiologic role in pregnancy by suppressing the mother's immune responses against the foetus. HLA-G is already expressed during implantation. The trophoblastic cells express mostly HLA-G1, but other isoforms are expressed as well. During pregnancy, the peripheral blood concentration of soluble HLA-G raises from two- to five-fold compared to non-pregnant women (Nilsson et al., 2014). Not surprisingly, HLA-G expression, as well as polymorphism in the context of pregnancy complications, have been investigated. Several studies have reported an association between HLA-G polymorphisms and the risk of pre-eclampsia and recurrent miscarriages; however, the results are partly inconsistent (Nilsson et al., 2014). Interestingly, one study showed that the father's HLA-G*01:06 allele increased the risk of pre-eclampsia in multigravida women (Tan et al., 2008).

2.3 HPV detection

HPV cannot be cultured; thus, the detection of HPV relies on the assessment of nucleic acids (DNA/RNA). In the last few decades, the identification of HPV has developed from hybridisation methods to polymerase chain reaction (PCR) -based assays, and most recently, next-generation sequencing (NGS) assays. Initially, nucleic acid hybridisation, such as Southern blotting, in situ hybridisation (ISH), and dot-blot hybridisation (DB), was the preferred method to detect HPV DNA from samples. Eventually, this time-consuming technique was replaced by new technologies. Modern HPV detection methods can be broadly divided to signal amplification and target amplification methods; most of the HPV assays are based on the latter. In 2020, over 250 distinct HPV assays were available on the global market (Poljak et al., 2020). In this chapter, the principles of HPV detection and genotyping methods are discussed, as well as a brief review of the serological and morphological methods.

2.3.1 HPV amplification

2.3.1.1 Signal amplification

Signal-amplification is a method to detect HPV DNA and differentiate HR- and LR-HPV types. To put it briefly, signal amplification methods are based on the hybridisation event of the target sequence with target-specific probes (i.e., RNA probes) in liquid or in situ on the sample. The signal (hybridisation event) is amplified, and it can be visualised with different methods. Hybrid Capture ® 2 (HC2) is an example of a clinically used HPV assay that is based on signal amplification.

2.3.1.2 Target amplification

In target amplification, the RNA/DNA target sequence is amplified to a level at which it can be easily detected with a specific read-out system. PCR is the most widely used target amplification method. In PCR, DNA polymerase, target-specific oligonucleotides (“primers”), and nucleotides are needed to amplify a target nucleic sequence. DNA polymerase recognises and extends a pair of primers, which target the DNA region, such as the L1 or E6/7 gene of HPV. With repetitive cycles of temperature switches, the DNA sequence can be amplified exponentially; theoretically, PCR can produce one billion copies from a single double-stranded DNA molecule after 30 cycles of amplification. Different primers, such as the commonly used PGMY09/11, GP5+/6+ and SPF10, amplify different-sized DNA fragments (Gradíssimo & Burk, 2017).

Nested-PCR is a form of PCR that entails two consecutive PCR reactions. In the first round, the external (MY) primers are used, after which the inner (GP) primers are used in the second round. Nested-PCR is highly sensitive, as it enables HPV detection in samples with a low copy number of HPV (Husnjak et al., 2000).

HPV RNA detection

Although HPV DNA is the most commonly used target of amplification, a few commercial RNA tests are also available. The idea of a RNA test, such as APTIMA®, is to detect the mRNA transcript for HPV E6/E7 oncogenes. The detection of mRNA can be performed by using 1) reverse-transcriptase PCR (RT-PCR) in which the reverse transcriptase generates complementary DNA (cDNA) from the mRNA template, or 2) nucleic acid sequence-based amplification (NASBA) (Snijders et al., 2010).

2.3.2 HPV genotyping

There are multiple PCR-based genotyping methods available; they are mostly for research uses, whereas only a proportion of them are used in screening. A traditional DNA sequencing method is Sanger sequencing. In recent years, the use of NGS has become a major development in HPV genotyping, as it is a massively parallel high-throughput method. NGS is a very sensitive, accurate, and reproducible method for HPV genotyping, as it allows a larger number of samples to be analysed at the same time (Arroyo et al., 2013). It also detects multiple infections with a high rate of sensitivity (Arroyo et al., 2013). Moreover, NGS has been applied to detect novel HPV types and variant lineages/sublineages (Ekström et al., 2011; Siqueira et al., 2016).

2.3.2.1 Multiplex HPV genotyping (MPG)

Multiplex HPV genotyping (MPG) is a quantitative and sensitive high-throughput method to identify multiple HR and LR genotypes in a single reaction. In the Finnish Family HPV Study, MPG was used for HPV genotyping. In MPG, HPV DNA is amplified by using PCR with general GP5+/6+ primers, followed by the detection of PCR products with type-specific oligonucleotide probes coupled to fluorescence-labelled polystyrene beads (Schmitt et al., 2006).

2.3.3 Serology

The detection of serum HPV-antibodies is one method to indicate ongoing or past HPV infection, as well as immunisation. A limitation of this method is that a

detectable immune response via seroconversion is seen only among the subset of patients infected with HPV (Carter et al., 2000). Although serum HPV-antibodies are an indication of ongoing or past HPV infection, the anatomical site of infection cannot be distinguished by using serological methods. Thus, serological methods have not been established a role in diagnostic purposes, in which the main goal is to detect ongoing HPV infection and HPV-induced lesions. However, serology has been increasingly used in research to assess the HPV-specific immune responses induced by either a natural infection or vaccination (D'Souza et al., 2019; Gray et al., 2021; Syrjänen et al., 2009).

2.3.4 Morphological methods

2.3.4.1 Visual examination (VIA, VILI)

HPV can cause cervical precancerous lesions. In a visual inspection of the cervix, acetic acid (VIA) or Lugol's iodine (VILI) is used to make the lesions visible to the naked eye. In low-resource settings, a visual examination may be the only available method to detect HPV infection (Sangwa-Lugoma et al., 2006; Sankaranarayanan, 2014; Santesso et al., 2016).

2.3.4.2 Colposcopy

Colposcopy is a procedure in which a magnifying instrument called a colposcope is used to examine the cervix (including its TZ), vagina, and vulva. A colposcopy may be indicated after the detection of an abnormal Papanicolaou smear (Pap smear) or HR-HPV. Applying 5% acetic acid to the cervix results a dehydration of the dysplastic cells, which is seen as an acetowhite staining in the abnormal region of the epithelium. Moreover, Lugol's solution, a brown iodine-containing solution, may be used to detect lesions as dysplastic epithelia becomes highlighted by the unabsorbed brown solution. After staining, punch biopsies are taken from the abnormal epithelia for the histopathological examination.

2.3.4.3 Pap smear cytology and liquid-based cytology (LBC)

The cervical cytology via a Pap smear is used in cervical cancer screening. In the Pap smear test, in its simplicity, a small brush (cytobrush) is used to gently remove cells from the surface of the cervix and the surrounding area, followed by the fixation of the smear onto the glass slide. Liquid-based cytology (LBC) is a modification of the traditional Pap test, in which the sample is transferred to a liquid medium. A

microscopic inspection is thereafter performed, and the abnormalities in sample cells are classified with the cytological classification system.

2.3.4.4 Histopathology

Histopathology is the gold standard in the diagnosis of precancerous/cancerous lesions. According to the World Health Organization (WHO), cervical precancerous lesions are classified into two grades by the severity of lesion: low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). Before the newer classification system, lesions were divided into three grades: cervical intraepithelial neoplasia (CIN) 1, 2, and 3. LSIL corresponds to CIN1, whereas HSIL includes CIN2 and CIN3.

2.4 Epidemiology of HPV infection

2.4.1 Modes of HPV transmission

Sexual intercourse, both penetrative and non-penetrative, is the primary route of mucosal HPV transmission. However, evidence from the last few decades suggest that mucosal HPVs have multiple non-sexual routes of transmission as well (Z. Liu et al., 2016). As HPV DNA has been widely detected in virgins and sexually unexperienced children from the very beginning of their life, non-sexual transmission seems evident (Z. Liu et al., 2016). The HPV transmission dynamics within an individual (autoinoculation), sexual couples, and persons with non-sexual contact, such as family members, are extremely complex. Thus, it is challenging to establish exact transmission routes. However, knowledge on this research area does evolve, albeit slowly.

The modes of non-sexual transmission include: 1) autoinoculation (i.e., self-inoculation), 2) non-sexual horizontal transmission, and 3) vertical transmission. Autoinoculation, i.e., transmission from one anatomic site to another site by scratching, is a feasible route for transmission within the individual. Concordant HPV types in fingers and genital/oral sites have been detected both among the sexually experienced and unexperienced cohorts (Fu et al., 2015; Houlihan et al., 2019; Partridge et al., 2007). One cohort study reported the autoinoculation of common hand warts to anogenital sites with 3/42 prepubertal children (Handley et al., 1993). Open-mouthed kissing has been associated with oral HPV infection even without a history of oral sex, indicating horizontal transmission via kissing (D'Souza et al., 2009; Fu et al., 2015). Person-to-person transmission via hands is a potential route for horizontal transmission as well (Hernandez et al., 2008; Malagón et al., 2021; L. Widdice et al., 2013; L. E. Widdice et al., 2010; Winer et al., 2010).

Although HPV has been detected in fomites, such as medical instruments and toilet seats, there is insufficient evidence on transmission via fomites. Interestingly, one recent meta-analysis estimated an airborne HPV transmission risk during the ablation procedures of HPV-related lesions; they suggested that medical staff using a CO2 laser have a higher risk for upper airway warts than controls (Palma et al., 2021).

2.4.1.1 Vertical transmission

The vertical transmission of an unknown virus was first reported in 1956 in a case of juvenile laryngeal papillomatosis (Hajek, 1956). Now the vertical HPV transmission from the mother to the newborn is thought to be the most significant transmission route of a perinatal HPV infection. There is no gold standard to confirm the vertical HPV transmission. However, detecting HPV DNA both in the genital site of the mother and the variating site of the newborn have been commonly used as an indication of vertical transmission. The vertical transmission rate describes that proportion of HPV-positive mothers, whose newborns have been detected with HPV at birth, i.e., the proportion of HPV-positive mothers transmitting HPV to their newborn offspring. According to one meta-analysis that comprised 446 HPV-positive mothers and their newborn offspring, the vertical transmission rate based on the *genotype-specific* HPV concordance was estimated to be approximately 25% (Chatzistamatiou et al., 2016). In a recent study with 422 HPV-positive pregnant women, 7.2% of newborns born to HPV-positive mothers were declared HPV-positive at birth and/or at 3 months; of those newborns, 85.2% had at least one of the same HPV genotypes that were detected in their mothers (Khayargoli et al., 2023).

Generally, vaginal delivery has shown to be a risk factor for vertical HPV transmission (Chatzistamatiou et al., 2016; Hahn et al., 2013). In the study by Hahn et al, vaginal delivery and multiple HPV types in the mother were associated with vertical HPV transmission; the gestational week, birth weight, mother's genital bacterial infection, length of labour, premature rupture of the membrane, or risk of HPV type (LR/HR) did not influence the risk of vertical transmission (Hahn et al., 2013). However, the sample size in the study was low; only 72 mothers and their 15 newborns were HPV-positive.

Although the mother-to-child HPV transmission has been investigated more thoroughly, the perinatal transmission from fathers to newborns may be another potential transmission route. To date, only a few studies have included fathers in their vertical transmission analyses (Rintala et al., 2005; Skoczyński et al., 2019; E. M. Smith et al., 2004). Hence, the father's role in perinatal HPV transmission remains still unclear. In addition to the vertical transmission, the horizontal

transmission to newborn from family members and other caregivers via hand contact and kissing seems possible.

Depending on when early-life transmission takes place, vertical transmission can be further categorised as 1) periconceptional, 2) prenatal, and 3) perinatal transmission. Periconceptional transmission occurs at the time around fertilisation, prenatal transmission during pregnancy, and perinatal transmission during childbirth, or immediately thereafter (Syrjänen, 2010). However, no exact definition for perinatal transmission exists; it can be also used to indicate the transmission taking place soon before or after birth. Moreover, there is no consensus on how many days after birth perinatal transmission can be considered, as the timeframe of the perinatal period is defined in diverse ways.

Periconceptional transmission

As HPV DNA has been isolated from the semen (Laprise et al., 2014) and the male reproductive tract, such as the testis, epididymis, vas deferens, and urethra (Martorell et al., 2005; Nielson et al., 2007; Švec et al., 2003), the periconceptional transmission from the father to the embryo seems at least theoretically possible. In semen, HPV DNA is documented to localise in the sperm head (Foresta et al., 2011) and its integration into the nucleus is less documented (Mastora et al., 2021). Although spermatozoa are generally considered transcriptionally and translationally inactive, certain HPV genes have shown to be expressed actively in sperm cells (Lai et al., 1997).

Interestingly, HPV has been detected in the female upper genital tract, including the endometrium, fallopian tubes, and ovaries, and in patients with upper genital tract carcinomas (Giordano et al., 2006; Ip et al., 2002). According to a meta-analysis with 2280 patients that had ovarian cancer, the pooled prevalence of HPV in ovarian cancer tissue was 15.9% (Cherif et al., 2021). However, the significance of HPV in the female upper genital tract remains unclear, and more studies are needed in this field. To date, no studies exist on HPV detection in oocytes.

Theoretically, sperm might function as a vector for transferring HPV into the oocyte during fertilisation. One in-vitro study has shown that human sperm cells transfected with E6/E7 genes, and sperm cells exposed to the HPV L1 capsid protein, are able to penetrate into the hamster oocyte, and HPV genes can be even actively transcribed by the penetrated oocyte (Foresta et al., 2011). Another study showed that mouse spermatozoa were able to internalise a construct of cloned HR-HPV DNA; subsequently, the in-vitro fertilisation of the mouse oocyte was made with these infected spermatozoa, and HPV was detected in the mouse embryo (Mastora et al., 2021). Moreover, sperm cells have shown to carry exogenous HPV DNA directly to the mouse blastocyst (Cabrera et al., 1997). These in-vitro findings call for further studies to explore the potential HPV transmission during fertilisation.

Prenatal transmission

Although the risk of vertical HPV transmission is shown to be higher in vaginal deliveries than in caesarean sections (Chatzistamatiou et al., 2016; Medeiros et al., 2005), a caesarean section does not eliminate the risk of vertical transmission. According to one meta-analysis, perinatal transmission occurs in 15% of newborns born via caesarean sections, suggesting that the transmission may happen prior to the delivery (Chatzistamatiou et al., 2016). As HPV has been detected in placental tissue, amniotic fluid, umbilical cord blood (Ambühl et al., 2016), and aborted products of conception (Skoczyński et al., 2011), an intrauterine transmission during the pregnancy seems evident. Placental HPV, as well as the mother's cervical HPV, have been associated with spontaneous abortions and preterm births (Ambühl et al., 2016; Niyibizi et al., 2021). However, the exact routes of intrauterine transmission are poorly understood. To date, at least two feasible explanations for intrauterine transmission exist: 1) transmission as an ascending infection from the maternal genital tract through microtears in the foetal membranes, and 2) transmission via blood circulation through the placenta (Freitas et al., 2013; Syrjänen, 2010). These alternative intrauterine HPV transmission routes are demonstrated in **Figure 8**.

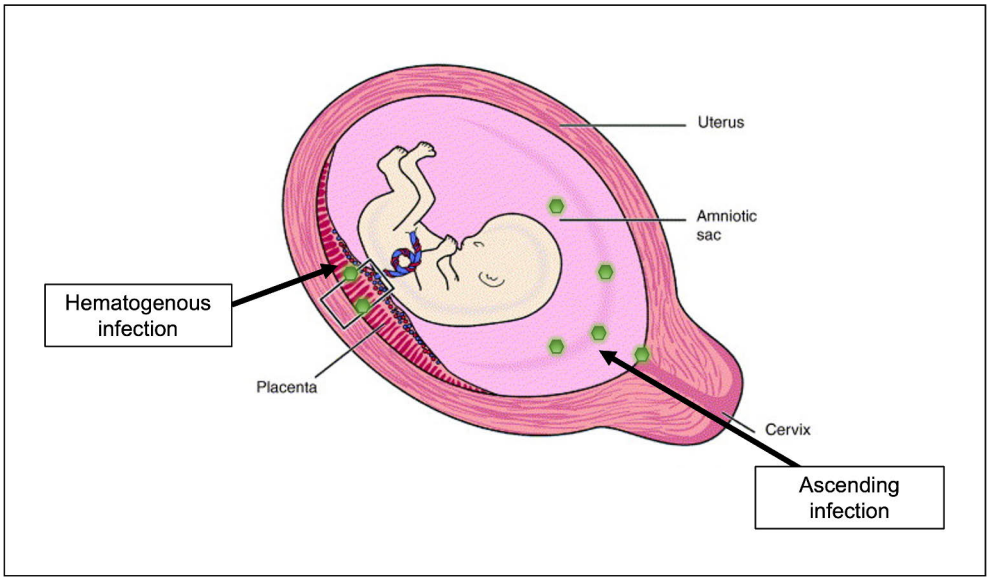

Figure 8. Two feasible routes for intrauterine HPV transmission. Modified from Pereira et al 2005 (Pereira et al., 2005). Transmission may happen as an ascending infection through the infected maternal genital tract. Alternatively, HPV may be transmitted from the mother to the placenta and trophoblastic cells via hematogenous transmission. The foetus may acquire HPV by ingesting infected cells along with the amniotic fluid or through the umbilical cord blood.

Perinatal transmission

Perinatal HPV transmission is thought to result mainly from close contact with the infected maternal genital tract during delivery. In fact, perinatal transmission is suggested to be the most evident mode of vertical transmission. However, it is partly controversial whether HPV detected in newborns represents a true infection or passive contamination from the infected birth canal. Moreover, it is suggested that HPV infection in newborns is transmitted vertically from the mother or father during everyday baby care routines via digital contacts. Although HPV has been detected in breast milk from lactating mothers, the vertical transmission to newborns through the breast milk does not seem evident (Louvanto et al., 2017; Yoshida et al., 2011).

2.4.2 HPV prevalence

2.4.2.1 HPV prevalence in adult population

HPV is the most common sexually transmitted infection, and nearly all sexually active women and men will become infected at some point during their lifetime. Most individuals are not aware they are infected with HPV since they are asymptomatic. The exact lifetime risk is difficult to estimate, as it is likely to have many transient infections without any clinical signs of infection. Overall, the prevalence of HPV varies greatly, depending on the population and age. The worldwide cervical HPV prevalence among women with normal cytology is estimated to be approximately 10% (Bruni et al., 2010). The prevalence is highest in women under the age of 25 years, just after their sexual debut (Bruni et al., 2010). In many populations, a second peak in HPV prevalence is seen at older ages (≥45 years) (Bruni et al., 2010). The reason for the second peak is unclear and might be multifactorial; at least, changes in sexual habits have suggested to play some role (Bosch et al., 2008). In a systematic review, the cervical HPV prevalence among 13,757 pregnant women with full-term pregnancies was estimated at 17.5% with a high variation between study populations (Ambühl et al., 2016).

In men, the genital HPV prevalence ranges widely, depending on the anatomic sample site and population. One meta-analysis estimated the prevalence to be 20% or higher (Dunne et al., 2006). In Europe, the male genital HPV prevalence is shown to be 12% with considerable heterogeneity between studies (Hebnes et al., 2014). Compared to women, the genital HPV prevalence among men remains more stable during their lifetime (Giuliano et al., 2008; J. S. Smith et al., 2011).

Oral prevalence in adults have been estimated at 4.5% and 7.5% in two systematic reviews, again with a high variation of prevalence between the studies (Kreimer et al., 2010; Wood et al., 2017). D'Souza et al. analysed the data of 13,089

people aged 20-69 in the National Health and Nutrition Examination Survey (NHANES) and reported an oral HR-HPV prevalence of 3.5%; the prevalence was higher in men than in women (D'Souza et al., 2017). However, Kreimer et al. showed a similar oral prevalence among both sexes (Kreimer et al., 2010).

HPV16 is the most common HPV type found in women's genital site (Bruni et al., 2010), and one of the most detected HPV types in men's genital site and both sexes' oral sites as well (Bettampadi et al., 2020; Giuliano et al., 2008; Hebnes et al., 2014; Kreimer et al., 2010).

