The seroepidemiology of human-papillomavirus in relation to non-melanoma skin cancer

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THE SEROEPIDEMIOLOGY OF HUMAN-PAPILLOMAVIRUS IN RELATION TO NON-MELANOMA SKIN CANCER

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I hereby certify that this thesis represents my own work.

D. Casabonne

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ABSTRACT

Non-melanoma skin cancer, comprising basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) is the most commonly diagnosed cancer in Caucasian populations. Established risk factors include exposure to solar ultra-violet radiation and immunosuppression, such as that experienced by organ transplant recipients (OTR). A role for cutaneous human papillomaviruses (HPV) in the aetiology of SCC has been suggested, but remains uncertain. The aims of this thesis were to examine the association between SCC and antibodies against the L1 antigen of 38 HPV types using Luminex technology among Caucasian individuals and to investigate the seroepidemiology of cutaneous HPV types. Data came from a small prospective study of 39 cases and 80 controls (the Oxford component of the European Prospective Investigation into Cancer and Nutrition) and from case-control studies nested among high-risk cohorts of OTR from London and from Oxford (119 prevalent cases and 425 controls).

Around 85% of controls were seroactive to at least one HPV type. In the prospective study, there were no statistically significant differences in the seroprevalence of antibodies against any of the HPV types examined between incident cases and controls. In the case-control studies, as expected, antibodies against HPV 16 were associated with a self-reported history of an abnormal cervical smear and antibodies against HPV 6 were associated with a self-reported history of genital warts, validating the methodology. However, no clear associations between any of the HPV types examined (including betaHPVs) and prevalent SCC were identified. Adjustment for potential confounding factors, such as self-reported history of sun exposure made no material difference to the results. Limitations of the studies are the low statistical power and the use of new serological assays. These serological data do not provide evidence in support of a role for HPV in the aetiology of cutaneous SCC.

Contents

List of abbreviations used in the thesis

- AK: *Actinic keratosis*
- BCC: *Basal Cell Carcinoma*
- BD: *Bowen's disease*
- CI: *Confidence interval*
- CIS: *Carcinoma in situ*
- CR: *Count ratio*
- HLA: *Human Leukocyte Antigens*
- HPV: *Human Papillomavirus*
- IC: *Immunocompetent*
- KS: *Kaposi's sarcoma*
- MM: *Malignant Melanoma*
- NHL: *Non-Hodgkin's lymphoma*
- NMSC: *Non-Melanoma Skin Cancers*
- OR: *Odds ratio*
- OTR: *Organ transplant recipient*
- SCC: *Squamous Cell Carcinoma*
- SIR: *Standardised Incidence Ratio*
- UV: *Ultraviolet*

List of Tables

List of Figures

CHAPTER 1

Rationale and outline of the work described in this thesis

1.1 Introduction, aims and outline of the thesis

Non-melanoma skin cancer (NMSC), comprising basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) is the most common cancer in populations of European descent. The main and well-established environmental factor associated with the development of NMSCs is ultraviolet (UV) radiation, causing genetic mutations which might subsequently lead to oncogenic transformations. Immunosuppressed patients, in particular organ transplant recipients (OTR), have a higher risk of NMSCs and especially SCCs than the rest of the population. The risk increases with increasing level of immunosuppression and with time since transplantation. Furthermore, squamous cell carcinomas occur significantly more frequently than basal cell carcinomas in transplant recipients, reversing the ratio usually found in the general population (4 BCCs to 1 SCC). In Chapter 2, there is a brief background on the history of transplantation, immunosuppressive

treatments and on the first reports of skin malignancies among OTR, together with basic descriptions of the anatomy of the skin and a short review of the epidemiology of skin cancer in the general population. Chapter 3 describes the epidemiology of non-melanoma skin cancer among OTR.

Results in allograft recipients suggest a viral involvement in the pathogenesis of SCC since highest incidences are also reported in the transplant population for tumours of viral origins such as non-Hodgkin's lymphoma (Epstein Barr virus) and Kaposi Sarcoma (human herpes virus 8). The main aim of this thesis is to investigate the role of HPV in the aetiology of NMSC using serological data from a small prospective study from the Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford) and from nested case-control studies among cohorts of high risk OTR in London and in Oxford.

A large body of research has been undertaken to examine the potential role of HPV in the aetiology of skin cancers. The oncogenic mechanism of HPV in cervical cancers is well understood and the causative association is now established; however, it is still uncertain what role, if any, HPV plays in the aetiology of non-melanoma skin cancers. The hypothesis tested in this thesis is therefore: Is human papillomavirus a cause of squamous cell carcinoma?

Chapter 4 gives a review of the current epidemiological evidence on the association between HPV and, SCC and BCC. In Chapter 5, I describe methods of the small prospective pilot study from EPIC-Oxford, together with new data from nested case-control studies (with questionnaire data and biological material) conducted among high risk cohorts of OTR from London and from Oxford. Patients with end-stage renal disease on dialysis, at increased risk of infections and cancers probably due to abnormalities of the immune system, and immunocompetent patients were also included to compare seroprevalence

across different immune status.

To date, few data are available on the seroprevalence and risk factors associated with HPV types other than those associated with cancer of uterine cervix. Chapter 6 shows results on the seroepidemiology of HPV among OTR and on an examination of the seroprevalence and epidemiology of HPV among different ethnic groups and among people with different immunological status. In chapter 7 results on the role of HPV and other factors in the development of NMSC and particularly SCC among OTR are described. The final chapter summarises the findings of this thesis and gives suggestions for future work (Chapter 8).

1.2 My role in the preparation of this thesis

The idea of this thesis on HPV and SCC was conceived by my supervisor Dr Robert Newton. The comparison between HPV seroprevalence of transplant, IC and dialysis patients were conceived by co-supervisors Dr Harwood and Dr Proby. Data used for this work include new data collected from London and Oxford and existing data from EPIC-Oxford. The main questionnaire was written by Dr Robert Newton.

The study in London was set up in 2002 by Dr Newton in collaboration with Dr Harwood and Dr Proby. I joined the study in January 2004 and took a prominent role in collaboration with the research nurse (Liza Mitchell) on collection, collation and cleaning of data. I liaised with the renal transplant and dermatology centres to obtain databases and I selected patients with end stage renal failure.

For the EPIC study, I selected plasma samples, organised the shipping to Heidelberg, performed statistical analyses and drafted the publication. In 2005, I conducted the case-control study among OTR from Oxford, in consultation with Dr Robert Newton. I obtained ethical approval, updated the London questionnaire, invited all patients to participate and

supervised the data and specimen collection and shipping for the Oxford study. I liaised with the Oxford Cancer Intelligence Unit to obtain incidence rates of malignant melanoma (MM) for the Oxford region by calendar year, age group and sex, and calculated standardised incidence ratio of MM. I conducted the vast majority of data entry (Krys Baker entered the first 100 patients from London) and cleaning for both OTR centres and, I did the quality control. I have checked all histological reports from hospital records. The literature reviews, all statistical analyses and the writing up of the studies conducted are also my own work.

1.3 Publications relating to this thesis

- Casabonne D, Waterboer T, Michael K.M, Pawlita M, Lally A, Mitchell L, Imko-Walczuk B, Wojnarowska F, Newton R, Proby C, Harwood c. The sero-epidemiology of human papillomavirus among Caucasian transplant recipients (Submitted).

- Casabonne D, Waterboer T, Mitchell L, Michael K.M, Pawlita M, Newton R, Harwood C, Proby C. The seroprevalence of human papillomavirus by immune status and by ethnicity in London (Submitted).

- Casabonne D, Lally A, Mitchell L, Michael K.M, Waterboer T, Pawlita M, Imko-Walczuk B, Wojnarowska F, Proby C, Harwood C, Newton R. A case-control study of cutaneous squamous cell carcinoma among Caucasian organ transplant recipients: the role of antibodies against human papillomavirus (HPV) and other risk factors. Int J Cancer. In press.

- Proby C.M, Wisgerhof H.C, Casabonne D., Green A.C, Harwood C.A., Bouwes Bavinck J-N. Chapter: The epidemiology of transplant-associated keratinocyte cancers in different geographical regions. In Cancer Treatment and Research Series:

Advances in Cutaneous Transplant Oncology; Edited by Eggert Stockfleth, Claas Ulrich, Charlotte Proby, Sylvie Euvrard and Jan Nico Bouwes Bavinck. In press.