2.4.2.2 HPV prevalence in children

HPV can cause a variety of mucosal and cutaneous infections in children as well. HPV is found in the oral and genital sites of children before sexual activity and even in early infancy, suggesting non-sexual modes of transmission as discussed in the Chapter *2.4.1. Modes of HPV transmission*. HPV prevalence among children varies highly, depending on the sample site, HPV detection methods used, and the age of children at the time of sampling. The oral HPV prevalence in children aged 0–17 ranges from 0% to 51.7% (Jenison et al., 1990; Koch et al., 1997; Puranen et al., 1997; Rice et al., 2000; Watts et al., 1998). In vertical transmission studies, newborns' oral HPV prevalence at the age of 0–4 days has shown to vary from 0.9% to 39.7% (Hahn et al., 2013; Khayargoli et al., 2023; Park et al., 2012; E. M. Smith et al., 2004, 2010; Tseng et al., 1998).

There is limited data on genital HPV prevalence among prepubertal children and sexually naïve adolescents. According to a few small studies, genital prevalence is reported to vary from 3.0% to 34.5% among >2 years old sexually naïve children and adolescents (Bacopoulou et al., 2016; Doerfler et al., 2009; Myhre et al., 2003). In vertical transmission studies, genital HPV prevalence is reported to vary from 0.6% to 30.9% among 0–4 days old newborns (Rintala et al., 2004; E. M. Smith et al., 2004, 2010; Tseng et al., 1998).

2.4.3 HPV clearance and persistence

Most HPV infections, even with the HR-HPV type, are transient and asymptomatic, and cleared by the host immune system within 12-24 months (de Sanjosé et al., 2018). The potential for spontaneous clearance is high, especially in younger populations in which infections are common. One cohort study of 2,065 patients between the ages of 18 and 29 showed that 61% of patients with a newly diagnosed HR-HPV infection cleared the infection within one year (Schmeink et al., 2013). However, if the immune system fails to clear the HPV, the infection becomes persistent. The persistence of a HR-HPV infection is commonly known to be a risk

factor of precancerous lesions and further invasive cancer. However, there is a lack of a generic definition for persistence. In literature, the definition of persistence varies; most commonly, it is defined as HPV positivity at a minimum of two time points (Rositch et al., 2013). HR infections are shown to persist longer than LR infections (Rositch et al., 2013; Trottier & Franco, 2006); HPV16 being the most persistent type (Schiffman et al., 2005).

HPV persistence in young children is more unlikely. The vast majority of the HPV infections detected at birth is shown to clear during the first months of life (Hahn et al., 2013; Khayargoli et al., 2023; Park et al., 2012; Rombaldi et al., 2009; Syrjänen et al., 2021; Tenti et al., 1999). However, in some children, HPV acquired in early infancy may persist; Syrjänen et al. showed that oral HPV detected at birth can persist for years with a mean duration of persistence of 20.6 months (Syrjänen et al., 2021). However, long-term follow-up studies are required to assess the clinical relevance of early-life HPV infection and its persistence.

2.4.4 HPV progression

Of all HPV-related cancers, the progression of cervical cancer is the best understood since virtually all cervical cancers are related to HPV (Walboomers et al., 1999). Invasive cervical cancer is developed from preinvasive lesions (LSIL, HSIL), which are diagnosed by a cervical biopsy and histological examination. LSIL has a low potential for progression and a high potential for regression. In fact, LSIL (CIN1) is a histological diagnosis of benign viral replication with the best spontaneous regression potential. On the other hand, HSIL (CIN2/3) has a higher potential for progression and a lower potential for regression. Among lesion severity, the patient's age influences the risk of progression; the risk of cervical cancer is lowest in younger age groups (Benard et al., 2012). The progression from histological LSIL to invasive cervical cancer is illustrated in **Figure 9**.

The rate of progression and regression varies, depending on the age of the population. One retrospective study of 680 patients with biopsy-proven LSIL showed that 49% of lesions regressed, 45% persisted, and 7% progressed to HSIL at their 6-month follow-up (Bansal et al., 2008). Of those patients who had persistent LSIL at 6 months, 50% regressed, 46% persisted, and 4% progressed to HSIL.

In a meta-analysis of 36 studies including a total of 3160 women with CIN2, the pooled rates were 50% for regression, 32% for persistence and 18% for progression to CIN3 or worse at the 24-month follow-up (Tainio et al., 2018). Among the subgroup of 1069 women under 30 years of age, 60% regressed, 23% persisted and 11% progressed. The data of the natural history of CIN3 is limited; as CIN3 is a direct precursor for cervical cancer, a follow-up in lieu of treatment is not recommended. The most valid direct estimates regarding the CIN3 progression to

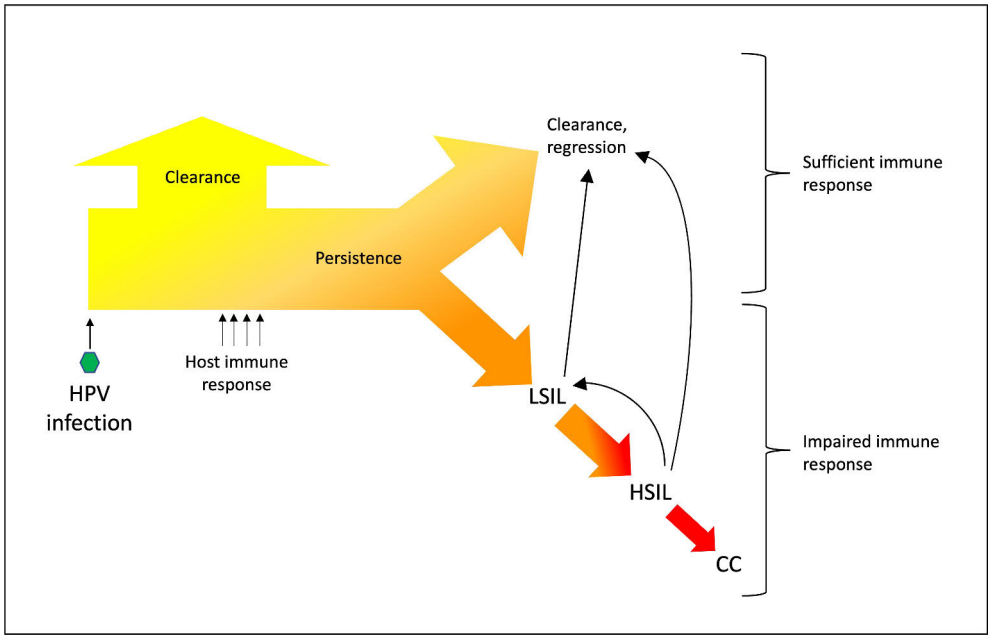

Figure 9. The progression from HPV infection to cervical precancerous lesions and invasive cervical cancer.

invasive cancer are from an unethical clinical study conducted at National Women's Hospital in Auckland, New Zealand (McCredie et al., 2008); among women with CIN3 (n=143) who were managed only with the follow-up without receiving treatment, the cumulative percentage for cancer of the cervix or vaginal vault was 20.0% by 10 years and 31.3% by 30 years.

2.4.5 HPV manifestations

2.4.5.1 HPV manifestations in adult population

HPV encompasses a great worldwide burden of diseases, including medical problems from benign warts to different HPV-related cancers – most importantly, cervical cancer. Cervical cancer is the fourth most common cancer and cause of cancer death in women, and HPV is responsible for nearly all cervical cancers (Sung et al., 2021). Across the globe, 604,000 new cervical cancer cases and 342,000 deaths occurred in 2020; the vast majority of those took place in underdeveloped or developing nations (Sung et al., 2021). The two most common histologic types of invasive cervical cancers are SCC and adenocarcinoma, accounting for approximately 60% and 30% of cervical cancer cases, respectively (Pankakoski et

al., 2022); especially the incidence of adenocarcinoma is increasing (Bray, Carstensen, et al., 2005; Parkin & Bray, 2006).

In addition to cervical cancer, HPV is responsible for a substantial fraction of other anogenital (anal, vulvar, vaginal, and penile), as head and neck (oropharyngeal, oral cavity, laryngeal) cancers affecting both women and men (de Martel et al., 2017). **Table 1** shows each HPV-related cancer with their HPV attributable fraction (AF) (i.e., the proportion of cancer cases that would not have occurred if HPV had been totally absent from the population) (de Martel et al., 2017). Examples of the most common HPV types related to cancer are shown in **Table 1**.

At the head and neck region, HPV is primarily associated with oropharyngeal cancer (OPC). Approximately 79,000 new OPC cases occur per year (Sung et al., 2021); a third of these cases worldwide are estimated to be caused by HPV with a high variation between nations (de Martel et al., 2017; Kreimer et al., 2005). The disease predominantly affects men; overall, OPCs and HPV-positive OPCs are 4-5 times more prevalent in men than in women, and the reason is not fully understood (de Martel et al., 2017; Sung et al., 2021). In recent decades, there has been a sharp increase in the incidence of oropharyngeal cancer in developed countries (Chaturvedi et al., 2013). This is presumed to be a result of the increase in HPV-positive cancers caused by oral oncogenic HPV – most importantly, HPV16-infections (Chaturvedi et al., 2013; Lundberg et al., 2011; Mehanna et al., 2013; Näsman et al., 2009; Nichols et al., 2013; Simard et al., 2014). The aetiology and prognosis of HPV-positive and HPV-negative OPCs differ from each other. Patients with HPV-positive cancer have a better rate of survival than patients with HPV-negative cancer (Ang et al., 2010), for which tobacco and alcohol consumption have been established as main risk factors (Blot et al., 1988).

Cancer is a rare manifestation of HPV. More commonly, the infection becomes clinically visible as a benign lesion. Common benign lesions with responsible LR types are shown in **Table 2**. Common benign manifestations are anogenital warts (condyloma acuminata), which are caused mainly by LR-HPV types HPV6 and HPV11 (Zhu et al., 2019). At the oral site, benign oral papillomas or condylomas, which cannot be histologically or clinically distinguished from each other, are often seen. While there are no population-based studies on the incidence or prevalence of these lesions, a Swedish cohort study encompassing 20,000 citizens showed a 0.1% prevalence of oral warty lesions (Axéll, 1976).

Many cutaneous βHPVs are responsible for benign skin warts frequently detected in children and adults. However, there is evidence that some βHPVs, together with UV-radiation, are involved in cutaneous SCC (Rollison et al., 2019).

Table 1. HPV-related malignant lesions.

MALIGNANT LESIONS		
ANATOMIC REGION	**AF[b] (%)**	**MOST IMPORTANT HPV-TYPES THAT ARE ATTRIBUTABLE TO CANCER**
anogenital cancers: -cervix -anus, -vulva -vagina -penis	 100.0 88.0 24.9 78.0 50.0	HPV 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68[a]
head and neck cancers: -oropharynx -oral cavity -larynx	 30.8 2.2 2.4	HPV 16 ja 18 (Kreimer et al., 2005)
skin cancers: -SCC of sun-exposed skin		Some βHPV types (e.g., HPV 5 and 8, but more evidence is needed) (Rollison et al., 2019)

[a]Classified as HR (group 1) and probable HR types (group 2A) by the IARC Monograph Working Group (Bouvard et al., 2009).
[b]The population attributable fraction (AF) for HPV-related cancers means the proportion of cancer cases that would not have occurred if HPV had been totally absent from the population (de Martel et al., 2017).

2.4.5.2 HPV manifestations in children

In the paediatric population, the most common clinical HPV manifestations include benign skin warts, oral papillomas, and anogenital warts. Also, JORRP and focal epithelial hyperplasia (FEH) are rarely seen. Formerly the presence of anogenital warts in children considered a sign of sexual abuse. However, it is now known that they can be presented without any sexual contact. In children, anogenital warts are caused by mucosal or cutaneous types of HPV (Braun et al., 2021).

Especially in school-age children, skin warts are a common manifestation of cutaneous HPV infections that are acquired mostly horizontally, but also vertically (Antonsson et al., 2003; Tamer et al., 2008; Weissenborn et al., 2009). Cutaneous HPV infections can persist for years on healthy skin (Hazard et al., 2007). Common warts account for 70% of all skin warts, and compared to plantar and flat warts, they are seen more commonly in younger children (Syrjänen, 2010). However, skin warts are rare among children under 5 years (Syrjänen, 2010). Common cutaneous HPV types responsible for these warts are shown in **Table 2**.

FEH, Heck's disease, is a rare benign disorder that is clinically shown as multiple exophytic lesions in the oral cavity (Said et al., 2013). HPV13 and HPV32 have been associated with the FEH lesions (Said et al., 2013). The disease is seen especially in

children and adolescents, as well as adults, and particularly among specific ethnic and racial populations; for example, a high prevalence is found among Eskimos in Greenland (Said et al., 2013).

Recurrent respiratory papillomatosis (RRP) is a rare benign but potentially severe condition in which squamous papilloma lesions repeatedly grow within the aerodigestive tract, most often in the larynx. It is mainly caused by a local infection of LR-HPV types HPV6 and HPV11. There has been a documented decline in the RRP incidence after the implementation of HPV vaccination programs (Novakovic et al., 2018). Depending on the patient's age at onset, the disease is considered adult-onset RRP (AORRP) and juvenile-onset RRP (JORRP), of which the latter is generally a more aggressive disease (Benedict & Derkay, 2021). In JORRP, the disease is suggested to result from an HPV infection transmitted vertically from the mother to the newborn (Shah et al., 1986). A younger age at the onset of the JORRP is related to more severe disease (Buchinsky et al., 2019; Xi et al., 2020). Common symptoms of JORRP are progressive hoarseness, stridor, and respiratory distress. The course of the disease is variable, as some patients may go into remission spontaneously, while others may develop an aggressive disease in which treatment requires repeated surgery, impairing the quality of life for children and their families (Derkay & Bluher, 2019; Lindman et al., 2005). In recent years, therapeutic HPV vaccinations and monoclonal antibodies as an adjuvant treatment beside surgery have shown promising results in the treatment of recurrent diseases (Rosenberg et al., 2019; Ryan et al., 2021).

Table 2. HPV-related benign lesions in adult and paediatric populations.

BENIGN LESIONS	
ANATOMIC REGION	**MOST IMPORTANT HPV-TYPES THAT ARE ATTBIBUTABLE TO LESION**
anogenital region: -anogenital warts	 HPV 6 and 11 (Zhu et al., 2019)
oral region: -oral papillomas/condylomas, RRP -laryngeal papillomas -focal hyperplasia (Heck's disease)	 HPV 6, 11 (Benedict & Derkay, 2021; Syrjänen, 2018) HPV 6,11 (Cubie, 2013) HPV 13, 32 (Said et al., 2013)
skin: -common warts (verruca vulgaris) -plantar warts (myrmecia) -flat warts (verruca plana)	 HPV 1, 2, 4, 27, and 57 (Doorbar et al., 2015) HPV1 and 4 (Doorbar et al., 2015) HPV 3 and 10 (Doorbar et al., 2015)

2.4.6 Risk factors for HPV infection and HPV-related pathology

Sexual behaviour and age are the main risk factors for incident HPV infection. The HPV type is the strongest factor that affects the absolute risk of viral persistence and disease progression given the viral persistence (Schiffman et al., 2005). A persistent HR-HPV infection is necessary, but not sufficient to induce cervical cancer (Cohen et al., 2019). The progression of HPV-induced lesions to malignancy requires additional cofactors, as only a small proportion of infections progress. Herein, the risk factors for HPV infection and HPV-related cancer are discussed.

Sexual behaviour

Sexual behaviour, such as the number of lifetime sexual partners and acquisition of a new sexual partner, is associated with incident genital HPV infection (Giuliano et al., 2011; Giuliano, Harris, et al., 2002; Moscicki et al., 2001; Muñoz et al., 2004; Pauli et al., 2022; Sellors et al., 2000, 2003; Vaccarella, Franceschi, et al., 2006; Velicer et al., 2009; Winer et al., 2003). The protective effect of condom usage against new HPV infection is controversial. However, a large meta-analysis did not observe an association between HPV presence and condom use (Vaccarella, Franceschi, et al., 2006). Anal HR-HPV infections have been shown to be more prevalent among men having sex with men (MSM) than in men having sex with women (MSW) (Wei et al., 2021).

Sexual behaviour is thought to affect the oral HPV risk as well; the number of sexual partners has been associated with oral HPV infection (Gillison et al., 2012) and oropharyngeal cancer (Heck et al., 2010). The association between oral sex and oral HPV infection is controversial (Beachler et al., 2015; D'Souza et al., 2016, 2017; Kreimer et al., 2013; Winer et al., 2003); however, the data is more consistent in recognising oral sex as a risk factor for oropharyngeal cancer (D'Souza et al., 2007; Heck et al., 2010; Herrero et al., 2003).

Smoking

Being a current smoker is associated with an increased prevalence of HPV and persistence at the genital, oral and anal sites (Beachler et al., 2015; D'Souza et al., 2017; Gillison et al., 2012; Giuliano, Sedjo, et al., 2002; Kero et al., 2014; Schabath et al., 2012; Sellors et al., 2000; Umutoni et al., 2022; Vaccarella et al., 2008). Moreover, both current and former smoking habits have been recognised as a risk factor for cervical cancer (Appleby, Beral, Berrington de González, Colin, Franceschi, Goodill, et al., 2006). The biological effect of smoking has been shown

to be associated with a dampening of local immune markers of the cervical mucosa (Barton et al., 1988).

Age

HPV prevalence is highly age-dependent, as women under the age of 25 hold the highest risk for incident infections, and the risk decreases until they are middle-aged (Bruni et al., 2010; de Sanjosé et al., 2007; Muñoz et al., 2004; Sellors et al., 2000; Velicer et al., 2009). Immature immune responses and the immature cervical epithelium of adolescents presumably play a role in HPV susceptibility.

Reproductive factors

The data on oral contraceptive (OC) use affecting the natural course of HPV infection is inconsistent (Appleby, Beral, Berrington de González, Colin, Franceschi, Green, et al., 2006; Molano et al., 2003; Moreno et al., 2002; Moscicki et al., 2001; Nielsen et al., 2010; Sellors et al., 2000; J. S. Smith et al., 2003; Vaccarella, Herrero, et al., 2006). However, according to the pooled analyses of the IARC HPV Prevalence Surveys with 15,145 women, it was reported that neither long-term OC use nor high parity was associated with HPV prevalence (Vaccarella 2006); hence, they seem to increase the risk of cervical cancer (Appleby, Beral, Berrington de González, Colin, Franceschi, Green, et al., 2006; Moreno et al., 2002; J. S. Smith et al., 2003; Vaccarella, Herrero, et al., 2006). The use of OCs and parity, especially the number of full-term pregnancies, expose the individual to high levels of sex hormones. The TZ contains high levels of oestrogen and progesterone receptors (Remoue et al., 2003), but the reason for the association between sex hormones and cervical carcinogenesis is not completely understood. As an example of many explanations, sex hormones are suggested to stimulate HPV gene expression, cell proliferation, and sensitise the TZ to carcinogenesis by altering the local cervical immune microenvironment (Delvenne et al., 2007). Moreover, not only does higher parity increase the hormone levels, but it also exposes the cervix to repeated tissue damage during delivery, which may contribute to oxidative stress and DNA damage (Castle, 2004).

Vaginal microbiome

The vaginal microbiome, such as bacterial vaginosis (BV) and chlamydia trachomatis infection, is also associated with HPV infection (Liang et al., 2019) and BV is even related to CIN (Kyrgiou et al., 2017; Liang et al., 2019). However, it is unclear whether HPV induces a change in the vaginal microbiome, or that the vaginal

microbiome makes the cervix more prone to HPV infection and its persistence. There may be some other unrecognised host factors predisposing the individual to both vaginal dysbiosis and HPV infection.

HIV and immunosuppression

Immunological cofactors facilitate the natural course of HPV infection, infection susceptibility, and carcinogenesis. Immunosuppressive conditions, such as HIV infection, organ or stem cell transplantation, and certain autoimmune diseases with immunosuppressive treatment, increase the risk of HPV infection and/or HPV-related cancers (Denny et al., 2012; Moscicki et al., 2019). The higher risk of an HPV infection in HIV patients is related to HPV susceptibility, the decreased ability to clear infections due to their impaired cell-mediated immunity, and the reactivation of a latent HPV infection. Compared to HIV-uninfected women, women living with HIV have a higher prevalence of genital HPV infection and persistence, as well as cervical precancer and invasive cancer (Denny et al., 2012). HIV is a risk factor for anal HPV infection, as well as anal cancer; in particular, HIV-infected MSM have a high risk for anal HPV infection and anal cancer (Machalek et al., 2012). Moreover, HIV increases the risk of an oral HPV infection (Beachler et al., 2015).

Host genetic factors – HLA-G

The reason behind the HPV infection progression is thought to be multifactorial, including the risk factors mentioned above. Host genetic immunological co-factors are supposed to facilitate the natural course of HPV infection as well. In particular, the HLA allele repertoire, which is crucial for cell-mediated immune responses, may be a critical factor in determining whether HPV will be cleared, or if it evades the immune system and persists. Thus, the susceptibility for infection may be influenced by the individual's HLA-allele combination.

As HLA-G has immunosuppressive properties, its role in the natural history of viral infections is of great interest. By now, the data of the impact of HLA-G in particular HPV infection and cervical pathology is limited and controversial. Studies evaluating the specific coding region of HLA-G gene has shown that certain HLA-G alleles/genotypes influence the HPV risk, but the results are partly inconsistent and further research is needed to make a consensus (Alves et al., 2015; Ferguson et al., 2011; Metcalfe et al., 2013). Moreover, the role of HLA-G 3'UTR polymorphism in HPV infection has been under research, although a recent meta-analysis did not show an association between HLA-G 3'UTR and HPV susceptibility (Moossavi et al., 2021). Regarding oral HPV, an association between HLA-G polymorphism and oral HPV infection in women has been shown (Jaakola et al., 2021).

HLA-G is also suggested to facilitate the cervical cancer progression. Thus, the association of HLA-G expression with cervical precancer and cancer has been increasingly studied in the past decade (Gimenes et al., 2014; Li et al., 2012; H.-H. Xu et al., 2018). As HLA-G is considered as an immune checkpoint molecule, its expression in malignant cells is suggested to enable escaping from the immunosurveillance. Polymorphism in the coding region of the HLA-G gene has been found to influence the risk of cervical cancer and its precursors, but the data is inconsistent (Ferguson et al., 2012; Simões et al., 2009). Moreover, HLA-G 3'UTR polymorphism has been associated with the progression of cervical lesions (Bortolotti et al., 2014; Ferguson et al., 2012; I. D. Silva et al., 2013; H.-H. Xu et al., 2018; Y. C. Yang et al., 2014). Interestingly, one case-control study with the North Indian cohort evaluated the role of HLA-G polymorphism in head and neck squamous cell carcinomas (HNSCC), suggesting that HLA-G polymorphism may influence the HNSCC risk (Agnihotri et al., 2017). However, more research is needed to understand the role of HLA-G in HPV-related pathogenesis.

2.5 HPV prevention strategies

Cervical cancer covers the highest burden of HPV-related diseases. In 2020, the WHO launched three steps for cervical cancer elimination: vaccination, screening, and treatment (*WHO*, 2020). By successfully implementing these three steps by 2030, more than 40% of new cervical cancer cases and 5 million related deaths could be reduced by 2050. The aim of cervical cancer screening is to prevent cervical and vaginal cancers, and no similar screening program for other HPV-related diseases exists. The effectiveness of anal cancer screening in certain risk groups is intensively studied (Palefsky et al., 2022). For now, evidence is insufficient to estimate the effectiveness of oral cancer screening (Brocklehurst et al., 2013). However, HPV vaccination is suggested to reduce the burden of non-cervical diseases in the future.

2.5.1 Screening

The cervical cytology by the Pap smear, developed by Dr. George Nicholas Papanicolaou (1883-1962), is one of the greatest achievements in cancer prevention history. It has been used for cervical cancer screening in many developed countries since the 1950s. In Finland, screening with the Pap smear was introduced in 1960s. Widespread screenings have led to a decrease in the incidence of cervical cancer and mortality in numerous high-income countries (Anderson et al., 1988; Arbyn et al., 2009; Bray, Loos, et al., 2005; Peto et al., 2004). For example, in British Columbia, Canada, the incidence of invasive cervical cancer decreased by 78% and mortality by 72% 30 years after the introduction of cervical cancer screening (Anderson et al., 1988).

Nowadays, HPV testing has replaced the Pap smear as the primary screening test in most countries, while the Pap smear is still used as a triage test for HPV-positive women. In four European randomised controlled trials, the HPV-based screening was shown to provide 60–70% greater protection against invasive cervical cancer compared to cytology (Ronco et al., 2014). Moreover, HPV assays, which can be performed via self-sampling, have been developed. They have shown to be as sensitive as clinical samples (Arbyn, Smith, et al., 2018). Self-collected HPV testing is promising in terms of expanding the screening in low- and middle-income countries (Kamath Mulki & Withers, 2021) and improving the participation of women who do not routinely attend screening (Arbyn, Smith, et al., 2018).

The effectiveness of screening in cancer prevention is based on early detection of HR-HPV infections and precancerous lesions (e.g. LSIL, HSIL, adenocarcinoma in situ (AIS)) to avoid the progression to invasive cancer. Aberrant findings in cytology are usually an indication for a colposcopy and biopsy to verify the histological diagnosis. Depending on the histological diagnosis and the patient's age, the appropriate management of these lesions are: 1) immediate treatment, or 2) follow-up and treatment only in cases of persistent lesions. The treatment is a surgical excision, usually performed by cervical conisation with a loop electrosurgical excision procedure (LEEP). Interestingly, in recent years, several studies have shown that the combination of HPV vaccination and surgical excision reduce a risk of recurrent cervical intraepithelial neoplasia compared to surgical excisions alone (Jentschke et al., 2020). Moreover, the efficacy of therapeutic HPV-vaccines in treating CIN2/3 lesions is intensively studied in phase II/III clinical trials (Ibrahim Khalil et al., 2023).

Although the early detection and treatment of precancerous lesions is possible due to screening, the other side of the coin is the overtreatment of these lesions. Recent evidence shows that only approximately 10% of CIN2 lesions progress in women under the age of 30 (Tainio et al., 2018), but as it cannot be distinguished which ones will progress, surgical excision of all the lesions is the preferred treatment. Excision increases the risk for adverse obstetric outcomes, such as premature birth (Kyrgiou et al., 2016). Hence, the overtreatment of lesions, especially in young women, should be avoided. There is a great need for a prognostic test to differentiate lesions that tend to progress from those that regress. The detection of epigenetic changes related to cancer progression, is proposed to serve as a predictive or diagnostic biomarker for cancer progression. Measuring DNA methylation at specific CpG sites in HPV and host genes has shown to be a promising marker for predicting progressive cervical diseases (Clarke et al., 2012; Hernández-López et al., 2019; Kalantari et al., 2014; Louvanto et al., 2020; Mirabello et al., 2013). Moreover, evidence suggests that a methylation classifier could be useful in cervical screening programs for the triage of HR-HPV-positive women to the

colposcopy (Brentnall et al., 2014; Lorincz et al., 2016). In the future, methylation classifiers may open new possibilities in screening by targeting more intensive follow-up and treatment to individuals with a higher risk for progression.

2.5.2 Vaccination

Since 2007, prophylactic HPV vaccination programs have been implemented worldwide in almost all high-income countries. There has been an increase in the introduction of these programs in low and middle-income countries, where the cervical cancer incidence is the highest (Bruni et al., 2021). HPV vaccination is suggested for girls and boys primarily before the initiation of sexual activity and their subsequent exposure to HPV. To date, three HPV vaccines are commercially available, all consisting of highly immunogenic virus-like particles (VLP) formed by the self-assembly of the L1 protein of targeted HPV types:

- bivalent (Cervarix ®) against HR-HPV 16/18
- quadrivalent (Gardasil®) against HR-HPV 16/18 and LR-HPV 6/11
- nonavalent (Gardasil9 ®) against HR-HPV 16/18/31/33/45/52/58 and LR-HPV 6/11.

Moreover, the bivalent vaccine has shown a cross-protection against HR-HPV types HPV31, HPV35, HPV45, and HPV51 (Lehtinen & Dillner, 2013). These three vaccinations, which cover the most important HR-types, could potentially prevent up to 90% of HPV-related cancers and precancerous lesions (de Sanjose et al., 2019).

A meta-analysis of the population-level vaccine impact has shown that after the implementation of girls' vaccination programs, there was a significant decrease in the prevalence of HPV infection and CIN2+ among young women, and in the prevalence of genital warts among both women and men (Drolet et al., 2019). Similarly, the reduction of the CIN2+ risk in young women have been shown in randomised control trials (Arbyn, Xu, et al., 2018). Yet, there is limited data on the vaccine's protection against oral HPV infections (Chaturvedi et al., 2019; Herrero et al., 2013). As it takes years or decades for the HPV infection to progress to invasive cancer, the actual change in HPV-related cancer rates will be seen in the coming years.

Although HPV vaccination, with its efficacy, immunogenicity and safety, could be described as a success story for the prevention of HPV-related disease, there are considerable challenges. In 2019, more than half of the countries worldwide had introduced the HPV vaccine, but due to different population sizes, 70% of girls still lived in countries without the HPV vaccination program (Bruni et al., 2021). Moreover, in many countries that have already introduced the HPV vaccination programs, the performance of HPV vaccination coverage is still low (Bruni et al., 2021).

3 Aims

This doctoral dissertation evaluated the HPV infection transmission among family members. Moreover, the role of HLA-G in men's HPV infection outcomes and the father-newborn HPV concordance were determined. The data from the original Finnish Family HPV Study were used. The three main aims of this dissertation were:

AIM 1: Investigate whether HLA-G alleles and genotypes impact men's oral and/or genital natural HPV infection outcomes.

AIM 2: Evaluate whether HLA-G alleles and/or genotypes have a role in the father-to-child HPV transmission during the perinatal period.

AIM 3: Assess whether the mother's and/or father's genital or oral HPV infection influences the risk of perinatal HPV infection transmission to their offspring.

Our hypothesis was that HLA-G would have an impact on the fathers' HPV infection outcomes, as well as the perinatal HPV transmission to the offspring. In addition, regarding the HPV transmission, our hypothesis was that the mother would most likely have a greater role in the vertical HPV transmission, but the father would also have some role in the HPV transmission.

4 Materials and Methods

4.1 Study population

4.1.1 The Finnish Family HPV Study

The Finnish Family HPV (FFHPV) Study is a longitudinal cohort study conducted at the Department of Gynaecology and Obstetrics, Turku University Hospital, and the Department of Oral Pathology, University of Turku. Originally a total of 329 families with 329 mothers, 132 fathers and their 331 newborns (including two sets of twins) were included in the study. Pregnant mothers who planned to have delivery at Turku University Hospital, and their spouses, were recruited between 1998 and 2002. The inclusion criteria for participation in the study were: mother's age ≥18 years and ≥36 week of pregnancy. Mothers were included regardless of their previous HPV status or planned mode of delivery (vaginal delivery or caesarean section). Mothers, fathers and newborns were followed up for six years to elucidate the natural history of HPV infection between family members. Follow-up visits included HPV testing during every visit and a questionnaire at the baseline and end of the follow-up. In this thesis, 319 mothers, 132 fathers and 321 newborn offspring (two sets of twins) were included from the original Finnish Family HPV Study cohort comprising 321 mother-newborn pairs and 134 father-newborn pairs.

The study was performed in line with the principles of the Declaration of Helsinki. The study protocol and its amendment (#3/1998, #2/2006 and 45/180/2010) have been approved by the Ethics Committee of Turku University Hospital. All adult participants gave their written informed consent to participate in the study. The written informed consent for the child's participation was obtained from both parents of the child.

4.1.2 Demographic data

At the baseline and end of the six years' follow-up, the mothers and fathers filled a structured questionnaire recording demographic data, including their socioeconomic status, general health, and risk factors for HPV infection. A history of possible HPV-related lesions was asked in detail in the baseline questionnaire of the study.

Participants' earlier history of anogenital or oral lesions or warts did not affect the study recruitment. The selected demographic data of fathers were used in this thesis: a self-reported history of infertility, HPV-related symptoms, previous chlamydia and mumps infections, asthma, allergy, and atopy, as well as signs of genital, skin, and oral warts.

4.2 HPV samples

4.2.1 Sample collection

Herein, the sample collection of the FFHPV Study is explained at the extent of data used in this thesis. Scraping samples from mothers, fathers, and newborns were collected for HPV genotyping. Mothers' oral and genital (cervical), as well as fathers' genital (urethral), oral and semen samples, collected at baseline (36 weeks of pregnancy) were included in the present study. Moreover, the follow-up data of fathers was included. Fathers' follow-up samples for HPV genotyping were available solely from the oral cavity; during the 6-year follow-up, oral brush samples were collected at baseline and at 2-, 6-, 12-, 24-, 36-, and 77-month follow-up visits.

Oral and genital (from the labia/prepuce and scrotum) scraping samples were collected from newborns immediately after birth in the delivery room and postpartum at day 3 (at discharge), as well as the 1-, 2- and 6-month follow-up visits. Placental and umbilical cord blood samples were taken immediately after delivery. From the placenta, two representative samples covering all tissue layers were taken. An umbilical venous cord blood sample was taken while the placenta was still in situ. Next, the sample technique will be explained by each anatomic site.

Scraping samples

All scraping samples from mothers (oral and genital=cervical), fathers (oral and genital=urethral), and newborns (oral, genital) were taken by using a small brush (MedScand, Malmö, Sweden). After sampling, the brush was placed in the test tube containing 70% ethanol except the brush used for cervical samples, which was placed in a tube with 0.05 M PBS (phosphate-buffered saline) with 100 µg gentamycin as a sampling medium. All samples were immediately frozen at -20°C and then stored at -70°C until HPV testing.

- Cervical samples from the mothers were taken from the uterine cervix with a cytobrush.
- Urethral samples from the fathers were collected from the distal part of urethral mucosa, at 1 cm depth from the urethral meatus.

- Oral samples from mothers, fathers, and newborns were collected from the buccal mucosa of both cheeks, and from the upper and lower vestibular area while avoiding contact with the tongue.
- Genital samples of newborns were collected from the labia of baby girls and from the prepuce and scrotum of baby boys.

Semen samples

The semen collection and analyses were done according to the guidelines of the Nordic Association for Andrology. At baseline, fathers delivered a semen sample taken into a plastic container via masturbation using gloves. At least two days of abstinence was required. If the sample was taken at home, it was transported to the laboratory within two hours after ejaculation. The centrifugation of samples was performed in a Sorval MC12V (Sorval Instruments, Zurich, Switzerland) at 3500 rpm for 15 minutes. Seminal plasma and cells were stored separately, at -20°C and -70°C, respectively.

Placental and umbilical cord blood samples

Two representative samples covering all tissue layers were taken from the central part of the maternal side of placenta immediately after delivery. Samples were stored at -70°C until analysed. One was immediately frozen at -70°C until analysed for HPV testing, and another was fixed in formalin for routine histology. Umbilical venous cord blood samples were collected into vacuum EDTA (Ethylenediaminetetra-acetic acid) tubes while the placenta was still in situ, and samples were frozen immediately at -70°C.

4.2.2 DNA isolation

DNA extraction from all samples, apart from the umbilical cord blood samples, was obtained with the high salt method (Miller et al., 1988). According to the high salt method, samples were lysed in a lysis buffer (10 mM Tris, 400 mM NaCl, 100 mM EDTA, 1 % SDS), followed by digestion with proteinase K (10 µg/ml) overnight at 37°C. The proteins were then precipitated with saturated NaCl and ethanol. Purified DNA was dissolved in 50 µl water, mixed for 15–30 minutes, and stored at -20°C. From the umbilical cord blood samples, DNA was extracted with the high pure PCR template preparation kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions.

During DNA extraction, contamination was carefully monitored by simultaneous DNA extraction from cultured human fibroblasts or HPV-negative

immortalised human gingival keratinocytes, which served as negative controls for contamination during DNA extraction. Only eight study samples were processed at the same time. For each set of eight samples, one fibroblast-negative control and one HPV16-positive control (Siha cell lines, human cervical epithelial carcinoma, HTB-35, American Type Culture Collection) were used.

4.2.3 HPV detection and genotyping

HPV amplification was done by nested PCR with MY09/MY11 primers first, and then with GP05+/GP06+ for all other samples except cervical samples, for which a single PCR with GP05+/GP06+ was used (Snijders et al., 1990). Contamination during HPV amplification was carefully controlled. In addition to the negative and positive controls described above, one no-DNA sample was included for each set of eight study samples to be processed for HPV amplification. The DNA extraction, master mix for PCR, and addition of the target DNA in the reaction mixture were all done in separate rooms that were exchanged regularly in the MediCity Research Laboratory, Faculty of Medicine, University of Turku, Finland. In these research rooms, neither any clinical samples nor HPV-positive cell lines were used.

For HPV genotyping, the earlier nested PCR products (amplified by outer MY09/MY11 and inner GP05+/GP06+ primers) were reamplified for biotinylation using GP05+/GP06+ primers. HPV genotyping was performed by the Luminex-based Multimetrix kit (Multimetrix, Progen Biotechnik GmbH, Heidelberg, Germany), which identifies 24 different LR- and HR-HPV genotypes (LR genotypes: 6, 11, 42, 43, 44 and HR genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82) (Schmitt et al., 2006). The test was performed according to the protocol, except that only half of the volumes were used. In the final step, the same volume as in the protocol (100 µl) was used for analysing the labelled hybrids with the Luminex LX-100 analyser (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, CA, USA). The median fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cut-off value for each run and HPV type was calculated as 1.5 times the background MFI (negative control) +5 MFI. To exclude possible contamination, all HPV16-positive samples were retested from the original samples by using nested PCR with an in-house bead-based HPV genotyping assay. Importantly, the samples collected for HPV testing were never analysed at the same time, including DNA extraction or HPV amplification. In addition, the samples were stored in separate boxes.

4.3 HLA-G determination

The DNA from fathers' and newborns' frozen whole blood samples were extracted for HLA-G typing by using the MagNAPure 96 System (Roche). The determination of HLA-G alleles was done by direct DNA sequencing exploring exons 2-4 (1718 bp) of the HLA-G gene regions, as described by Ferguson et al. (Ferguson et al., 2011). This DNA fragment was produced by using G-25: 5′=-TCCATGAGGTATTTCAGCGC-3′= and G-4R: 5′=-CACCACCGACCCTGTTAAAG -3′= primers. Amplicons were generated in a total volume of 30 µl using 200 ng of genomic DNA, 1.0 U of Taq Platinum DNA polymerase high fidelity (Invitrogen, Carlsbad, CA), 250 µM of each deoxynucleoside-5′=-triphosphate (Invitrogen), 0.5 µM of each primer, 1xPCR buffer (Invitrogen), and 1.5 mM MgSO4 (Invitrogen). After amplification by PCR, the PCR products were determined using the BigDye terminator cycle sequencing reactions (Applied Biosystems, Foster City, CA). The PCR products were run in an automated DNA sequencing ABI Prism 3100 capillary sequencer (Applied Biosystems). The sequencing of PCR products was performed in both directions.

4.4 Definition of variables

HLA-G alleles and genotypes (I, II)

As the child inherits one allele from the mother and another from the father, there should be at least one common allele between the mother/father and the newborn. Together, these two inherited alleles determine individuals' HLA-G genotype. Only those HLA-G alleles and genotypes that were ≥ 3% prevalent among fathers were included in the analyses in the first phase (I). In the second phase (II), when the father-newborn HLA-G concordances were analysed, only those HLA-G alleles and genotypes that 1) were identified both among the fathers and newborns and 2) were ≥3 % prevalent among the fathers and/or newborns were included in the analyses. In the father-newborn analyses (II), HLA-G alleles and genotypes were explored both in high- and low-resolution groups. Each specific allele/genotype represented one high-resolution group, whereas low-resolution groups consisted of sets of similar alleles/genotypes. The generation of low-resolution groups for HLA-G alleles and genotypes is shown in **Table 3**.

Table 3. HLA-G allele and genotype low-resolution groups. Among alleles, only one low-resolution allele group, 01:01+, could be generated. In the HLA-G genotype level, four low-resolution genotype groups were generated.