- Casabonne D, Michael KM, Waterboer T, Pawlita M, Forslund O, Burk RD, Travis RC, Key TJ, Newton R. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. Int J Cancer 2007; 121: 1862-1868. [1]

- Imko-Walczuk B, Lally A, Le Mire L, Casabonne D, Hollowood K, Bordea C, Wojnarowska F. Melanomas in renal transplant recipients: the London experience, and invitation to participate in a European study: reply from authors. Br J Dermatol 2007; 156 (1):167-169. [2]

CHAPTER 2

Background on transplantation and skin cancer

2.1 Introduction

This chapter gives a short history of transplantation, immunosuppression and describes the first reports on skin cancer in the transplant recipient population. It also includes basic definitions of skin cancer and particularly non-melanoma skin cancer. It ends with a short description of the epidemiology of skin cancers in the general population.

2.2 A brief history of transplantation

The earliest report on transplantation came from China from the 5th century BC [3]. The physician Pien Ch'iao exchanged hearts of two soldiers 'to balance their opposite personality'. However the best known record of early transplantation has been attributed to Cosmas and Damian (the patron saints of surgeons). These twin Arabs, converted to Christianity, were born in Cilicia in Asia Minor during the 3rd century [4]. According to

the legend they amputated the gangrenous leg of the sacristan Deacon Justanian and grafted the leg of an Ethiopian Moor gladiator recently buried (Figure 2.1).

Figure 2.1: Oil painting, attributed to the Master of Los Balbases, Saints Cosmas and Damian (1495) *(Source: http://library.wellcome.ac.uk)*

Since this period, many unsuccessful transplantations took place and it is only during the 20th century that transplantation expanded remarkably. In 1906, cornea was successfully grafted by Eduard Zirm in Austria, however for organ transplantations the problem of rejection seemed insurmountable. In 1912, Alexis Carrel earned the Nobel Prize for his work on transplantation and sutures of blood vessels. He also noticed that organs were failing because of 'biological' factors. Around the world, multiple allograft and xenograft attempts were performed and surgical problems were gradually overcome but patients still had a very short survival [4].

An important step forward for patients with end-stage renal diseases was the development of the first renal dialysis machine during the Second World War by Willem Johan Kolff in the Netherlands. One of his later achievements was a heart-lung machine [5].

Frank Macfarlane Burnet and Peter Medawar brought a major insight into the transplantation world with the concept of 'acquired immunological tolerance'. They demonstrated that individuals acquire early in life the faculty to tolerate their own cells and to reject non-self or foreign cells. Hence, the exposure to an antigen at a fetal stage could induce tolerance later in life or inhibit the production of antibodies against this foreign organism. They won the Nobel Prize in 1960 for their work [6].

In 1954, Joseph Murray and his team at the Peter Bent Birmingham Hospital in Boston performed a kidney transplantation between homozygotic twins. The kidney was placed in the iliac fossa and the ureter was connected to the recipient bladder. The patient survived 9 years. This was the first successful organ transplantation [7]. The first successful transplantation of cadaveric kidney, lung, liver, pancreas, intestine, heart and heart-lung were achieved respectively in 1961, 1963, 1963, 1966, 1967, 1967 and 1981 [7].

None of these could have been achieved without the prevention of graft rejection: the discovery of corticosteroids (1936), X-ray irradiation and the introduction of immunosuppressive treatments. From 1960, the first efficient drug regimen consisted of azathioprine and prednisone. During this period, the first-year survival rate for liver transplants was only around 24-33%. With the discovery of ciclosporin in 1972, a new era started [8]. For instance, two year survival rate for liver recipients were only estimated at around 70% in most centres [7]. Progressively, newer immunosuppressive drugs such as OKT3 (1987), tacrolimus (1997), mycophenolate mofetil (1998), sirolimus (1999) have been introduced with better efficiency against rejection than the triple therapy (azathioprine, prednisone and ciclosporin) [9].

Nowadays, as reported by UK Transplant (NHS), between April 2006 and March 2007, 3,087 solid-organ transplantations were performed from 702 living donors (23%) and 2385 from cadaveric donors (77%) [10]. These figures include donors with multiple recip-

ients.

2.3 First reports on malignancies among OTR

In 1909, Paul Ehrlich postulated that the immune system protects humans and other species from cancer development. Fifty years later, Lewis Thomas and Frank MacFarlane Burnet proposed the concept of immunological surveillance [11]. The idea was that lymphocytes could identify and kill malignant or foreign cells and that cancers would originate in cells which had not been destroyed [12]. Hence, an increased incidence of malignancies would be expected in patients with weaker immune system. Transplantation was a good opportunity to test this hypothesis.