LOW-RESOLUTION GROUP	HIGH-RESOLUTION ALLELES AND/OR GENOTYPES INCLUDED
HLA-G ALLELE	
01:01+	G*01:01:01 G*01:01:02 G*01:01:03 G*01:01:14
HLA-G GENOTYPES	
01:01+/01:01+	G*01:01:01/01:01:01 G*01:01:01/01:01:02 G*01:01:01/01:01:03 G*01:01:01/01:01:14 G*01:01:02/01:01:02 G*01:01:02/01:01:03 G*01:01:02/01:01:14
01:01+/01:03+	G*01:01:01/01:03:01 G*01:01:02/01:03:01
01:01+/01:04+	G*01:01:01/01:04:01 G*01:01:02/01:04:01 G*01:01:03/01:04:01
01:01+/01:06+	G*01:01:01/01:06 G*01:01:02/01:06

Father-newborn HLA-G allele concordance (II)

The HLA-G allele concordance/discordance between the father and the newborn were determined for each HLA-G allele separately. The *HLA-G concordance* for a specific HLA-G allele was considered if the father and his offspring both had at least one common HLA-G allele. In other words, the HLA-G allele concordance was defined if both parties were heterozygous or homozygous for this specific allele. If one party had an allele and the other did not, the pair was counted as *HLA-G discordant* for the allele. The HLA-G allele/genotype concordance/discordance is described in **Table 4**.

In the high-resolution analyses, two groups, HLA-G allele concordance (≥2 shared alleles) and discordance, were used. However, in low-resolution analyses, the HLA-G allele concordance was further divided into three groups by the number of shared alleles (2 = 2 shared alleles, 3 = 3 shared alleles, 4 = 4 shared alleles within the father-newborn pair).

Father-newborn HLA-G genotype concordance (II)

Concordance/discordance between the father and the newborn was determined for each HLA-G genotype as well. If both parties had the HLA-G genotype, the pair was counted as *HLA-G concordant* for a specific HLA-G genotype. If only one had the HLA-G genotype but another one did not, the pair was counted as *HLA-G discordant* for that genotype.

Table 4. Simple overview of HLA-G allele/genotype sharing between father-newborn pairs. Allele/genotype prevalent (+) or absent (-).

HLA-G SHARING	ALLELE/GENOTYPE PREVALENCE	
	FATHER	NEWBORN
CONCORDANCE	+	+
DISCORDANCE	+	-
	-	+
ABSENT	-	-

HPV grouping (I, II, III)

In HPV genotyping analyses, HPV types were classified as any-, LR- or HR-HPV-groups. The any-HPV group counted HPV positivity regardless of the detected HPV type. The LR-group counted HPV genotypes 6, 11, 42, 43, 44 and HR-group HPV genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82. Two or more different HPV genotypes detected in the same sample were defined as a multiple infection. Multiple-type HPV infections were sorted out as individual HPV genotypes.

HPV concordance between parents and newborns (III)

In assessing the HPV concordance, parent's baseline samples and newborn's samples taken at birth were included. Only those parent-newborn pairs both having an HPV sample available (i.e., for mother-newborn pairs both mother's and her newborn's sample available, and for father-newborn pairs both father's and his newborn's sample available) were considered. Any-, LR- or HR-HPV concordance were defined if the mother/father and her/his offspring both were detected positive for any-, LR- or HR-HPV, respectively. Genotype-specific HPV concordance was defined if the mother/father and her/his offspring both had the same type-specific HPV genotype detectable at any anatomic site.

HPV concordance between fathers and newborns (II)

The influence of HLA-G on the father-newborn HPV concordance was analysed not only at birth, but also during the postpartum period. Thus, the HPV concordance between fathers' HPV status at baseline and the newborn's oral HPV status 1) at birth and 2) at postpartum were determined. In those analyses, the newborn's oral HPV status was counted as the HPV point prevalence as followed: 1) *at birth* covering oral samples at delivery and at day 3 at discharge, and 2) *at postpartum* covering samples taken at 1-, 2- and 6-month follow-ups.

Fathers' oral outcomes (I)

Among fathers, four oral HPV outcome variables were defined:

1. Always HPV negative – included fathers who tested negative for HPV at baseline and at every follow-up visit.
2. Incident HPV – included fathers who tested negative at baseline and then positive at some point during the follow-up.
3. Clearance – included fathers that tested HPV-positive at baseline or at some point in the follow-up, then turned HPV-negative before the end of the follow-up and remained negative until the end of the follow-up.
4. Persistence – included fathers that tested positive for the same HPV genotype with two or more consecutive visits. This definition also included fathers who tested consecutively positive with the same HPV genotypes as part of a multiple infection.

4.5 Statistical analyses

STATA SE15.1 (StataCorp College station TX, USA) and IBM SPSS Statistics version 26.0 for Windows software (SPSS Inc. Chicago, Illinois) were used for all statistical analyses. All tests were two-sided, and p-values <0.05 were considered statistically significant.

HLA-G in fathers' HPV infection outcomes (I)

A total of 130 fathers who had both completed HLA-G allele testing and had at least one oral HPV genotyping result available were included to evaluate the impact of HLA-G on fathers' HPV infection outcomes. Baseline HPV samples for two fathers were missing, leaving 128 fathers with HPV results of their semen, urethra, and oral HPV baseline samples for analyses.

Only those HLA-G alleles and genotypes that were ≥ 3% prevalent among fathers were included in the analyses. To estimate the effect of each allele, subjects who tested positive for an allele (homozygous or heterozygous) were compared to those who tested negative for that allele. Unconditional logistic regression analyses were used to determine the associations of HLA-G alleles and genotypes with semen, genital (urethral), and oral HPV positivity and oral HPV infection outcomes (always HPV negative, incident HPV, clearance, and persistence). Moreover, the associations of HLA-G alleles and genotypes with possible risk factors (demographic data) were determined by the same statistical method. The fathers missing the allele/genotype were used as a reference. Results were reported by odds ratios (OR) with 95% confidence intervals (CI).

HLA-G in father-newborn HPV concordance (II)

A total of 134 father-newborn pairs, with both parties having their HLA-G allele determination available, were included in the father-newborn HPV concordance analyses comprising 132 fathers and their 134 offspring since two fathers had twins. Only those HLA-G alleles and genotypes that 1) were identified both among the fathers and newborns and 2) were ≥3% prevalent among the fathers and/or newborns were included in these analyses. HLA-G alleles were explored both in high- and low-resolution groups. In assessing the HPV concordance between the father and his newborn, only the father-newborn pairs that both had HPV samples available were considered. Unconditional logistic regression was used to determine the associations between 1) within the father's and his newborn's shared HLA-G alleles or genotypes and 2) the father-newborn HPV concordance and prevalence. Results were reported by OR with 95% CI. In high-resolution HLA-G allele analyses, the father-newborn pairs with both parties being negative for these specific alleles served as a reference, whereas in low-resolution group analyses, the HLA-G allele discordant father-newborn pairs served as a reference.

HPV concordance between parents and newborns (III)

In these analyses, we included 319 mother-newborn pairs, 132 fathers and their 321 newborns (two sets of twins), who all had HPV genotyping results available at least from the oral and/or genital samples. The newborns included two sets of twins, comprising a total of 321 mother-newborn pairs and 134 father-newborn pairs.

The genotype-specific HPV concordance was defined when both the parent and the newborn tested positive for the specific HPV genotype. The vertical transmission rate (the proportion of HPV-positive mothers/fathers having a concordant HPV type with her/his child) was calculated for the mother–newborn pairs and father–newborn

pairs. The proportion of mothers and fathers having a concordant HPV genotype with her/his offspring was calculated by each HPV genotype as well.

Associations of type-specific HPV presence between the mother–newborn pairs and father–newborn pairs were determined by using the univariable logistic regression analysis and reporting results by OR with 95% CI. The reference category for the specific HPV genotype positivity was the negativity for that HPV genotype. Concordance between mothers'/fathers' and newborns' genotype-specific HPV status was also assessed by using the Cohen's kappa (κ) method. The following benchmark scale for interpreting the κ statistics by Landis and Koch was used to describe the degree of agreement (HPV concordance) between the mother-newborn pairs and father-newborn pairs: <0.00, poor; 0.00–0.20, slight; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, almost perfect degree of agreement (Landis & Koch, 1977).

Associations of newborns' LR- and HR-HPV presence with mothers' and fathers' corresponding LR- and HR-HPV presence were determined by using univariable and another-parent adjusted (mothers' HPV adjusted by fathers' HPV and vice versa) multinomial (LR- and HR-HPV types) logistic regression analyses, and reporting results by ORs and another-parent adjusted odds ratios (aORs) with 95% CI. The reference category for LR- and HR-HPV was HPV-negative.

5 Results

5.1 Study cohort

At the baseline of the study, the mean age of the 319 mothers was 25.5 years (standard deviation (SD)±3.4, range 18–46), and of the 132 fathers, 28.8 years (SD±5.0, range 19–46). These mothers and fathers with baseline samples were included in the evaluations of the parent-newborn HPV transmission (III) and in the evaluations of the father-newborn HLA-G concordance and HPV concordance (II). When evaluating the impact of HLA-G on the father's oral HPV infection outcomes (I), 76.2% (99/130) of the fathers completed the first 3-year follow-up and 42.3% (55/130) the full 6-year follow-up with oral samples available from each follow-up visit.

5.2 HPV prevalence among mothers, fathers and newborns (III)

HPV prevalence

When HPV prevalence at any anatomic site was calculated, the person was counted as HPV-positive if his/her HPV sample was positive in at least one anatomic site. Among newborns, mothers, and fathers, HPV prevalence at any anatomic site was 31.2% (n=100), 31.2% (n=100), and 45.5% (n=61), respectively (**Figure 10**). Newborns' *oral* HPV prevalence was 23.0% (n=74) and *genital* HPV prevalence 10.0% (n=32).

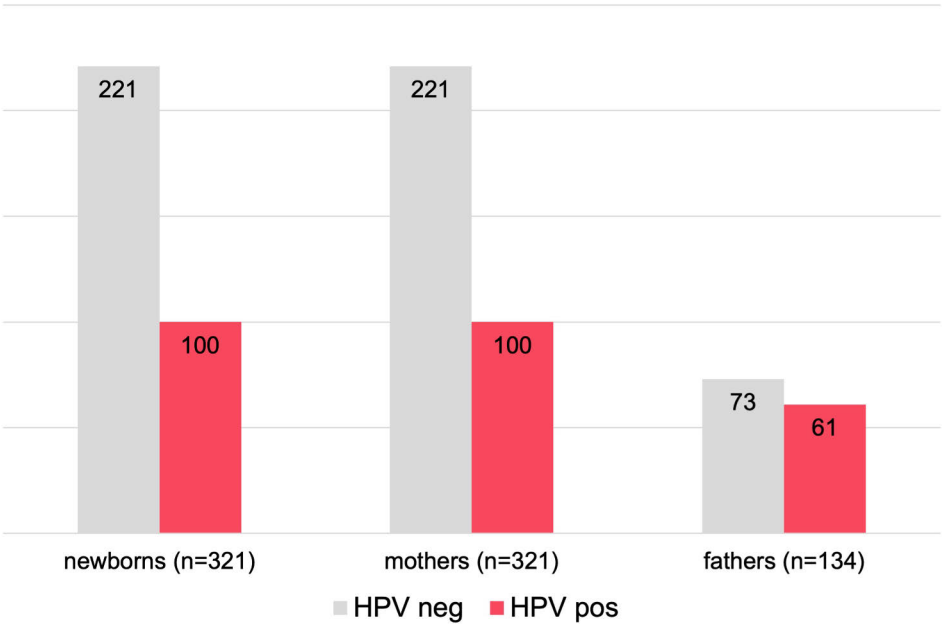

Figure 10. The frequency of any HPV positivity/negativity in newborns' (oral/genital/umbilical cord blood/placenta) samples collected at birth, and mothers' (oral/genital) and fathers' (oral/genital/semen) samples collected at baseline (i.e., before birth).

Type-specific HPV prevalence

When type-specific HPV prevalence among mothers, fathers and newborns were utilised, HPV samples at specific anatomic sites were pooled together (mother's oral and genital; father's oral, genital and semen; newborn's oral, genital, umbilical cord blood and placenta). Multiple-type infections were sorted out as individual HPV genotypes. Type-specific HPV prevalence at any anatomic site among newborns, mothers, and fathers is shown in **Table 5**. HPV genotype distributions by the different anatomic sites are shown in **Table 6.** HPV16 was the most common HPV genotype identified in all groups (newborns, mothers, fathers), followed by HPV6. Among newborns, HPV16 and HPV6 were found in 15.9% (n=51) and 4.7% (n=15) of samples, respectively. HPV16 accounted for 20.9% (n=67) of mothers' and 23.9% (n=32) of fathers' HPV infections, whereas HPV6 was found in 4.7% (n=15) of mothers' and in 9.7% (n=13) of fathers' samples. The most common HPV type, HPV16, was found in all anatomic sites of newborns (oral, genital, umbilical cord blood, and placenta), mothers (oral and genital), and fathers (oral, genital and semen), as shown in **Table 6**.

Two or more different HPV genotypes detected in the same sample was defined as a multiple infection, which accounted for 5.9% (n=19) of newborns', 10.3%

(n=33) of mothers', and 18.2% (n=24) of fathers' HPV infections. In multiple infection samples, up to four different HPV genotypes were detected in mothers and fathers, and up to three different HPV genotypes in newborns (**Table 6**). Fifteen newborns with multiple infections had two different HPV genotypes, while three different HPV genotypes were found in four newborns.

Table 5. Prevalence (%) by HPV genotypes in newborns' (oral/genital/umbilical cord blood/placenta) samples collected at birth, and mothers' (oral/genital) and fathers' (oral/genital/semen) samples collected at baseline (i.e., before birth). Modified from Original Publication III.

	Newborn (N=321)		Mother (N=321)		Father (N=134)	
HPV genotype	n	(%)	n	(%)	n	(%)
HPV neg	221	(68.8)	221	(68.8)	73	(54.5)
Any HPV	100	(31.2)	100	(31.2)	61	(45.5)
6	15	(4.7)	15	(4.7)	13	(9.7)
11	1	(0.3)	6	(1.9)	5	(3.7)
16	51	(15.9)	67	(20.9)	32	(23.9)
18	7	(2.2)	9	(2.8)	4	(3.0)
31	3	(0.9)	3	(0.9)	3	(2.2)
33	13	(4.0)	3	(0.9)	10	(7.5)
35	0		2	(0.6)	0	
39	4	(1.2)	1	(0.3)	0	
42	0		4	(1.2)	0	
43	0		2	(0.6)	2	(1.5)
45	2	(0.6)	5	(1.6)	1	(0.7)
51	0		2	(0.6)	2	(1.5)
52	0		1	(0.3)	0	
53	1	(0.3)	0		4	(3.0)
56	5	(1.6)	6	(1.9)	2	(1.5)
58	2	(0.6)	8	(2.5)	0	
59	5	(1.6)	4	(1.2)	1	(0.7)
66	9	(2.8)	9	(2.8)	2	(1.5)
68	1	(0.3)	0		0	
70	2	(0.6)	2	(0.6)	5	(3.7)
73	1	(0.3)	0		0	
82	1	(0.3)	1	(0.3)	5	(3.7)
Multiple type HPV	19	(5.9)	33	(10.3)	24	(18.2)

Multiple-type HPV infections were sorted out as individual HPV genotypes.
N=Number of newborn and their mother or father, n=number of HPV-types found. HPV types were missing for father n=187.

Table 6. Distribution of HPV genotypes by the anatomic sites among newborns and their mothers and fathers. Modified from Original Publication III.

	HPV genotype positivity																
	6	11	16	18	31	33	39	45	53	56	58	59	66	68	70	73	82
Newborns (N=321)[a]																	
Number of HPV+ newborns at any site	**15**	**1**	**51**	**7**	**3**	**13**	**4**	**2**	**1**	**5**	**2**	**5**	**9**	**1**	**2**	**1**	**1**
Oral	11	1	31	6	3	6	3	2	1	4	2	3	7	1	2	1	1
Genital	3		20	1		5	1			1	1		1				
Umbilical cord blood	5		5				1										
Placenta	5		7													1	
Mothers (N=321)[b]																	
Number of HPV+ mothers at any site	**15**	**6**	**67**	**9**	**3**	**3**	**1**	**5**	**0**	**6**	**8**	**4**	**9**	**0**	**2**	**0**	**1**
Oral	9	1	39	3			1			1	5		3				
Genital	6	5	31	6	3	3		5		5	3	3	6		2		1
Fathers (N=134)[c]																	
Number of HPV+ fathers at any site	**13**	**5**	**32**	**4**	**3**	**10**	**0**	**1**	**4**	**2**	**0**	**1**	**2**	**0**	**5**	**0**	**5**
Oral	1	2	12	1	1	4									1		5
Genital	8	1	13		1	5			3	2			1		3		
Semen	8	2	12	3	1	5		1	1			1	2		1		

[a]**Newborns (N=321):** HPV negative n=221; one HPV type found in 81 newborns; 2 different HPV types found in 15 newborns; 3 different HPV types found in 4 newborns

[b]**Mothers (N=321):** HPV negative n=221; one HPV type found in 67 mothers; 2 different HPV types found in 20 mothers; 3 different HPV types found in 9 mothers; 4 different HPV types found in 4 mothers

[c]**Fathers (N=134):** HPV negative n=73; one HPV type found in 37 fathers, 2 different HPV types found in 19 fathers, 3 different HPV types found in 4 fathers; 4 different HPV types found in 1 father

HPV prevalence in fathers' semen, genital and oral samples at baseline

HPV prevalence by HPV genotypes at baseline in semen-, genital (urethral), and oral samples among 128 fathers are shown in **Table 7**.

Table 7. HPV prevalence by HPV genotypes at baseline in semen-, genital (urethral), and oral samples among the 128 fathers from the Finnish Family HPV Study. Modified from Original Publication I.

HPV genotype	semen n=86 (%)	genital n=122 (%)	oral n=128 (%)
LR-HPV			
HPV6	7 (8.1)	7 (5.7)	1 (0.8)
HPV11	2 (2.3)	1 (0.8)	2 (1.6)
HPV43	…	1 (0.8)	1 (0.8)
total LR-HPV	9 (10.5)	9 (7.4)	4 (3.1)
HR-HPV			
HPV16	12 (14.0)	10 (8.2)	12 (9.4)
HPV18	3 (3.5)	…	1 (0.8)
HPV31	1 (1.2)	…	1 (0.8)
HPV33	4 (4.7)	5 (4.1)	4 (3.1)
HPV45	1 (1.2)	…	…
HPV51	…	1 (0.8)	…
HPV53	1 (1.2)	3 (2.5)	…
HPV56	…	2 (1.6)	…
HPV59	1 (1.2)	…	…
HPV66	2 (2.3)	1 (0.8)	…
HPV70	1 (1.2)	3 (2.5)	1 (0.8)
HPV82	…	…	5 (3.9)
total HR-HPV	23 (26.7)	20 (16.4)	21 (16.4)
Any HPV+	27 (31.4)	27 (22.1)	24 (18.8)
HPV negative	59 (68.6)	95 (77.9)	104 (81.3)
Multiple infection[a]	6 (7.0)	6 (4.9)	4 (3.1)

[a]Infection with two or more different HPV genotypes detected defined as a multiple infection.

5.3 HPV concordance between parents and newborns (III)

5.3.1 Genotype-specific HPV concordance between parents and newborns

HPV genotype-specific concordance of parent-newborn pair were determined if parent and newborn were positive for the same HPV genotype at any anatomic site. The rate of vertical transmission was 37.0% (37/100) and 35.1% (33/94) from mothers' *any anatomic site* to newborns' *any anatomic site*, and from mothers' *genital site* to newborns' *any anatomic site*, respectively. From fathers' *any anatomic site* to newborns' *any anatomic site*, the vertical transmission rate was shown to be 19.7% (12/61). With HPV16, the mother-newborn transmission rate was 31%; 21 of 67 HPV16-positive mothers transmitted HPV16 to their children.

Table 8 shows the association of type-specific HPV presence (ORs) and the degree of HPV concordance (κ) between mother-newborn pairs and father-newborn pairs. Statistically significant HPV concordances were observed with HPV6, HPV16, HPV18, HPV31, and HPV56; ORs ranged from HPV16 OR of 3.41 (95% CI 1.80–6.48) to HPV31 OR of 634 (95% CI 28.5–14087). The father-newborn HPV concordances were statistically significant with HPV6 (OR 4.89, 95% CI, 1.09–21.9) and HPV31 (OR 65.0, 95% CI 2.92–1448). A total of 60% (9/15), 31% (21/67), 22% (2/9), 67% (2/3), and 50% (3/6) of HPV6-, HPV16-, HPV18-, HPV31- and HPV56-positive mothers, respectively, had a concordant HPV genotype with their newborns. Of HPV6- and HPV31-positive fathers, 23% (3/13) and 33% (1/3), respectively, had a concordant HPV genotype with their newborns. The degree of HPV concordance between newborns' and mothers' genotype-specific HPV status was substantial with HPV31 (κ =0.66), and moderate both with HPV6 (κ =0.58) and HPV56 (κ =0.54), as seen in **Table 8**.

5.3.2 LR- and HR-HPV-specific concordance between parents and newborns

Table 9 shows the associations of the presence of newborns' LR- and HR-HPV *at any anatomic site* with their mother's or father's corresponding LR- and HR-HPV *at specific anatomic sites*. **Table 10** summarises the association of the presence of newborns' *oral* LR- and HR-HPV with their mothers' and fathers' corresponding LR- and HR-HPV *at specific anatomic sites*. When the impact of parents' *oral* HPV presence on the newborns' *oral* HPV presence was analysed, both LR- and HR-HPV detected at the mothers' *oral site* was related to the newborns' *oral* LR- and HR-HPV presence, whereas at the father's *oral site*, only HR-HPV was related to the

newborns' *oral* HR-HPV presence. These associations also remained statistically significant in another parent -adjusted model. The presence of the father's *genital* LR-HPV was related to the newborn's *oral* LR-HPV infection, and the association was also seen in the mother-adjusted model. There was no association between mothers' *genital* LR- or HR-HPV presence and newborns' *oral* LR- or HR-HPV presence.

The influence of mothers' and fathers' HPV status on newborns' *genital* HPV infections is shown in **Table 11**. Only mothers' *genital* HR-HPV presence showed to be the significant risk factor for newborns' *genital* HR-HPV presence, which strengthened in the father-adjusted analysis.

Table 8. Association of type-specific HPV presence (ORs) and the degree of HPV concordance (κ) between mother-newborn pairs and father-newborn pairs among family members from the Finnish Family HPV Study. Modified from Original Publication III.

	Newborn and mother (N=321)				Newborn HPV due to his/her mother (N=321)				Newborn and father (N=134)				Newborn HPV due to his/her father (N=134)			
HPV type	Both negative	Child positive	Mother positive	Both positive	%	κ	OR	(95% CI)	Both negative	Child positive	Father positive	Both positive	%	κ	OR	(95% CI)
6	300	6	6	9	60	**0.58**	**75.0**	**(20.2–278)**	114	7	10	3	23	0.19	**4.89**	**(1.09–21.9)**
11	314	1	6	0					128	1	4	0				
16	224	30	46	21	31	0.21	**3.41**	**(1.80–6.48)**	86	16	24	8	25	0.10	1.79	(0.67–4.69)
18	307	5	7	2	22	0.23	**17.5**	**(2.89–106)**	130	0	4	0				
31	317	1	1	2	67	**0.66**	**634**	**(28.5–14087)**	130	1	2	1	33	0.39	**65.0**	**(2.92–1448)**
33	305	13	3	0					121	3	10	0				
39	317	3	0	1					133	1	0	0				
45	314	2	5	0					132	1	1	0				
53	320	1	0	0					129	1	4	0				
56	313	2	3	3	50	**0.54**	**157**	**(18.8–1304)**	132	0	2	0				
58	313	0	6	2					133	1	0	0				
59	312	5	4	0					133	0	1	0				
66	303	9	9	0					128	4	2	0				
68	320	1	0	0					136	0	0	0				
70	317	2	2	0					129	0	5	0				
73	320	1	0	0					136	0	0	0				
82	319	1	1	0					128	1	5	0				

% indicates the proportion of mothers/fathers that have a concordant HPV genotype with their offspring. Cohen's kappa (κ) shows the degree of HPV concordance between mothers/fathers and their newborns for each HPV genotype. The following benchmark scale describes the degree of agreement (HPV concordance): <0.00 Poor, 0.00–0.20 Slight, 0.21–0.40 Fair, 0.41–0.60 Moderate, 0.61–0.80 Substantial, 0.81–1.00 Almost perfect degree of agreement. Moderate or higher degrees of agreement (κ≥0.41) and statistically significant ORs (P<0.05) are shown in bold. Empty cells indicate results that cannot be computed due to the zero frequencies.

Table 9. Association of newborns' LR- and HR-HPV presence *at any anatomic site* with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* among family members from the Finnish Family HPV Study. Modified from Original Publication III.

	Newborns' HPV prevalence at birth							
					Univariable model		**Adjusted model[a]**	
	HPV negative n=221	LR-HPV[b] n=13	HR-HPV[b] n=87		LR-HPV[b]	HR-HPV[b]	LR-HPV[b]	HR-HPV[b]
	n (%)	n (%)	n (%)		OR (95% CI)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)
Mother								
Negative	167 (74.9)	6 (46.2)	48 (54.5)		1.00	1.00	1.00	1.00
Oral	21 (9.4)	3 (23.1)	19 (21.6)		3.98 (0.92–17.1)	**3.15 (1.58–6.33)**	**4.75 (1.05–21.5)**	**3.57 (1.75–7.30)**
Genital	30 (13.5)	2 (15.4)	13 (14.8)		1.86 (0.36–9.63)	1.51 (0.73–3.12)	2.29 (0.43–12.2)	1.55 (0.74–3.27)
Multiple site	3 (1.3)	2 (15.4)	7 (8.0)		**18.6 (2.60–132.5)**	**8.12 (2.02–32.6)**	**13.2 (1.68–103.5)**	**7.52 (1.82–31.0)**
Father								
Negative	51 (22.9)	5 (38.5)	17 (19.3)		1.00	1.00	1.00	1.00
Oral	6 (2.7)	1 (7.7)	8 (9.1)		1.70 (0.17–17.1)	**4.00 (1.21–13.2)**	1.99 (0.19–21.1)	**4.58 (1.34–15.6)**
Genital	8 (3.6)	2 (15.4)	5 (5.7)		2.55 (0.42–15.4)	1.88 (0.54–6.51)	2.37 (0.32–17.3)	1.86 (0.49–6.99)
Semen	9 (4.0)	0	5 (5.7)		…	1.67 (0.49–5.66)	…	1.89 (0.54–6.63)
Multiple site	14 (6.3)	0	3 (3.4)		…	0.64 (0.16–2.51)	…	0.61 (0.15–2.49)

Newborns' (N=321) HPV prevalence at birth are shown with their mothers' and fathers' baseline HPV status *at specific anatomic sites*. Associations of newborns' LR- and HR-HPV presence *at any anatomic site* with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* were calculated by using univariable- and another parent -adjusted multinomial logistic regression analyses. Results are shown by ORs and another-parent aORs with 95% CIs. The reference category for HPV positive was HPV negative. Statistically significant results (P<0.05) are shown in bold.

[a]Mothers' HPV adjusted by fathers' HPV and vice versa

[b]LR-HPV: 6, 11, 42, 43, 44; HR-HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82

Table 10. Association of newborns' *oral* LR- and HR-HPV presence with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* among family members from the Finnish Family HPV Study. Modified from Original Publication III.

	Newborns' oral HPV prevalence at birth			Univariable model		Adjusted model[a]	
	HPV negative n=247	LR-HPV[b] n=12	HR-HPV[b] n=62	LR-HPV[b]	HR-HPV[b]	LR-HPV[b]	HR-HPV[b]
	n (%)	n (%)	n (%)	OR (95% CI)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)
Mother							
Negative	184 (74.5)	5 (41.7)	32 (51.6)	1.00	1.00	1.00	1.00
Oral	23 (9.3)	3 (25.0)	17 (27.4)	**4.80 (1.08–21.4)**	**4.25 (2.05–8.83)**	**7.12 (1.44–35.1)**	**4.87 (2.29–10.4)**
Genital	36 (14.6)	2 (16.7)	7 (11.3)	2.04 (0.38–11.0)	1.12 (0.46–2.73)	2.76 (0.48–15.7)	1.16 (0.47–2.90)
Multiple site	4 (1.6)	2 (16.7)	6 (9.7)	**18.4 (2.71–126)**	**8.63 (2.30–32.3)**	**10.4 (1.30–82.9)**	**8.28 (2.12–32.3)**
Father							
Negative	60 (24.3)	3 (25.0)	10 (16.1)	1.00	1.00	1.00	1.00
Oral	7 (2.8)	1 (8.3)	7 (11.3)	2.86 (0.26–31.3)	**6.00 (1.73–20.8)**	3.89 (0.33–45.9)	**7.72 (2.10–28.3)**
Genital	9 (3.6)	3 (25.0)	3 (4.8)	**6.67 (1.16–38.2)**	2.00 (0.46–8.68)	**7.93 (1.12–56.1)**	2.10 (0.43–10.2)
Semen	12 (4.9)	0	2 (3.2)	…	1.00 (0.19–5.15)	…	1.29 (0.24–6.98)
Multiple site	14 (5.7)	0	3 (4.8)	…	1.29 (0.31–5.29)	…	1.34 (0.30–5.87)
Unknown	145 (58.7)	5 (4.7)	37 (59.7)	0.69 (0.16–2.98)	1.53 (0.72–3.28)	0.72 (0.17–3.29)	1.63 (0.74–3.63)

Newborns' (N=321) *oral* HPV prevalence at birth are shown with their mothers' and fathers' baseline HPV status *at specific anatomic sites*. Associations of newborns' *oral* LR- and HR-HPV presence with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* were calculated by using univariable- and another parent -adjusted multinomial logistic regression analyses. Results are shown by ORs and another-parent aORs with 95% CIs. The reference category for HPV positive was HPV negative. Statistically significant results (P<0.05) are shown in bold.

[a]Mothers' HPV adjusted by fathers' HPV and vice versa

[b]LR-HPV: 6, 11, 42, 43, 44; HR-HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82

Table 11. Association of newborns' *genital* LR- and HR-HPV presence with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* among family members from the Finnish Family HPV Study. Modified from Original Publication III.

	Newborns' genital HPV prevalence at birth			Univariable model		Adjusted model[a]	
	HPV negative n=289	LR-HPV[b] n=3	HR-HPV[b] n=29	LR-HPV[b]	HR-HPV[b]	LR-HPV[b]	HR-HPV[b]
	n (%)	n (%)	n (%)	OR (95% CI)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)
Mother							
Negative	204 (70.6)	0	17 (58.6)	1.00	1.00	1.00	1.00
Oral	41 (14.2)	0	2 (6.9)	…	0.59 (0.13–2.63)	…	0.63 (0.14–2.87)
Genital	34 (11.8)	2 (66.7)	9 (31.0)	…	**3.18 (1.31–7.70)**	…	**3.34 (1.35–8.30)**
Multiple site	10 (3.5)	1 (33.3)	1 (3.4)	…	1.20 (0.14–9.94)	…	1.06 (0.12–9.18)
Father							
Negative	66 (22.8)	0	7 (24.1)	1.00	1.00	1.00	1.00
Oral	13 (4.5)	1 (33.3)	1 (3.4)	…	0.73 (0.08–6.40)	…	0.74 (0.08–6.71)
Genital	12 (4.2)	1 (33.3)	2 (6.9)	…	1.57 (0.29–8.50)	…	1.70 (0.30–9.67)
Semen	11 (3.8)	0	3 (10.3)	…	2.57 (0.58–11.5)	…	2.03 (0.43–9.55)
Multiple site	17 (5.9)	0	0	…	…	…	…
Unknown	170 (58.8)	1 (33.3)	16 (55.2)	…	0.89 (0.35–2.56)	…	0.87 (0.34–2.24)

Newborns' (N=321) *genital* HPV prevalence at birth are shown with their mothers' and fathers' baseline HPV status *at specific anatomic sites*. Associations of newborns' *genital* LR- and HR-HPV presence with their mothers' and fathers' corresponding LR- and HR-HPV presence *at specific anatomic sites* were calculated by using univariable- and another parent -adjusted multinomial logistic regression analyses. Results are show by ORs and another-parent aORs with 95% CIs. The reference category for HPV positive was HPV negative. Statistically significant results (P<0.05) are shown in bold.

[a]Mothers' HPV adjusted by fathers' HPV and vice versa

[b]LR-HPV: 6, 11, 42, 43, 44; HR-HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82

5.4 HLA-G polymorphism among fathers and newborns

5.4.1 HLA-G allele and genotype distribution among fathers (I)

Overall, eight different HLA-G alleles with 15 different HLA-G genotype combinations were identified among fathers. **Figure 11** shows the distribution of HLA-G alleles and genotypes among fathers. The most common HLA-G allele was the wild-type G*01:01:01 (86.2%, n = 112), followed by G*01:01:02 (36.2%, n = 47). The most common genotype found was G*01:01:01/01:01:01 (37.7%, n = 49), followed by G*01:01:01/01:01:02 (23.1%, n = 30), respectively.

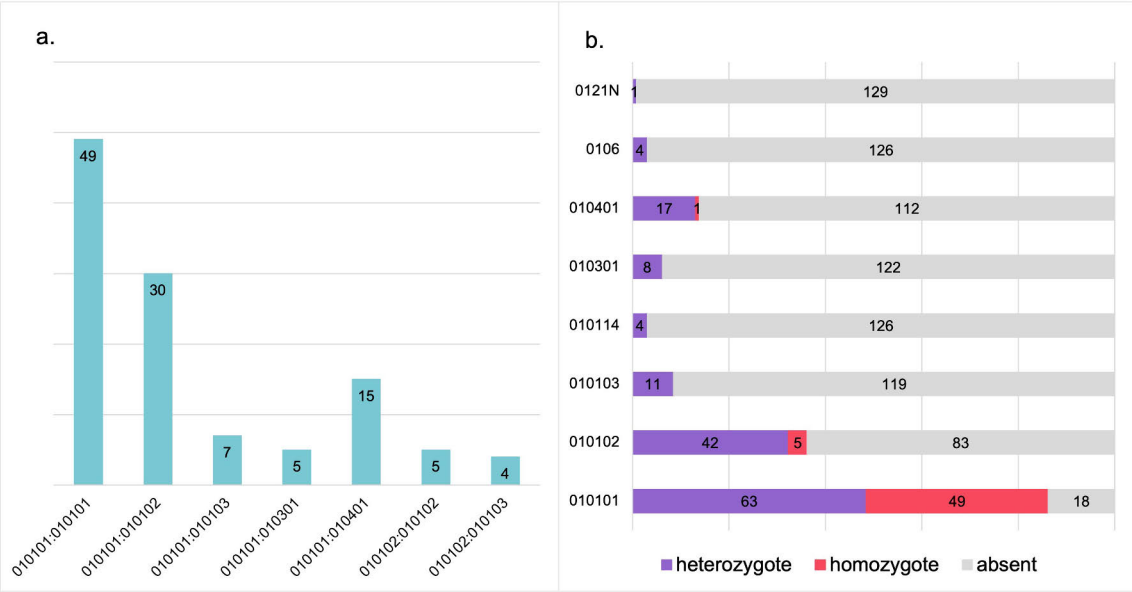

Figure 11. HLA-G genotype (**a**) and allele (**b**) distribution among the 130 fathers from the Finnish Family HPV Study. Those alleles and genotypes that were ≥3% prevalent were included. Different HLA-G genotypes (a) are marked on the x-axis, while the number of fathers with a certain genotype is presented on the y-axis. Stacked bar columns represent the distribution of different alleles (b) (homozygote=having two alleles, heterozygote=having one allele, absent=missing allele).

5.4.2 HLA-G allele and genotype distribution among newborns (II)

The most common HLA-G allele found among newborns was the wild-type G*01:01:01, followed by G*01:01:02; 85.8% (n=115) and 32.8% (n=44) of

newborns had the allele, respectively. The most common HLA-G genotype among the newborns was G*01:01:01/01:01:01, followed by G*01:01:01/01:01:02; 36.6% (n=49) and 21.6% (n=29), had the HLA-G genotype, respectively.

5.4.3 HLA-G allele and genotype sharing among father-newborn pairs (II)

Figure 12 shows allele sharing (Figure 12a) and genotype concordance (Figure 12b) among 134 father-newborn pairs. The most shared allele was the G*01:01:01, for which 73.9% (n=99) of pairs shared at least two common alleles, (i.e., both were heterozygous/homozygous for the allele), followed by other alleles that were shared between 2.2% (n=3) and 22.4% (n=30) among the father-newborn pairs. The most commonly concordant HLA-G genotype between the father-newborn pairs was the G*01:01:01/01:01:01; 25.4% (n=34). Overall, 37.3% (n=50) of the father-newborn pairs had any concordant HLA-G genotype.

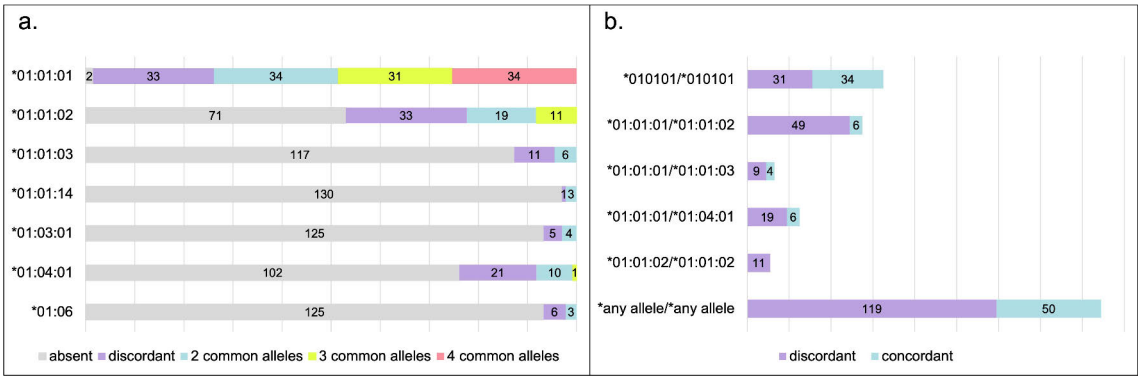

Figure 12. HLA-G (**a**) allele and (**b**) genotype concordance among the 134 father-newborn pairs from the Finnish Family HPV Study. Stacked bar columns show the HLA-G (a) allele sharing (absent = both missing the allele; discordant = one homozygous or heterozygous for the allele and the other absent; 2 common alleles = both heterozygous for the allele; 3 common alleles= one heterozygous for the allele and other homozygous for the allele; 4 common alleles=both homozygous for the allele) and (b) genotype concordance (discordance = one having the genotype and the other missing the genotype; concordance = both having the same genotype) between the 134 father-newborn pairs from the Finnish Family HPV Study. Those HLA-G alleles and genotypes that were ≥3% prevalent among the father-newborn pairs were included.

5.5 The role of HLA-G polymorphism in father's HPV infection (I)

5.5.1 HLA-G polymorphism and HPV positivity in fathers

To evaluate the influence of fathers' HLA-G alleles and genotypes on HPV prevalence, the association of different HLA-G alleles/genotypes with the frequency of any-, LR-, and HR-HPV positivity in all three baseline-tested anatomical samples (semen, genital=urethral, oral) were determined, as seen in **Table 12.** Allele G*01:01:02 was shown to be protective for any oral HPV infection with an OR of 0.20 (95% CI 0.06–0.72), and for oral HR-HPV infection as well, with an OR of 0.24 (95% CI 0.07–0.85). Allele G*01:01:03 was associated with an increased risk for genital (urethral) HR-HPV infection (OR 4.94, 95% CI 1.34–18.27). Regarding the HLA-G genotypes, no significant associations were found between HLA-G genotypes and HPV positivity at the evaluated anatomic sites.