At the end of the sixties and the early seventies, strong evidence of an increase in cancers among transplant recipients was accumulating through case reports [13, 14, 15]. In the following decade, cohort studies were set up to confirm these observations. To do this, cancer incidence rates from transplant recipients were compared with the 'expected' ones in the general population. In 1973, Fraumeni and Hoover conducted the first study in 6297 patients followed for at least one month after transplantation between 1951 and 1971 [16]. Based on low incidence rates from the Third National Cancer Survey in the USA, they reported a statistically significant 4-fold increased risk of skin and lip cancers in transplant recipients compared with the general population. A non statistically significant increase of 30% was also reported using high incidence rates. In 1979, Kinlen *et al.* established a collaborative study between United Kingdom, Australia and New Zealand and corroborated results of the previous cohort [17, 18].

Overall a two-fold to six-fold increased incidence of all cancers has been found in organ transplant recipients compared with the general population [16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44]

[15-43]. Higher incidences have been reported for lymphomas, Kaposi sarcoma (KS), ano-genital, skin and lips cancers and in cancers associated with the type of organ transplanted [20, 17]. Most of these cancers, with the exception of skin cancers, have a proven viral etiology. Non-Hodgkin's lymphoma (NHL) has been associated with Epstein Barr virus, KS with human herpes virus 8, cervical and anal cancers with human papillomavirus.

In HIV-infected patients, another secondary immunodeficiency condition, an excess risk is also mainly found for those cancers with a viral aetiology [40, 45] and an excess of non-melanoma skin cancers are also reported.