5.5.2 HLA-G polymorphism and oral HPV infection outcomes in fathers

The associations of HLA-G alleles and genotypes with oral HPV infection outcomes (always HPV negative, incident HPV, HPV clearance, and HPV persistence) are shown in **Table 13**. Allele G*01:01:01 showed to be protective for an incident oral HPV infection (OR 0.30, 95% CI 0.11–0.84). Interestingly, allele G*01:01:01 was also associated with a lower risk of persistent oral infection (OR 0.24, 95% CI 0.08–0.69). No significant associations between any HLA-G genotypes and the oral HPV outcomes were observed.

5.5.3 Association of HLA-G polymorphism with demographic data of fathers

The association of the fathers' self-reported demographic data with fathers' HLA-G alleles and genotypes are given in **Table 14**. A total of 33.9% (40/118) of fathers reported allergy to pollen and/or animals. Allele G*01:03:01 was significantly associated with an increased risk of allergy (OR 13.59, 95% CI 1.57–117.25). Food allergies were excluded from this variable. 6.3% (7/112) of fathers reported a history of oral warts, for which HLA-G genotype G*01:01:01/01:01:03 appeared to be a significant risk factor (OR 8.00, 95% CI 1.23–51.89).

Table 12. Associations of different HLA-G alleles and genotypes with the semen-, genital- (urethral) and oral HPV positivity among 128 fathers from the Finnish Family HPV Study at baseline. Modified from Original Publication I.

	semen OR (95 % CI)			**genital (urethral) OR (95 % CI)**			**oral OR (95 % CI)**		
HLA-G	any HPV+ (n=27)	LR (n=9)	HR (n=23)	any HPV+ (n=27)	LR (n=9)	HR (n=20)	any HPV+ (n=24)	LR (n=4)	HR (n=21)
Alleles[a,b]									
***010101**	1.83 (0.47–7.20)	0.80 (0.15–4.40)	2.41 (0.49–11.82)	0.70 (0.22–2.17)	1.27 (0.15–10.99)	0.63 (0.18–2.20)	4.49 (0.57–35.56)	NA	3.91 (0.49–31.11)
***010102**	0.73 (0.28–1.89)	2.92 (0.66–12.81)	0.51 (0.18–1.49)	1.06 (0.43–2.56)	0.90 (0.21–3.82)	1.20 (0.45–3.21)	**0.20 (0.06–0.72)**	NA	**0.24 (0.07–0.85)**
***010103**	0.25 (0.03–2.07)	0.80 (0.09–7.25)	0.29 (0.03–2.46)	3.37 (0.94–12.07)	NA	**4.94 (1.34–18.27)**	1.71 (0.42–7.01)	NA	2.00 (0.48–8.27)
***010114**	1.10 (0.10–12.64)	NA	1.30 (0.11–15.02)	3.72 (0.50–27.74)	5.81 (0.47–71.29)	2.45 (0.21–28.38)	NA	NA	NA
***010301**	NA	NA	NA	0.48 (0.057–4.11)	NA	0.66 (0.077–5.70)	NA	NA	NA
***010401**	3.13 (0.77–12.73)	NA	3.82 (0.92–15.78)	0.46 (0.098–2.18)	0.72 (0.08–6.24)	0.30 (0.038–2.46)	2.56 (0.85–7.70)	2.56 (0.25–26.58)	2.40 (0.74–7.72)
***0106**	2.28 (0.30–17.11)	3.56 (0.29–43.92)	2.71 (0.36–20.52)	1.18 (0.12–11.82)	3.83 (0.36–41.24)	NA	1.46 (0.15–14.72)	11.22 (0.89–141.98)	NA
Genotypes[b]									
010101/010101	0.99 (0.39–2.54)	0.48 (0.09–2.52)	1.08 (0.40–2.91)	0.78 (0.32–1.93)	0.78 (0.18–3.33)	0.84 (0.31–2.31)	1.89 (0.77–4.63)	1.89 (0.26–13.97)	2.08 (0.81–5.36)
010101/010102	1.24 (0.43–3.57)	2.83 (0.66–12.09)	0.98 (0.31–3.16)	1.01 (0.36–2.82)	1.01 (0.19–5.21)	1.17 (0.38–3.61)	0.26 (0.06–1.18)	NA	0.30 (0.07–1.37)
010101/010103	NA	NA	NA	2.84 (0.60–13.57)	NA	4.01 (0.82–19.57)	3.57 (0.74–17.15)	NA	4.17 (0.86–20.21)
010101/010401	3.25 (0.67–15.66)	NA	3.93 (0.81–19.17)	0.27 (0.03–2.14)	0.86 (0.10–7.54)	NA	2.47 (0.76–8.06)	3.13 (0.30–33.03)	2.21 (0.62–7.87)
010102/010102	NA	NA	NA	0.88 (0.09–8.17)	2.84 (0.28–28.58)	NA	NA	NA	NA
010102/010103	0.72 (0.07–7.24)	2.33 (0.22–25.24)	0.85 (0.08–8.60)	3.72 (0.50–27.74)	NA	5.17 (0.68–39.10)	NA	NA	NA
010101/010301	NA	NA	NA	0.88 (0.09–8.17)	NA	1.20 (0.13–11.32)	NA	NA	NA

[a] Being homozygote or heterozygote for the HLA-G allele.

[b] Only those alleles and genotypes that were ≥3 % prevalent among fathers were included in the analyses.

Table 13. Associations of HLA-G alleles and genotypes with oral HPV infection outcomes among 130 fathers from the Finnish Family HPV Study. Modified from Original Publication I.

HLA-G	always HPV- OR (95 % CI)	incidence OR (95 % CI)	clearance OR (95 % CI)	persistence OR (95 % CI)
Alleles[a,b]				
***010101**	1.50 (0.53–4.28)	**0.30 (0.11–0.84)**	1.38 (0.36–5.20)	**0.24 (0.08–0.69)**
***010102**	1.07 (0.52–2.20)	1.77 (0.85–3.71)	0.54 (0.19–1.57)	2.06 (0.86–4.92)
***010103**	1.78 (0.51–6.15)	0.37 (0.08–1.77)	0.55 (0.09–3.57)	1.57 (0.38–6.36)
***010114**	NA	NA	1.16 (0.11–11.89)	NA
***010301**	2.48 (0.57–10.87)	1.06 (0.24–4.66)	NA	1.36 (0.26–7.17)
***010401**	1.15 (0.42–3.13)	0.46 (0.14–1.48)	3.98 (0.47–33.64)	0.77 (0.21–2.90)
***0106**	NA	5.59 (0.56–55.35)	0.36 (0.05–2.75)	4.25 (0.57–31.71)
Genotypes[b]				
010101/010101	0.83 (0.40–1.72)	0.78 (0.37–1.65)	1.26 (0.43–3.68)	0.54 (0.21–1.41)
010101/010102	1.31 (0.58–2.98)	1.03 (0.44–2.40)	0.63 (0.18–2.17)	1.00 (0.36–2.77)
010101/010103	1.95 (0.42–9.08)	NA	0.76 (0.07–8.88)	1.65 (0.30–9.03)
010101/010401	1.27 (0.43–3.73)	0.40 (0.11–1.51)	2.96 (0.34–25.70)	0.26 (0.03–2.05)
010102/010102	0.94 (0.15–5.80)	2.76 (0.44–17.15)	0.76 (0.07–8.88)	2.81 (0.44–17.73)
010102/010103	1.42 (0.19–10.43)	1.80 (0.25–13.21)	0.37 (0.02–6.26)	1.35 (0.13–13.50)
010101/010301	6.00 (0.65–55.26)	0.43 (0.05–3.96)	NA	NA

[a] Being homozygote or heterozygote for the HLA-G allele.

[b] Only those alleles and genotypes that were ≥3 % prevalent among fathers were included in the analyses.

Table 14. Association of HLA-G alleles and genotypes with the demographic data of 130 fathers collected via the questionnaire at baseline. Modified from Original Publication I.

				HLA-G allele[a,b]			
	***010101**	***010102**	***010103**	***010114**	***010301**	***010401**	***0106**
infertility	0.51 (0.10–2.68)	2.50 (0.63–9.87)	3.68 (0.65–20.71)	4.50 (0.42–48.35)	6.06 (0.99–36.99)	NA	NA
history of chlamydia infection	1.38 (0.30–6.28)	0.74 (0.21–2.57)	0.58 (0.06–6.06)	NA	0.90 (0.08–10.77)	9.33 (0.94–92.47)	0.90 (0.08–10.77)
history of genital warts	0.50 (0.14–1.77)	1.55 (0.57–4.25)	1.26 (0.25–6.48)	5.41 (0.71–41.09)	0.81 (0.09–7.19)	1.07 (0.28–4.16)	1.69 (0.17–17.13)
history of oral warts	0.35 (0.06–2.01)	2.78 (0.59–13.14)	4.85 (0.81–29.09)	NA	2.75 (0.28–26.66)	NA	5.67 (0.51–62.98)
history of skin warts	2.64 (0.79–8.86)	1.08 (0.50–2.35)	1.85 (0.49–6.95)	0.38 (0.04–3.75)	0.45 (0.08–2.41)	0.79 (0.28–2.25)	NA
history of mumps	0.59 (0.17–2.01)	1.35 (0.60–3.05)	1.07 (0.24–4.71)	0.63 (0.09–4.62)	0.45 (0.10–2.14)	0.79 (0.27–2.31)	NA
asthma	NA	NA	3.93 (0.37–41.72)	NA	NA	NA	NA
allergy	0.39 (0.13–1.18)	1.27 (0.57–2.82)	2.09 (0.57–7.68)	0.64 (0.06–6.37)	**13.59 (1.57–117.25)**	0.37 (0.10–1.38)	NA
atopy	0.40 (0.07–2.20)	0.61 (0.12–3.15)	4.25 (0.74–24.57)	5.10 (0.47–55.54)	NA	2.11 (0.39–11.45)	NA
	HLA–G genotypes[b]						
	010101/010101	**010101/010102**	**010101/010103**	**010101/010401**	**010102/010102**	**010102/010103**	**010101/010301**
infertility	0.19 (0.02–1.58)	1.81 (0.42–7.79)	6.06 (0.99–36.99)	NA	NA	NA	4.50 (0.42–48.35)
history of chlamydia infection	1.58 (0.43–5.77)	0.73 (0.16–3.30)	0.90 (0.08–10.77)	NA	NA	NA	NA
history of genital warts	0.55 (0.18–1.66)	0.99 (0.29–3.30)	2.09 (0.38–11.69)	0.73 (0.15–3.55)	1.69 (0.17–17.13)	NA	NA
history of oral warts	0.26 (0.03–2.24)	1.51 (0.27–8.31)	**8.00 (1.23–51.89)**	NA	NA	NA	NA
history of skin warts	1.29 (0.61–2.74)	1.87 (0.75–4.64)	0.87 (0.19–4.07)	1.03 (0.35–3.05)	0.38 (0.04–3.75)	NA	1.18 (0.16–8.65)
history of mumps	0.93 (0.43–2.01)	1.17 (0.47–2.95)	1.29 (0.23–7.37)	0.60 (0.19–1.84)	1.95 (0.20–19.41)	0.63 (0.04–10.38)	0.63 (0.09–4.62)
asthma	5.21 (0.53–51.74)	NA	6.06 (0.55–67.27)	NA	NA	NA	NA
allergy	0.96 (0.44–2.11)	0.83 (0.33–2.13)	1.50 (0.32–7.05)	0.26 (0.06–1.23)	2.00 (0.27–14.75)	4.05 (0.36–46.11)	6.24 (0.63–62.08)
atopy	0.54 (0.10–2.80)	0.46 (0.05–3.93)	2.48 (0.26–23.52)	0.98 (0.11–8.57)	NA	7.71 (0.62–95.80)	NA

[a] Being homozygote or heterozygote for the HLA-G allele.

[b] Only those alleles and genotypes that were ≥3 % prevalent among fathers were included in the analyses.

5.6 The role of HLA-G allele/genotype sharing in HPV concordance between father-newborn pairs (II)

5.6.1 Association of HLA-G allele and genotype sharing with HPV concordance among father-newborn pairs

Table 15 shows the associations of the father-newborn HLA-G discordance (only the father or the newborn had the allele, 1 shared allele) and concordance (both tested positive for a particular allele, ≥2 shared alleles) with the father-newborn HPV concordances; HPV concordances were determined between the father's three anatomic (semen, genital, and oral) HPV status at baseline and the newborn's oral HPV status at birth and postpartum. The father-newborn pairs with both parties missing the allele served as a reference.

The HLA-G allele G*01:01:03 *concordance* was associated with the father's genital (urethral) and newborn's oral HR-HPV concordance at birth (OR 17.00, 95% CI 1.24–232.22). Controversially, during the postpartum period, the G*01:01:03 *discordance* was associated with the father's genital (urethral) and newborn's oral HR-HPV concordance (OR 6.67, 95% CI 1.08–40.97). The HLA-G allele G*01:04:01 concordance increased the father's oral and newborn's postpartum oral any- and HR-HPV concordance, with OR 7.50 (95% CI:1.47–38.16) and OR 7.78 (95% CI:1.38–43.85), respectively. In this case, the HPV type-specific concordance was further evaluated. HPV type-specific concordance was seen in two father-newborn pairs with HR-types HPV33 and HPV70 (50% of concordant pairs had HPV genotype-specific concordance, data not shown). Further evaluations showed that if the mother's oral HPV status was also considered, the adjusted ORs for the father-newborn G*01:04:01 concordance and the father's oral and newborn's postpartum oral any- and HR-HPV concordance remained statistically significant; adjusted ORs 11.50 (95% CI 1.77–74.87) and 9.86 (95% CI 1.49–65.12), respectively (data not shown).

When the impact of the father-newborn HLA-G genotype concordance on the father-newborn HPV concordance was evaluated, no association was observed between HLA-G genotype concordance and HPV concordance among the father-newborn pairs at birth nor the postpartum period.

5.6.2 Association of HLA-G allele and genotype sharing with HPV prevalence among father-newborn pairs

The association between father-newborn HLA-G allele sharing and common HPV prevalence among fathers and/or newborns is shown in **Table 16.** The HLA-G allele

G*01:01:03 discordance increased the risk of fathers' genital (urethral) and/or newborn's oral any- and HR-HPV prevalence at birth with OR 5.47 (95% CI 1.11–26.89) and 6.76 (95% CI 1.37–33.35), whereas G*01:01:03 concordance increased the risk of fathers' oral and/or newborn's oral HR-HPV prevalence at birth with OR 8.81 (95% CI 1.00–77.94). The prevalence in fathers' baseline samples and newborns' oral samples taken at birth were analysed separately as well; G*01:01:03 allele discordance seemed to increase solely the father's genital (urethral) any- and HR-HPV positivity but not the newborn's oral HPV positivity at birth (data not shown). The G*01:01:03 allele concordance lost its statistical significance when fathers' oral HR-HPV prevalence and newborns' oral HR-HPV prevalence at birth were explored separately; thus, it did not show an impact on one or the other's oral HR-HPV prevalence alone (data not shown).

G*01:06 allele concordance associated with the LR-HPV prevalence in fathers' semen/genital and/or newborn's oral sites at birth; OR ranging between 12.00 (95% CI 1.03–140.19) and 16.15 (95% CI 1.37–190.72). When exploring this association with the fathers' and newborn's prevalence alone, G*01:06 concordance did not associate with the father's semen alone nor the newborn's oral LR-HPV positivity at birth. However, G*01:06 concordance increased the father's genital LR-HPV positivity, but not the newborn's oral LR-HPV positivity at birth, when the prevalence among fathers and newborns were analysed separately (data not shown). No association was seen between the father-newborn HLA-G allele concordance/discordance and the fathers' and/or newborns' postpartum HPV prevalence.

When the association of the father-newborn HLA-G genotype concordance (in both high- and low-resolution groups) and HPV prevalence among the fathers and/or newborns were analysed, no significant association was found in the high-resolution group, whereas in the low-resolution group, G*01:01/01:04 concordance associated with a greater HR-HPV prevalence in fathers' semen and/or newborn's oral sites at the postpartum period, OR 7.00 (95% CI 1.14–42.97). Similarly, G*01:01/01:04 concordance increased the fathers' any genital and/or newborns' postpartum any oral HPV prevalence with OR 6.50 (95% CI 1.05–40.13).

Table 15. Association of HLA-G allele sharing with HPV concordance among 134 father-newborn pairs from the Finnish Family HPV Study. The father-newborn pairs with both parties being negative for these specific alleles served as a reference in high-resolution HLA-G allele groups. In low-resolution HLA-G allele groups, discordant father-newborn pairs served as a reference. Modified from Original Publication II.

		Newborn's oral HPV[b] OR (95 % CI)					
		Father semen[b]		Father genital[b]		Father oral[b]	
HLA-G allele concordance[a]	allele sharing	any	HR	any	HR	any	HR
At birth							
01:01:02	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	0.27 (0.03–2.53)	NA	0.39 (0.07–2.15)	0.32 (0.03–3.14)	1.18 (0.28–4.96)	1.04 (0.21–5.20)
	≥2	0.36 (0.04–3.52)	0.43 (0.04–4.26)	1.18 (0.24–5.70)	0.59 (0.06–6.18)	NA	NA
01:01:03	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	2.22 (0.20–25.00)	NA	0.60 (0.07–5.54)	NA	NA	NA
	≥2	NA	NA	8.44 (0.69–103.70)	**17.00 (1.24–232.22)**	1.08 (0.11–10.89)	1.18 (0.11–12.21)
01:04:01	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	2.67 (0.23–30.80)	8.50 (0.44–163.89)	0.46 (0.05–4.15)	1.06 (0.10–10.84)	0.52 (0.06–4.74)	0.56 (0.06–5.39)
	≥2	2.67 (0.23–30.80)	2.83 (0.24–34.14)	3.70 (0.21–64.51)	NA	0.83 (0.08–8.08)	1.13 (0.11–11.95)
01:06	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	NA	NA	NA	NA	NA	NA
	≥2	3.58 (0.28–45.80)	3.50 (0.27–46.05)	3.91 (0.23–67.57)	NA	4.89 (0.28–85.63)	NA
Low-resolution							
01:01+	1	1.00	1.00	1.00	1.00	1.00	1.00
	2	NA	NA	0.33 (0.01–11.94)	NA	NA	NA
	3	NA	NA	0.18 (0.01–4.26)	0.10 (0.003–3.15)	NA	NA
	4	NA	NA	0.26 (0.01–4.59)	0.14 (0.01–2.67)	NA	NA
Postpartum							
01:01:02	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	0.83 (0.20–3.54)	1.60 (0.33–7.65)	1.55 (0.40–5.99)	2.50 (0.56–11.21)	1.63 (0.41–6.51)	1.92 (0.46–7.95)
	≥2	0.15 (0.02–1.39)	0.32 (0.03–3.15)	0.58 (0.10–3.23)	NA	0.40 (0.04–3.79)	0.51 (0.05–5.00)
01:01:03	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	3.90 (0.49–31.20)	3.88 (0.47–31.91)	4.90 (0.86–27.88)	**6.67 (1.08–40.97)**	NA	NA
	≥2	NA	NA	NA	NA	NA	NA
01:04:01	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	1.54 (0.14–16.80)	NA	2.25 (0.36–14.03)	3.08 (0.46–20.70)	1.25 (0.13–12.24)	0.97 (0.10–9.57)

		Newborn's oral HPV[b] OR (95 % CI)					
		Father semen[b]		Father genital[b]		Father oral[b]	
HLA-G allele concordance[a]	allele sharing	any	HR	any	HR	any	HR
	≥2	6.94 (0.99–48.55)	6.43 (0.90–46.06)	0.75 (0.08–6.94)	1.23 (0.12–12.47)	**7.50 (1.47–38.16)**	**7.78 (1.38–43.85)**
01:06	0	1.00	1.00	1.00	1.00	1.00	1.00
	1	NA	NA	4.91 (0.28–84.58)	NA	NA	NA
	≥2	3.55 (0.20–61.38)	3.56 (0.20–62.63)	NA	NA	NA	NA
Low-resolution							
01:01+	1	1.00	1.00	1.00	1.00	1.00	1.00
	2	NA	NA	0.13 (0.004–4.00)	NA	NA	NA
	3	NA	NA	0.57 (0.03–11.85)	0.33 (0.01–8.18)	NA	NA
	4	NA	NA	0.18 (0.01–3.22)	0.18 (0.01–3.32)	NA	NA

[a]Only those alleles that were ≥3 % prevalent were included in the analyses.