2.4 Skin cancers in the general population

2.4.1 Definition

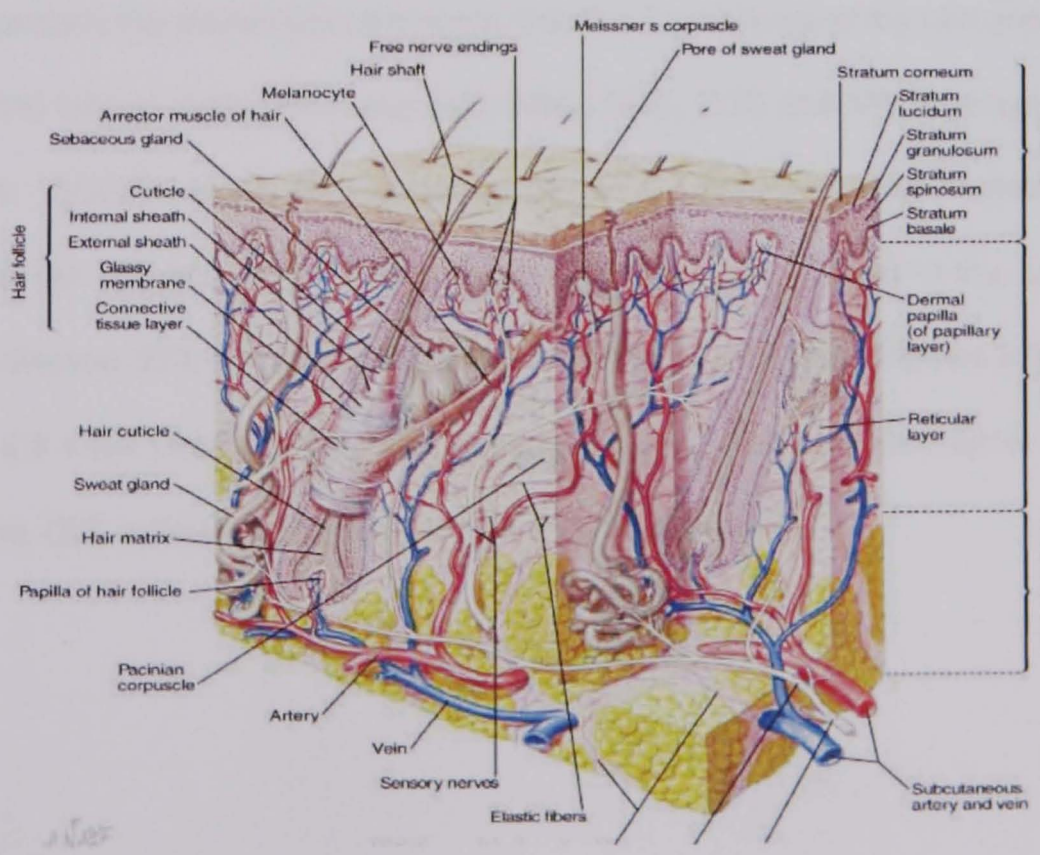


Figure 2.2: The skin *(Source: http://www.healthandage.com)*

The skin acts as a barrier and is constituted of three layers (Figure 2.2): epidermis, dermis and subcutaneous tissue. The epidermis, the outer layer of the skin, mainly consists of keratinocytes, melanocytes, Langerhans cells and Merkel Cells. The outermost layer, called the stratum corneum, is composed of dead and dying cells. The dermis is the middle layer of the skin located between the epidermis and subcutaneous tissue. The major cells in the dermis are fibroblasts which produce and secrete collagen and elastin fibers. The dermis also contains capillaries, lymphatic tissues, sebaceous glands, sweat glands, hair follicles as well as a relatively small number of nerve and muscle cells. The primary function of the dermis is to sustain and support the epidermis. Subcutanous tissue or hypodermis is the innermost layer of the skin located under the dermis and mainly consists of fat. Subcutaneous fat acts as a shock absorber and heat insulator. There are two main types of skin cancers. Non-melanoma skin cancer (NMSC) includes basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and some less common types of cancers like Merkel cell carcinoma, KS, T-cell lymphoma of the skin and sarcoma. The second type is malignant melanoma (MM). SCC, BCC and MM all originate in the epidermis. SCC develops from keratinocytes, BCC from basal keratinocytes possibly located in hair follicles and MM from the melanocytes also located in the basal layer. Bowen's disease (BD) is a squamous cell carcinoma in situ (CIS). Figures 2.3, 2.4, 2.5, 2.6, 2.7, 2.8 show photos of squamous cell carcinoma, basal cell carcinoma, malignant melanoma, CIS, actinic keratoses and viral warts respectively.

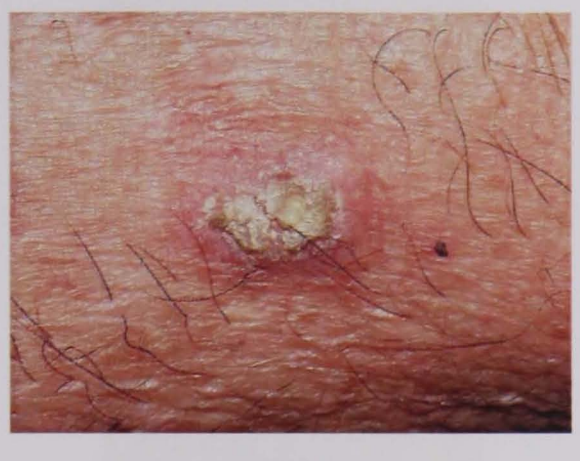


Figure 2.3: Squamous cell carcinoma

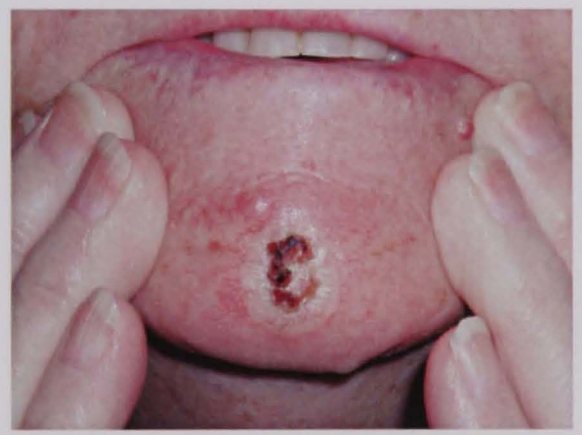


Figure 2.4: Basal cell carcinoma

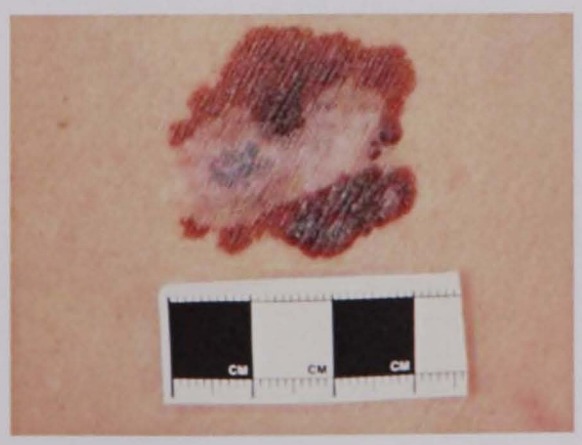


Figure 2.5: Malignant melanoma

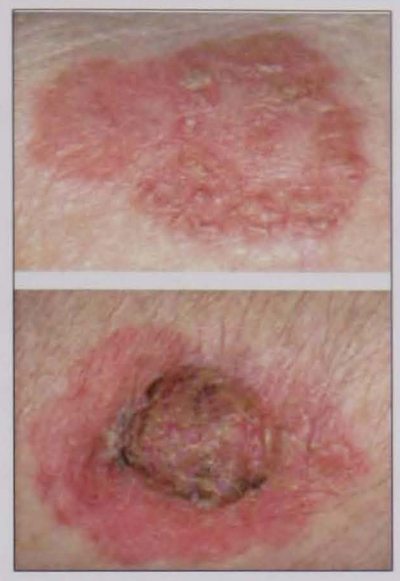


Figure 2.6: Carcinoma in situ - Bowen's disease

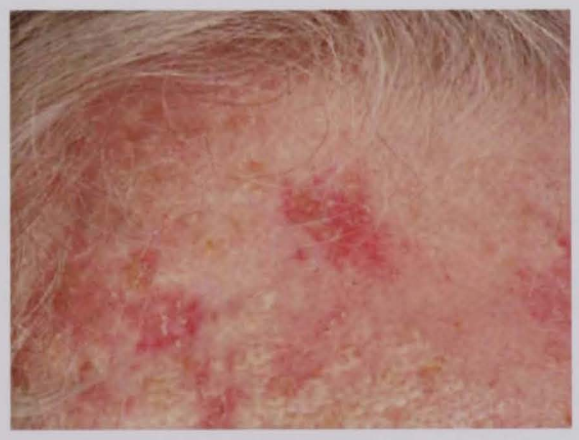


Figure 2.7: Actinic keratoses

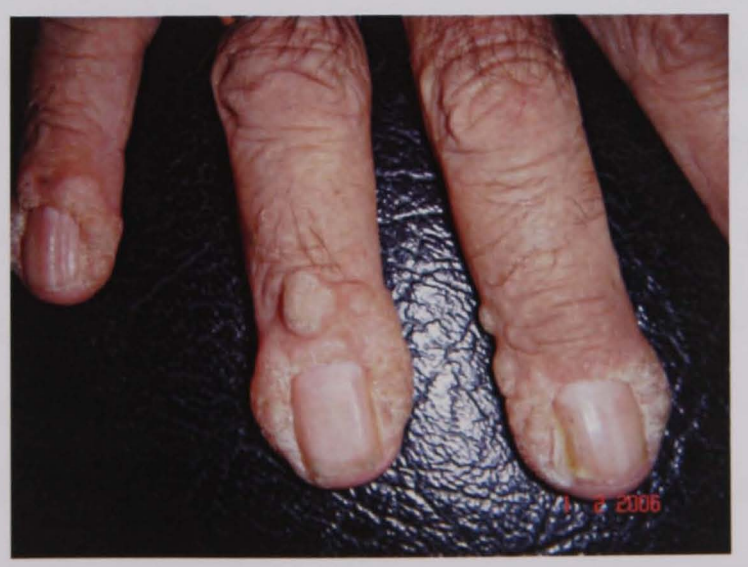


Figure 2.8: Viral warts

2.4.2 Skin cancer epidemiology in the general population

Skin cancer is the commonest cancer in Caucasian populations worldwide [46]. Hispanics and Asians have fewer skin cancers and the lowest occurrence rates are reported among populations with black or type VI skin. In 2005, in the UK, over 85,000 new skin cancers were reported of whom around 9583 people were diagnosed with MM. Over the same period, there were respectively around 45,500 and 38,500 new breast and lung cancer registrations. These data were obtained from Cancer Research UK [47]. It is important to note that these statistics are underestimating the true number of NMSC since these malignancies are poorly registered in many tumour registries [1]. NMSC is the commonest cancer in white populations, but being rarely fatal and badly registered it is often omitted or excluded from cancer reports.