[b]Father's semen, genital or oral HPV at baseline and newborn's oral HPV at birth and postpartum.

0 = no shared alleles; 1 = discordant for the allele; ≥2 = at least 2 shared alleles, i.e., both heterozygous or homozygous for the allele; 2 = 2 shared alleles, 3 = 3 shared alleles, 4 = 4 shared alleles

LR-HPV groups as well as HLA-G alleles 01:01:01, 01:01:14 and 01:03:01 were taken out due to the small number of cases.

NA=not applicable.

Table 16. Association of HLA-G allele sharing with HPV prevalence at birth among 134 father-newborn pairs from the Finnish Family HPV Study. The father-newborn pairs with both parties being negative for these specific alleles served as a reference in high-resolution HLA-G allele groups. In low-resolution HLA-G allele groups, discordant father-newborn pairs served as a reference. Modified from Original Publication II.

		HPV: father semen/newborn oral[b] OR (95 % CI)		
HLA-G allele concordance[a]	allele sharing	any	LR	HR
***01:01:01**	0	1.00	1.00	1.00
	1	0.60 (0.03–10.51)	NA	0.45 (0.03–8.02)
	≥2	1.06 (0.06–17.47)	NA	0.81 (0.05–13.40)
***01:01:02**	0	1.00	1.00	1.00
	1	1.46 (0.64–3.35)	1.32 (0.39–4.44)	1.41 (0.61–3.25)
	≥2	1.14 (0.48–2.70)	1.56 (0.46–5.30)	0.92 (0.38–2.23)
***01:01:03**	0	1.00	1.00	1.00
	1	1.09 (0.30–3.96)	3.03 (0.68–13.45)	1.47 (0.40–5.35)
	≥2	1.09 (0.21–5.62)	NA	1.47 (0.28–7.59)
***01:01:14**	0	1.00	1.00	1.00
	1	NA	NA	NA
	≥2	0.54 (0.05–6.11)	2.94 (0.25–34.25)	NA
***01:03:01**	0	1.00	1.00	1.00
	1	0.69 (0.11–4.26)	NA	0.91 (0.15–5.64)
	≥2	0.34 (0.03–3.40)	NA	0.46 (0.05–4.50)
***01:04:01**	0	1.00	1.00	1.00
	1	0.60 (0.23–1.58)	0.76 (0.16–3.70)	0.51 (0.18–1.42)
	≥2	0.82 (0.23–2.85)	0.59 (0.07–5.03)	1.06 (0.30–3.70)
***01:06**	0	1.00	1.00	1.00
	1	0.21 (0.02–1.88)	NA	0.28 (0.03–2.49)
	≥2	NA	**12.00 (1.03–140.19)**	NA
Low- resolution				
***01:01+**	1	1.00	1.00	1.00
	2	3.43 (0.29–40.95)	NA	3.43 (0.29–40.95)
	3	2.29 (0.21–24.68)	NA	1.50 (0.14–16.32)
	4	3.00 (0.30–30.02)	NA	2.30 (0.23–23.02)
		HPV: father genital/newborn oral[b] OR (95 % CI)		
HLA-G allele concordance[a]	allele sharing	any	LR	HR
***01:01:01**	0	1.00	1.00	1.00
	1	0.68 (0.04–11.95)	NA	0.63 (0.04–11.08)
	≥2	0.88 (0.05–14.55)	NA	0.71 (0.04–11.76)
***01:01:02**	0	1.00	1.00	1.00
	1	1.66 (0.72–3.85)	0.81 (0.23–2.83)	1.87 (0.80–4.38)
	≥2	0.79 (0.33–1.91)	0.44 (0.09–2.14)	0.92 (0.37–2.29)
***01:01:03**	0	1.00	1.00	1.00
	1	**5.47 (1.11–26.89)**	0.73 (0.09–6.15)	**6.76 (1.37–33.35)**
	≥2	1.37 (0.26–7.06)	NA	1.69 (0.33–8.76)

***01:01:14**	0	1.00	1.00	1.00
	1	NA	NA	NA
	≥2	2.41 (0.21–27.30)	3.60 (0.31–42.16)	1.42 (0.09–23.27)
***01:03:01**	0	1.00	1.00	1.00
	1	0.80 (0.13–4.94)	NA	0.97 (0.16–6.00)
	≥2	1.20 (0.16–8.77)	NA	1.45 (0.20–10.64)
***01:04:01**	0	1.00	1.00	1.00
	1	0.90 (0.34–2.37)	0.34 (0.04–2.76)	1.09 (0.41–2.87)
	≥2	0.41 (0.10–1.65)	1.62 (0.31–8.46)	0.30 (0.06–1.44)
***01:06**	0	1.00	1.00	1.00
	1	0.60 (0.11–3.39)	1.62 (0.17–14.92)	0.28 (0.03–2.47)
	≥2	2.39 (0.21–27.09)	**16.15 (1.37–190.72)**	2.80 (0.25–31.73)
Low- resolution				
***01:01+**	1	1.00	1.00	1.00
	2	0.50 (0.05–4.67)	NA	0.36 (0.04–3.52)
	3	0.81 (0.10–6.58)	NA	0.71 (0.09–5.73)
	4	0.91 (0.12–6.76)	NA	0.76 (0.10–5.67)

		HPV: father oral/newborn oral[b] OR (95 % CI)		
HLA-G allele concordance[a]	allele sharing	any	LR	HR
***01:01:01**	0	1.00	1.00	1.00
	1	0.68 (0.04–11.95)	NA	0.60 (0.03–10.51)
	≥2	0.75 (0.05–12.34)	NA	0.58 (0.04–9.57)
***01:01:02**	0	1.00	1.00	1.00
	1	1.64 (0.71–3.80)	1.34 (0.36–4.98)	1.62 (0.70–3.79)
	≥2	0.76 (0.31–1.88)	0.31 (0.04–2.65)	0.83 (0.33–2.09)
***01:01:03**	0	1.00	1.00	1.00
	1	0.61 (0.15–2.47)	1.02 (0.12–8.83)	0.44 (0.09–2.17)
	≥2	7.08 (0.80–62.57)	NA	**8.81 (1.00–77.94)**
***01:01:14**	0	1.00	1.00	1.00
	1	NA	NA	NA
	≥2	0.66 (0.06–7.51)	5.09 (0.43–60.73)	NA
***01:03:01**	0	1.00	1.00	1.00
	1	0.88 (0.14–5.46)	NA	1.12 (0.18–6.93)
	≥2	0.44 (0.04–4.35)	NA	0.56 (0.06–5.52)
***01:04:01**	0	1.00	1.00	1.00
	1	1.20 (0.46–3.15)	NA	1.54 (0.58–4.08)
	≥2	1.76 (0.50–6.14)	1.93 (0.37–10.23)	1.57 (0.45–5.52)
***01:06**	0	1.00	1.00	1.00
	1	0.26 (0.03–2.33)	NA	0.33 (0.04–2.96)
	≥2	2.64 (0.23–29.91)	4.86 (0.41–58.04)	3.35 (0.30–37.95)
Low- resolution				
***01:01+**	1	1.00	1.00	1.00
	2	3.43 (0.29–40.95)	NA	2.63 (0.22–31.35)
	3	2.44 (0.23–26.30)	NA	2.44 (0.23–26.30)
	4	2.04 (0.20–20.44)	NA	1.50 (0.15–15.08)

6 Discussion

6.1 HPV prevalence and concordance between newborns and parents (III)

6.1.1 HPV prevalence among newborns

In the literature, HPV prevalence in newborns varies highly, depending on the sample size, anatomic site of the sampling, timing of the sampling, and sample technique. Moreover, the observed HPV prevalence depends on the study design. For example, the inclusion criteria of mothers might influence the newborns' HPV prevalence; in some vertical transmission studies, only HPV-positive mothers have been included, while in other studies, mothers have been included regardless of their HPV status. A meta-analysis of 20 vertical transmission studies with 3,128 mother-newborn pairs reported an HPV prevalence of 7.6% among newborns' nasopharyngeal aspirate, cord blood, genital or oral samples taken within the first week after birth (Merckx et al., 2013). However, the heterogeneity between studies was high, as the HPV prevalence varied from 0% to 61%.

In the present study, 31.2% of newborns' samples taken at birth at any anatomic sites (HPV-positive in at least one anatomic site) were tested HPV-positive. This prevalence is higher than the reported HPV prevalence in newborns in most of the studies (Merckx et al., 2013); as discussed above, the studies differ greatly in their designs, and no completely comparable studies exist to compare our results of newborns' HPV prevalence. As an example, Rombaldi et al. reported an HPV prevalence of 22.4% (11/49) among newborns born to Brazilian mothers with a high probability for HPV infection, while Hong et al. found a prevalence of 23.5% (55/233) among newborns born to HPV-positive Chinese mothers (Y. Hong et al., 2013; Rombaldi et al., 2008). In both latter studies, the newborns' HPV prevalence was based on at least two different anatomic sample sites, as was in our study. However, the selection criteria of mothers differed from ours as in our study, pregnant women were recruited regardless of their HPV history. The differences in HPV prevalence can also be explained by the sensitivity of the methods used for HPV testing. In our study, nested PCR was used, followed by HPV genotyping, while in the study by Rombaldi et al., the PCR-positive sample was further amplified

by the genotype-specific PCR. In the study by Hong et al., HPV DNA was first amplified by PCR, followed by HPV genotyping using an HPV DNA Chip. In contrast, a recent study by Khayargoli et al. found an even lower HPV prevalence of 6.0% (22/368) based on the conjunctival/oral/pharyngeal/genital samples taken at birth among newborns born to HPV-positive mothers (self-collected vaginal sample) (Khayargoli et al., 2023). In that study, newborns' samples were collected with a Dacron swab, and HPV genotyping was performed by using the Linear Array genotyping assay, detecting 36 different genotypes. In our study, HPV genotypes were detected using the bead-based Multiplex Genotyping Assay, which has been shown to have a higher analytical sensitivity than Linear Array, which might partly explain the higher prevalence observed in our study (Comar et al., 2012).

In the previous vertical transmission studies, specifically the *oral* HPV prevalence in newborns at the age of 0-4 days have shown to vary from 0.9% to 39.7% (Hahn et al., 2013; Khayargoli et al., 2023; Park et al., 2012; E. M. Smith et al., 2004, 2010; Tseng et al., 1998). The literature on HPV prevalence, particularly in newborns' *genital* site, is sparser; among 0-4 days old newborns, the genital prevalence is reported to vary from 0.6% to 30.9% (Khayargoli et al., 2023; E. M. Smith et al., 2004, 2010; Tseng et al., 1998). In the present study, 23.0% (74/321) of oral samples and 10.0% (32/321) of genital samples taken at birth were tested positive for HPV, which is comparable to the observed range of other studies.

In our study, HPV16 was the most common HPV type detected in all sites of the newborns, as well as the mothers and fathers, which is consistent with other papers reported (Castellsagué et al., 2009; Hahn et al., 2013; Y. Hong et al., 2013; Park et al., 2012; E. M. Smith et al., 2010). The number of different genotypes identified among the cohort of this study appear to be realistic, as genotyping was performed with the Multimetrix® assay, which covers a broad range of different LR- and HR-types.

6.1.2 HPV concordance

We discovered that there is a genotype-specific HPV concordance between newborns and their mother and/or father with five different HPV genotypes (HPV6, HPV16, HPV18, HPV31, HPV56). This finding indicates the likelihood of a vertical HPV transmission between the parents and their newborn offspring. Furthermore, the parents' oral HR-HPV presence seems to impact the newborns' oral HR-HPV infection risk more than the parents' genital HR-HPV presence. On the other hand, the newborns' genital HR-HPV risk was only predicted by the mother's HR-HPV presence prior to the delivery.

To date, the studies have focused to establish a vertical transmission solely from mother to the child; few meta-analyses have been published on the mother-to-child

HPV transmission (Chatzistamatiou et al., 2016; Medeiros et al., 2005; Merckx et al., 2013; Zouridis et al., 2018), and the transmission rate is often used to indicate the likelihood of a vertical transmission. A meta-analysis including 2,111 mothers and 2,113 newborns estimated the vertical HPV transmission rate from mothers (genital sample) to newborns (oral/genital sample) to be 6.5%, with a wide range from 1.5% to 46.6% (Medeiros et al., 2005). Unfortunately, the vertical transmission rate was not based on the genotype-specific concordance data in this meta-analysis.

The data on vertical transmission according to the *genotype-specific* HPV concordance relies on relatively small studies (Bandyopadhyay et al., 2003; Castellsagué et al., 2009; Hahn et al., 2013; Y. Hong et al., 2013; Khayargoli et al., 2023; Park et al., 2012; Puranen et al., 1997; Rombaldi et al., 2008, 2009; E. M. Smith et al., 2004, 2010; Tenti et al., 1999). Two meta-analyses have collected data on vertical HPV transmission based on the genotype-specific HPV concordance. One meta-analysis, including eight clinical non-randomised studies with 446 HPV-positive mothers and their newborns, estimated the vertical transmission rate of 24.6% with significant heterogeneity between studies (Chatzistamatiou et al., 2016). Interestingly, another meta-analysis evaluated specifically the vertical *intrauterine* transmission by including only studies that reported data regarding the HPV status of 1) the mother, her uterus (placenta, amniotic fluid, or membrane samples), and the newborn, or 2) the mother and cord blood samples. The rate of intrauterine transmission was estimated to be 4.9% but again, the rate of transmission in selected studies varied highly between 0% and 46.7% (Zouridis et al., 2018).

We observed vertical transmission rates of 37.0% (37/100) and 35.1% (33/94) from the mothers' *any anatomic site* to the newborns' *any anatomic site*, and from the mothers' *genital site* to the newborns' *any anatomic site*, respectively. A Chinese study by Hong et al., including 233 HPV-positive mothers and their newborns, observed a vertical transmission rate of 11.2% (26/233), which is lower than the rate we reported (Y. Hong et al., 2013). In their study, the vertical transmission rate was based on the genotype-specific HPV concordance between the mother's cervical sample and the newborn's oral/genital sample, which is comparable to our study, but instead, the HPV DNA Chip was used for HPV genotyping, as discussed above. Moreover, the study design differed from ours as the mothers' samples were collected at the 22nd-26th week of their pregnancies, and the newborns' samples within the first 24 hours of life. In our study, the mothers' samples were collected at 36 weeks of pregnancy and the newborns' samples immediately after delivery. The mother's HPV detected earlier during the pregnancy might have already cleared before the onset of labour, which might partly explain the lower transmission rate observed in the study by Hong et al. In addition, the sample collection technique on the oral site differed; in the study by Hong et al., the newborn's exfoliated oral cells were collected by allowing the newborn to suck on the sampler, whereas we

collected the scraping samples from the newborn's oral cavity. In a study by Khayargoli et al., the evaluation of vertical HPV transmission was based on the mother's self-collected vaginal samples during pregnancy and the newborn's conjunctival/oral/pharyngeal/genital samples at birth and/or at 3 months of age. 7.2% (27/374) of newborns born to HPV-positive mothers were HPV-positive, and of those, 23/27 had at least one concordant HPV genotype with their mother (Khayargoli et al., 2023). Thus, the vertical transmission rate in that study was 6.1% (23/374).

In one meta-analysis, the newborn's risk of acquiring an HPV infection was calculated according to their mother's genital HPV status; newborns born to HPV-positive mothers had a 33% higher risk of an HPV infection than newborns born to HPV-negative mothers, and the risk was even higher (45%) when only the HR-HPV infection was considered (Merckx et al., 2013). In our study, newborns that were born to mothers with an oral HR-HPV infection or multiple site (≥2 anatomic site detected positive) HR-HPV infection, showed to be at a higher risk of a HR-HPV infection. Unexpectedly, the mother's genital HPV positivity was not associated with the newborn's HPV positivity at any anatomic site or solely at the oral site. This finding is against the general assumption that the majority of newborns' HPV infections are transmitted during delivery from infected maternal genital tract. However, we observed that mothers' genital HR-HPV positivity associated with newborns' genital HR-HPV positivity.

Besides the Finnish Family HPV Study, the current data on the vertical father-to-child HPV transmission relies on two small studies, in which both mothers and fathers were included (Skoczyński et al., 2019; E. M. Smith et al., 2004). Skoczyński et al. investigated the co-occurrence of HPV 16/18 infections between 146 Polish parental couples and their newborns. They determined the mothers' genital and oral HPV status, and the fathers' oral HPV status, at the onset of labour, and the newborns' oral HPV status immediately after delivery. According to their results, the mother's any site, genital, and oral HPV 16/18 infection, as well as the father's oral HPV 16/18 infection, increased the newborn's risk of an oral HPV infection at birth. These findings are in line with our results, as we also showed that the mother's and the father's oral HR-HPV infections increase the risk of the newborn's oral HR-HPV infection at birth.

Smith et al. examined the vertical HPV transmission from both parents to the newborns with an US population in Iowa; 574 mothers and newborns and 68 fathers participated in the study, and genotype-specific HPV concordances between the mother-newborn pairs and father-newborn pairs were determined, as was done in our study (E. M. Smith et al., 2004). Contradictorily to our findings, only one mother-newborn pair and no father-newborn pairs showed an HPV genotype-specific concordance. Thus, they suggested the vertical transmission from the parents to their

newborn to be as rare as < 1%. However, their study design differed slightly from ours, as the HPV samples from fathers' oral site and newborns' oral and genital sites were taken 65 hours after delivery. Moreover, in their study, the oral samplings were performed by an oral rinse for parents and an oral scraping for newborns, whereas in our study, oral scraping samples were taken from both parents and newborns.

Possible routes for vertical transmission

Although our results on the HPV concordance between parents and newborns suggest vertical transmission, the exact routes of transmission are still unknown and a matter of speculation. The father-newborn HPV concordance could result from a periconceptual transmission, as an infected spermatozoon may potentially serve as a vector to transfer the HPV to the oocyte during fertilisation (Foresta et al., 2011). Interestingly, the father's genital LR-HPV infection was associated with the newborn's oral LR-HPV presence. Unfortunately, due to a low sample size of semen samples, the statistical power did not reach to show an association between the father's seminal HPV and the newborn's HPV. In literature, HPV presence in the endometrium has been documented as well, so it cannot be excluded that the transmission might have happened during a trophoblast invasion (Ip et al., 2002; Syrjänen, 2010). However, we can only speculate the routes for periconceptional transmission due to limited data on this subject.

Transmission from the mother to the newborn during pregnancy could result from transplacental transmission, as we and others have shown HPV to be detected in placental and cord blood samples (Ambühl et al., 2016; Khayargoli et al., 2023; Sarkola et al., 2008). However, HPV detection from mothers' peripheral blood samples, which was not performed in this study, could have given more information on hematogenous transmission from the mother to the foetus.

Another possible source of HPV is the mother's infected genital tract, from which the transmission may happen during pregnancy as an ascending infection or during a vaginal delivery. Caesarean section is associated with significantly lower rate of vertical HPV transmission than vaginal delivery (14.9% vs. 28.2%); the transmission still occurs in 15% of the children born by caesarean section (Chatzistamatiou et al., 2016). According to our results, the mother's genital HR-HPV infection was associated with the newborn's genital HR-HPV presence. However, the mother's genital infection did not show an impact on the newborn's HPV risk at the oral site nor any anatomic site. This could be due to the fact that the mother's HPV detected at 36 weeks of pregnancy might have already been cleared prior to the delivery. Moreover, the mother's oral HPV might play a more important role in the newborn's oral HPV risk than the mother's genital HPV.

6.2 HLA-G allele distribution and concordance among fathers and newborns (I, II)

To date, 157 HLA-G alleles have been identified (*IPD-IMGT/HLA Database*, 2023). Among the fathers and newborns of this cohort, nine different HLA-G allele- and 19 different genotype combinations were identified. The relatively low number of identified alleles reflects the characteristic of this cohort as it represents a Finnish, Caucasian population with a quite restricted and homogenous gene pool due to historical isolation. Wildtype G*01:01:01 has shown to be the most common allele in many populations, and not surprisingly, it was the most common allele both among the fathers and newborns, and the most shared allele between the father-newborn pairs in our cohort as well (Castelli et al., 2014).