In the Caucasian population, BCC, a slow-growing and locally destructive lesion, is the most frequent skin cancer representing around 75% of all NMSC whereas 20% are SCCs. In the Black population, SCC is more common [48]. NMSC are usually easy to treat with early diagnosis and BCCs rarely metastasize [49] whereas SCCs have higher potential to do so [50]. The overall rate of metastasis from SCC depends on the time of presentation, the size, the site, the depth, the use of previous treatments, the immunosuppressed status and the histologic differentiation of the lesion [51]. Rates of metastasis have been estimated as 2% to 5% for low risk SCC but between 10% and 30% for high risk SCC [52, 53, 51, 54]. Malignant melanoma is less common (around 3% of all skin cancers) but is the most dangerous type of skin cancers as they have significant metastatic potential. Malignant melanoma is the third most common cancer death in 15-39 years old in the UK and accounts for 75% of skin cancer related deaths [47]. Patients with skin cancers have a very good prognosis when lesions are detected early and promptly removed.

[1] www.statistics.gov.uk

Treatments range from local surgical excision, cryotherapy, curettage, radiotherapy, skin grafts, Mohs' micrographic surgery and chemotherapy, depending on severity and site. Five-year survival for patients with MM are closely linked with Breslow thickness. In the UK, for in situ melanoma, more than 95% of patients are alive after 5 years whereas only 30-50% of patients with MM greater than 4mm survive the first five years [47]. Mortality from malignant melanoma represents 1% of all deaths from cancers [47]. In contrast, NMSC are only rarely fatal. For instance, in Australia, mortality rates are 2 per 100000 for males and 0.6 per 100000 in females whereas for lung cancer in males and breast cancer in female mortality rates are 36.4 and 18.2 per 100000 respectively (2001 data standardised to the 2000 World Standard Population) [55]. In 2005, 511 people died in the UK from NMSC [47].

Around the world, skin cancer incidence in White populations has been dramatically increasing over the last two-three decades [56, 57, 46, 58]. Ko *et al.* (1994) looked at age-standardised incidence rates in North Humberside for BCC, SCC, Bowen's disease (BD) and MM in the years 1978, 1980 and 1984 and all years between 1987 and 1991. They found that age-standardised incidences between 1978 and 1991 increased by 2.5 for BCC, 5 for BD and 1.5 for SCC and MM [46]. In 2000, in the United Kingdom, MM rates increased by 16% in a year and 24% over the last 5 years [47]. The ageing population or improved diagnostics cannot solely explain this increase and sun exposure behaviour changes, due to greater affluence, increased leisure time and facility to travel to sunny countries, may partly explain this rise [47]. In some parts of the world, for instance Australia, there is some evidence that the skin cancer increase has stopped or started to decline in some populations due to successful public awareness campaigns [56].

The main and well-established risk factor associated with the development of skin cancers is UV radiation exposure, mainly UV-B radiation (280 to 315nm) but also UV-A radiation

(UV-A1: 340 to 400nm and UV-A2: 315 to 340) [50] but not UV-C (100 to 280nm). UV radiation promotes mutations in DNA, including those in critical tumour suppressor gene, such as p53 [59, 60]. DNA damaged cells normally undergo apoptosis, but failure to destroy them may lead to development of malignancies [61]. UV radiation can induce local cutaneous immunosuppression (for a review [62]). The conclusion from the International Agency for Research on Cancer's monograph on solar and ultraviolet radiation was that "there is sufficient evidence in humans for the carcinogenicity of solar radiation. Solar radiation causes cutaneous malignant melanomas and non-melanocytic skin cancer" [63]. The sun exposure pattern seems however to differ between cancer types. A recent meta-analysis concluded that MM seems to be associated with intermittent sun exposure [64], whereas SCC risk would increase with continuous sun exposure [61]. For BCC, it is unclear and one hypothesis is that after reaching a plateau of sun exposure individuals would not be at further increased risk of BCC with increase of sun exposure [65, 61] or that it could also be due to intermittent sun exposure [66, 67]. Other risk factors ensuing from UV radiation are:

- Skin phototype (cf Fitzpatrick classification on Page 45): People with low ability to tan and who burn easily are more likely to develop skin cancer. Light eye colour, red hair and fair complexion have shown a less conclusive association [61, 63].