6.3 The role of HLA-G in father-newborn HPV concordance (II)

The present study was the first to evaluate the role of HLA-G in the father-newborn HPV concordance (possible vertical father-to-child HPV transmission). We showed that certain HLA-G alleles appear to have an impact on the father-newborn HPV concordance and prevalence during the perinatal period. However, HLA-G genotypes did not show an impact on the HPV concordance among the father-newborn pairs. There are no previous studies on HLA-G in the father-to-child HPV transmission to compare our results. A previous study with the same Finnish Family HPV Study cohort has evaluated the HLA-G and vertical mother-to-child HPV transmission and is thus comparable with the present study (Louvanto et al., 2018).

In the study by Louvanto et al., the association of the mother-newborn HLA-G gene concordance with the mother-newborn genotype-specific HPV concordance (i.e., HPV transmission) was evaluated (Louvanto et al., 2018); the HLA-G allele or genotype concordance did not show any impact on the mother-to-newborn HPV transmission. These findings are interesting as the father-newborn HLA-G allele concordance, but not the mother-newborn HLA-G allele concordance appears to have an impact on the mother/father-newborn HPV concordance.

Louvanto et al. showed that the mother-newborn high-resolution HLA-G genotype concordance of G*01:01:01/01:04:01 associated with HR-HPV positivity in the cord blood and the newborn's oral site, whereas the concordance of G*01:01:02/01:01:02 associated with both the mother's and the newborn's oral HR-HPV positivity. Conversely, we did not observe any association of the father-newborn high-resolution HLA-G genotype concordance with HPV positivity among fathers and newborns. However, in our study, an association was seen at the low-resolution level; the low-resolution group G*01:01+/01:04+ concordance associated with HR-HPV positivity in the father's semen and/or the newborn's oral sites at the

postpartum period. Moreover, G*01:01+/01:04+ concordance associated with any HPV positivity of the father's genital and/or the newborn's oral sites at the postpartum period. A greater sample size of father-newborn pairs might have shown an association in the high-resolution group as well, as was shown in the study by Louvanto et al.

In the present study, the father-newborn HLA-G*01:04:01 concordance associated with the father-newborn any- and HR-HPV concordance of the father's oral and the newborn's postpartum oral samples. Although, Louvanto et al. did not show an association between the mother-newborn HLA-G*01:04:01 concordance and the mother-newborn HPV concordance; they observed that the mother-newborn discordance with the same HLA-G allele G*01:04:01 increased the risk of the newborn's oral LR-HPV infection at birth. However, the different findings of G*01:04:01 concordance/discordance between these two studies cannot be logically explained, as the associations were observed with LR-HPV types in the study by Louvanto et al. and with any- and HR-types in the present study. Interestingly, one previous study with Italian cohort has shown an association of the HLA-G*01:04:01 allele with the vertical mother-to-child HCV transmission; allele G*01:04:01 was more frequently detected in HCV-positive children than in HCV-negative children (22.5% vs 6.8%) (Martinetti et al., 2006).

When the associations of the father-newborn allele G*01:06 concordance with HPV prevalence in fathers' semen samples, fathers' genital samples, and newborns' oral samples (at birth), were analysed separately, the father-newborn HLA-G*01:06 concordance associated solely with the father's genital HPV prevalence. Interestingly, the study by Louvanto et al. with mother-newborn pairs showed that G*01:06 mother-newborn discordance increased the newborn's oral LR-HPV positivity but had no impact on the mother's site (Louvanto et al., 2018).

6.4 The role of HLA-G in father's HPV infection (I)

As the HLA-G molecule is a novel biomarker and proposed as a potential therapeutic target in various types of cancers, those HLA-G polymorphism studies that concern HPV infection have focused on cervical precancer and cancer. Thus, the knowledge on HLA-G and HPV infection relies on female data (Alves et al., 2015; Ferguson et al., 2011, 2012; Jaakola et al., 2021; Louvanto et al., 2018; Metcalfe et al., 2013). The few studies in which male participants have been included have mainly focused on exploring HLA-G polymorphism in the HLA-G untranslated region (UTR) and disclosed an association with other viral infections, such as HCV (Catamo et al., 2017; Cordero et al., 2009; G. K. da Silva et al., 2014), HBV (Eskandari et al., 2017; Laaribi et al., 2015), and HIV (G. K. da Silva et al., 2014; Larsen et al., 2013), but not with HPV. As oropharyngeal cancer is related to HPV and affects especially

men, we found it important to evaluate the role of HLA-G in men's HPV infection, and particularly in men's *oral* HPV infection outcomes. To our knowledge, this is the first study reporting on the association of HLA-G polymorphism with HPV infection in men. Thus, the results of our study could be compared only to studies with female data.

Jaakola et al. have investigated the same topic with female data from the FFHPV Study (mothers from the FFHPV Study cohort); they evaluated the impact of HLA-G polymorphism on genital and oral HPV infection outcomes among women (Jaakola et al., 2021). HPV outcomes were determined similarly, as was done in our study (always HPV negative, incidence, clearance, persistence). In that study, several HLA-G alleles and genotypes showed to associate with women's oral infection outcomes, but none were associated with genital infection outcomes. In our study, the presence of the wild-type G*01:01:01 allele was shown to be protective for incident and persistent oral HPV infections in men. Conversely, Jaakola et al. did not show any association between allele G*01:01:01 and women's oral HPV infection outcomes. Metcalfe et al. reported an association of G*01:01:01 allele with an increased risk of genital LR-HPV infection among 548 Inuit women, which is partly inconsistent with our finding as we showed this allele to be protective for oral infection (Metcalfe et al., 2013). However, in the case-control study (539 cases with high-grade CIN/invasive cancer and 833 controls with normal cytology) by Ferguson et al., the heterozygotic form of the wild-type G*01:01:01 allele was reported to associate with a lower risk of cervical cancer in the Canadian population, supporting our suggestion for the protective role of this allele (Ferguson et al., 2012).

Allele G*01:01:02 is one of the several examples of polymorphism in the coding region that have shown an inconsistent influence on the HPV risk. In the present study, we showed G*01:01:02 allele to associate with a lower risk of any- and HR-HPV infections at oral site. Consistent with our results, Metcalfe et al. reported that the G*01:01:02 allele was associated with a lower risk of genital HPV infection (Metcalfe et al., 2013). Contradictorily, in the study by Ferguson et al., an association of HLA-G*01:01:02 with an increased risk of HPV16 and any HPVs from α species 1, 8, 10, and 13 was observed among 636 female university students (Ferguson et al., 2011). Moreover, in a study including 140 Brazilian pregnant women, no association was observed between the HLA-G allele polymorphism and HPV infection, but G*01:01:02 showed to be protective for CIN (Alves et al., 2015). Lastly, Jaakola et al. showed G*01:01:02 to reduce the probability of oral HPV clearance in women (Jaakola et al., 2021).

We showed that the allele G*01:01:03 increased the risk of genital HR-HPV infection in men. Interestingly, Jaakola et al. observed this allele to increase the risk of multiple infection at the oral site of women (Jaakola et al., 2021). Moreover, we observed an association between allele G*01:01:03 and men's self-reported history

of oral warts. However, Jaakola et al. did not observe a similar association of this allele G*01:01:03 with oral warts among women but showed an association between the genotype G*01:01:01/01:01:03 and oral warts.

In addition, we found that allele G*01:03:01 in men was significantly associated with an increased risk of allergies to pollen and/or animals. Several studies have shown that HLA-G is associated with allergic diseases (Murdaca et al., 2016). HLA-G is a tolerance-inducing molecule, but it is also a stimulus for T helper 2 cell (Th2) responses and regulatory T-cell (Treg) activation. Allergic diseases are driven by a Th2-polarised inflammation, and allergic patients display a defect in Treg cells, which may be restored through specific immunotherapy (Murdaca et al., 2016).

Lastly, in the present study, no association was observed between HLA-G genotypes and men's oral HPV infection outcomes, whereas Jaakola et al. observed an association of several genotypes and oral HPV infection outcomes in women. However, as the sample size was lower in our study (n=130 vs n=306), the statistical power might not be strong enough to show those associations with men.

6.5 Study strength and limitations

The Finnish Family HPV Study was originally designed to elucidate the dynamics of HPV infection between family members. The unique cohort with three family members - mothers, fathers, and their newborn offspring - and the extensive follow-up timeframe gave an opportunity to study not only the transmission chains, but also the epidemiology and natural history of HPV. Here, we investigated the HPV concordance between the parent-newborn pairs to represent the vertical transmission from the parents to their offspring. Although the FFHPV Study was not originally planned to explore the role of HLA-G polymorphism in HPV infections, the HLA-G determination from participants' serum samples extended the data to fit well in this purpose as well.

6.5.1 Strengths

The present study has several strengths. Firstly, the study cohort of FFHPV Study is unique as it represents Finnish families comprising mothers, fathers, and their newborn offspring with the same ethnic background. In some studies, the vertical transmission rate has been calculated by the mother-newborn concordance of any HPV types (Medeiros et al., 2005). This might lead to an overestimation of the mother-to-child HPV transmission, as subset of the mother-newborn HPV-positive samples may be discordant by the HPV genotype. In these cases, the newborn's source of HPV may be other than the mother, such as the father or another caregiver, or it may be sample contamination or a false HPV test result. We determined the

HPV concordance by each HPV genotype separately, which is a more representable way to establish the transmission than the concordance between any HPV types. Thus, we suggest that the observed genotype-specific concordance between mothers' or fathers' HPV status and newborns' HPV status at birth most likely represent a vertical transmission, including the possibility of an intrauterine transmission.

Moreover, the majority of studies have focused on evaluating the vertical transmission only between the mother and the newborn. In our study, we also included fathers to elucidate the possible role of a periconceptual transmission, which remains mostly unexplored. As we showed the HPV6 and HPV31 genotype-specific concordance between the father's baseline HPV status and the newborn's HPV status at birth, we suggest that fathers may be involved in a periconceptual HPV transmission.

Another strength of this study is the sampling technique. Accurate nested PCR for HPV testing was selected from the very beginning of the FFHPV Study as the collection of sufficient amounts of nucleated epithelial cells from the newborn's oral or genital mucosa is demanding, and the viral copies in these cells are estimated to be low. Moreover, the determination of newborns' HPV status was based on multiple anatomical sample sites, including the cord blood and placenta, which enabled us to observe the signs of an intrauterine transmission. The oral HPV outcomes of fathers were determined by using the follow-up data collected over a relatively long period (six years), which we can consider a strength as well. Although the follow-up time was long, the attendance of fathers was relatively high, especially for the first three years, and it did not drop lower than 40% by the end of the six-year follow-up.

6.5.2 Limitations

We acknowledge several limitations of the present study. Firstly, the sample size of the study was relatively low. Thus, the results presented are approximate, and should be validated by other studies. Moreover, the study cohort included not only complete families, but also incomplete families because the number of attending fathers (N=132) were lower compared to the mothers (N=319) and newborns (N=321). The reason for the fathers' unwillingness to participate in the study is unknown. However, we might speculate that males may not be as willing to attend, as they are not as familiar with HPV testing as females, who are used to participating in routine cervical cancer screening.

When investigating the vertical transmission, parental samples were collected only at 36 weeks of pregnancy, which we consider a limitation. The perinatal transmission, i.e., the transmission happening during childbirth, could have been demonstrated more specifically if the parents' samples could have been collected at the onset of labour. However, the most precise information of the three different

modes of vertical transmission - periconceptual, prenatal, and perinatal - could have been shown with multiple consecutive samplings of both parents right before fertilisation to the end of pregnancy.

As the majority of HPV infections detected at birth is shown to clear during the first months of life (Hahn et al., 2013; Khayargoli et al., 2023; Park et al., 2012; Rombaldi et al., 2009; Syrjänen et al., 2021; Tenti et al., 1999), it is a matter of debate whether HPV DNA detected in newborns represent a true infection or passive contamination/passage from the infected maternal birth canal or delivery room. We did not examine transcriptionally active HPV, which would have been one method to verify the state of HPV infection. Some researchers prefer newborns' sampling to take place 1-3 days after delivery to avoid contamination detection from the infected birth canal (E. M. Smith et al., 2010; Trottier et al., 2016). On the other hand, immediate sampling after birth minimises the risk of the newborn's exposure to other HPVs prior to the sampling. Repeated positive detections of HPV DNA at the same anatomic site at different time points would indicate a true infection rather than passive contamination. When we evaluated the vertical transmission from parents to newborns, the determination of the newborn's HPV status at birth was based only on a single sample taken immediately after delivery. In the HLA-G and father-newborn HPV concordance analyses, the determination differed slightly; the newborn's HPV status at birth was determined by pooling together the HPV samples taken at birth and at day 3.

However, clearance soon after birth does not necessarily mean that the detected HPV is a contamination. Maternal antibodies transferred to the foetus may induce a silent neutralisation of the newborn's HPV infection. Interpreting the mother's and newborn's serology may be helpful in the detection of perinatal infection, although it is known that seroconversion does not occur in all individuals infected with HPV (Carter et al., 2000). The recent study with the FFHPV Study cohort showed the seroconversion to HPV6, HPV11, HPV16, and HPV18 among newborns born to HPV-seronegative mothers (Syrjänen et al., 2022). This finding indicates that newborns have acquired HPV somewhere in their bodies, and even created the immune response to HPV, supporting the view of a true infection rather than contamination. As the mothers of these newborns were seronegative, the finding strengthens the assumption that the mother may not be the only source of the newborn's HPV, as was suggested in our study as well.

We used genotype-specific data to determine HPV concordances between the parents' and newborns' HPV status at any anatomic site. Although we showed HPV concordance between fathers and newborns, it does not verify direct father-to-newborn transmission. As an alternative to the direct father-to-newborn transmission, the transmission to the newborn might have been vertical from the mother, as the mother and father are expected to share the same HPV genotypes

through sexual transmission. Even if the mother's HPV sample is negative, it might be a false-negative result. However, as we used accurate nested PCR for HPV detection, the possibility of false-negative results is low, particularly in mothers' cervical samples in which the viral load is supposed to be high. In addition, we did not have information on the type-specific HPV variant via sequencing, which would provide more proof of the transmission between the mother-newborn pairs and father-newborn pairs.

When the HPV status was determined at each anatomic site separately, the sample size was too low to stratify an analysis by each HPV genotype. Thus, we had to group HPV types into LR- and HR groups when we evaluated which parents' anatomic HPV positivity affected the most on the likelihood of the newborn's HPV infection. Similarly, instead of the genotype-specific concordance, HPV types were grouped into any-, LR- and HR-groups when evaluated the role of HLA-G in father-newborn HPV concordance. As we did not run genotype-specific concordance analyses in this part of study (II), it is questionable whether our results on the HLA-G and father-newborn HPV concordance indicate the true HPV transmission from the father to the newborn.

Because of the restricted gene pool in this cohort, the low number of fathers and newborns with uncommon HLA-G alleles might have interfered with the detection rate of significant associations between HLA-G and HPV infections of fathers and newborns and their HPV concordance. The associations of the father-newborn HLA-G *allele* concordance with the HPV concordance was seen, but the same was not true with the HLA-G *genotype* concordances. This is possibly due to the fact that the sample size may have been too low to show the statistically significant associations in the HLA-G *genotype* level. The demographic data of fathers used in the present study was self-reported, which can create some bias especially with the self-reported wart diagnosis. As we had no similar follow-up data regarding fathers' genital samples as we had for oral samples, the evaluation of the role of HLA-G in HPV infection outcomes had to be restricted to only the oral site.

6.6 Impact of the study and future directions

HPV research has been mainly focused on infections among adolescents and adults, and its sexual transmission. Now that it is known that HPV can also be found in children without necessarily indicating sexual abuse, the data on HPV infections in the paediatric population and non-sexual transmission are slowly becoming available. However, many gaps still exist in the understanding of HPV transmission between different anatomic sites via sexual and non-sexual horizontal transmissions, autoinoculation, and vertical transmission.

We showed an HPV concordance between mothers/fathers and newborns, which suggests that parents' HPV infection may play an important role in newborns' susceptibility to perinatal HPV infection, but it does not answer the question about the exact routes of vertical transmission. More in vitro and clinical studies are required to understand the periconceptional and intrauterine transmissions.

The importance of vertical transmission in juvenile recurrent respiratory papillomatosis is widely accepted (Benedict & Derkay, 2021), but the significance of perinatally-acquired HPV infection for individuals later in life is unclear. Due to the lack of long-term follow-up studies, we can only speculate whether perinatal infection influences the HPV susceptibility and the risk of HPV-related diseases in adulthood. It has been even speculated that perinatal HPV infection could cause HPV-specific immunologic tolerance for the child as the newborn is exposed to HPV before the maturation of his/her immune system (Cason, 1996). More studies on children's immune responses are needed to bring light to this suggestion. Despite the high tendency for clearance, some of the early-life infections persist as well. Perinatal oral and genital HR-HPV infections are shown to occur from birth up to 26 months (Rintala et al., 2005). A recent follow-up study with the FFHPV Study cohort showed that oral HPV infection detected at birth can persist for a mean duration of 20.6 months (Syrjänen et al., 2021). Further studies with a longer follow-up timeframe are required to establish the natural course of perinatal infection.

Among the FFHPV Study cohort, the most common HPV type detected among newborns was HPV16, the most carcinogenic HPV type. The findings of early-life HR-HPV infections raise concern for a potential cancer risk later in life. The concordant HPV types (except HPV56) found in the present study could be vaccinated against with a 9-valent vaccine. In sight of achieving lifelong HPV protection, a future mode of preventing HPV could be early-infancy vaccination or vaccinating parents. As maternal HPV-IgG is transferred to the offspring (Syrjänen et al., 2022), the vaccination of pregnant mothers could be also considered. However, further studies on the vertical HPV transmission and consequences of perinatal infection are needed to extend the prevention of HPV in the long run. Moreover, it could be interesting to establish whether the vertical transmission of HPV also occur among parents who have been HPV-vaccinated prior to their reproductive ages. At the very least, it has shown that HPV-antibodies induced by 4-valent or 9-valent vaccines can be transferred from the mother to the cord blood (Guevara et al., 2019; Matys et al., 2012). In clinical practice, there is not enough evidence to suggest caesarean section as a method to reduce HPV transmission for several reasons. It has been estimated that one in every six children born by caesarean section from HPV-positive mothers are HPV-positive (Chatzistamatiou et al., 2016). Furthermore, JORRP, a consequence of the HPV6/11 infection, is an extremely rare event. And

most importantly, the consequences of perinatal HPV infection later in the individual's life are not known due to the lack of long-term follow-up studies.

Another main aim of this thesis was to shed light on the role of HLA-G in the natural course of HPV infection, particularly in men's HPV infections and the father-to-child transmission. To date, these two specific topics have remained unstudied. According to our findings, several HLA-G alleles might play a role in men's HPV infections and the father-to-child transmission. Although the sample size was low and presented results are approximate, we provided unique data in this research field. Overall, little is known about HLA-G in relation to the natural history of HPV infection, and the available data relies on small studies with partly controversial results. Because of the unpredictable natural course of HPV infection, more data on the potential diagnostic or prognostic biomarkers, such as HLA-G, are greatly needed to make screening more effective. Comparable studies are required to determine the evident HLA-G risk alleles and -genotypes for HPV infection and its progression. Due to the paucity of data, it is too early to assess the HLA-G gene testing in clinical use. However, in the future, HLA-G gene testing may be a potential tool to predict the individual's genetical risk of an HPV infection and its progression. In the long term, the future target is to establish the potentiality of HLA-G as a preventive tool in the detection of precancerous lesions, as well as an immunotherapeutic target for cancer treatment.

7 Conclusions

1. We showed the HPV concordance with five HPV genotypes between parent-newborn pairs, suggesting that parents' HPV infections may play an important role in newborns' susceptibility to perinatal HPV infections. Thus, the role of early-life HPV infection as a risk factor or protective factor for HPV-related cancer later in life should be further explored.
2. Against the general assumption, parents' oral HR-HPV infections seem to impact the newborns' oral HR-HPV infections more than parents' genital HR-HPV infections.
3. The HLA-G *allele* concordance appears to impact the HPV concordance between the father and his offspring. The father might have an important regulatory role in the natural history of his child's oral HPV infection.
4. The HLA-G polymorphism seems to play a role in oral and genital HPV infections in males.

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