- Ethnicity: Caucasians are more likely to develop non-melanoma skin cancers than people with darker pigmentation. Cancer in Black or other darkly pigmented populations do not necessarily occur on non sun-exposed areas [68, 69].

- Anatomic location: SCCs tend to occur on sun exposed areas, mainly the head and neck, whereas BCCs and MM also develop on intermittent or non sun-exposed body sites such as the trunk and legs [57, 56, 61, 63, 12].

- Geographical location: Highest skin cancer risks have been reported in people of European origin living near the Equator, with incidences declining with increasing latitudes [57, 56, 61, 63, 12]. In 2002, a survey found that non-melanoma skin cancers accounted for around 374,000 new cases of cancers in Australia (256,000 and 118,000 people with BCCs and SCCs respectively). The age-standardised incidence estimates using the 2000 World Standard Population were, for BCC, 1,150 and 820 per 100,000 in males and females respectively and for SCC, 560 and 320 per 100,000 in males and females respectively (2001 data standardised to the 2000 World Standard Population) [55]. In developing countries, NMSC occur mainly in patients of European origin [70].

- Migration: In countries with high ambient sun exposure, such as Australia, the highest risk of NMSC appears in the Australian-born Caucasian individuals rather than Caucasian immigrants who moved to Australia later in life [63, 61], probably reflecting the importance of early life UV exposure.

- Occupation: Outdoor workers have a higher risk of skin cancers, particularly of SCC, than those who work indoors [63]

- Solar skin damage: Actinic or solar keratoses and Bowen's disease (partial and full thickness epidermal dysplasia respectively) are precursors of SCC [61, 71, 72] and their presence is indicative of high risk for NMSC.

- Age: Incidence of all skin cancers increases with age and they occur mainly among the elderly. NMSC is most common in those over the age of 50 years [47].

Non-melanoma skin cancers are more common in men than women, whereas MM is more common among women [47]. Other risk factors for NMSC include exposure to chemicals (principally arsenic and tar derivatives), chronic inflammation such as burns

and scars, psoralen and UV-A (PUVA) treatment or previous exposure to ionising radiation [73, 74, 69, 75, 76, 77, 52, 12, 50]. Patients with previous skin cancers or precursor lesions such as actinic keratoses or Bowen's disease are also at increased risk of developing a subsequent cutaneous malignancy [78]. Family history is also a risk factor, especially for melanoma [72, 79, 80, 81]. Patients with genetic diseases resulting in reduced repair of UV induced DNA damage or reduced melanin synthesis (xeroderma pigmentosa and albinism respectively) have a higher risk of developing skin cancers [12]. An association between smoking and SCC has been reported in some [82, 45] but not all studies [83]. Immunocompromised patients such as transplant recipients (Chapter 3) and to a lesser extent HIV sero-positive patients [45, 84, 85, 86, 87] are more likely to develop skin cancers [40]. Nutritional studies have looked at the association between diet and NMSC. It is unclear if fat intake is associated with the development of skin malignancies [88].

CHAPTER 3

Literature review: Epidemiology of squamous cell carcinoma and basal cell carcinoma among organ transplant recipients

3.1 Introduction

The purpose of this chapter is to give an overview of the published literature on the epidemiology of non-melanoma skin cancer in transplant recipients. This chapter is particularly important in assessing all factors which could potentially confound or affect the association between HPV and the development of NMSC in transplant recipients.

3.2 Limitations

Cancer Registries in the United Kingdom often fail to register non-melanoma skin cancers (ICD 10_C44) since these lesions are rarely life threatening and often not reported [1].

[1] www.statistics.gov.uk

This problem occurs in almost all countries; indeed, Cancer Incidence in Five Continents (Volume VIII) reports cancer incidence for all sites excluding non-melanoma skin cancers. For instance, since 1995 the Oxford Cancer Intelligence Unit records only the first NMSC of each type occurring in a patient. In other words a patient having a BCC and a SCC will have both recorded whereas a patient with two BCC only or two SCC will only have the first lesion recorded. Prior to this date, all lesions were recorded. However, nationally the registration of non-melanoma skin cancer is very variable with some registries only recording SCC.

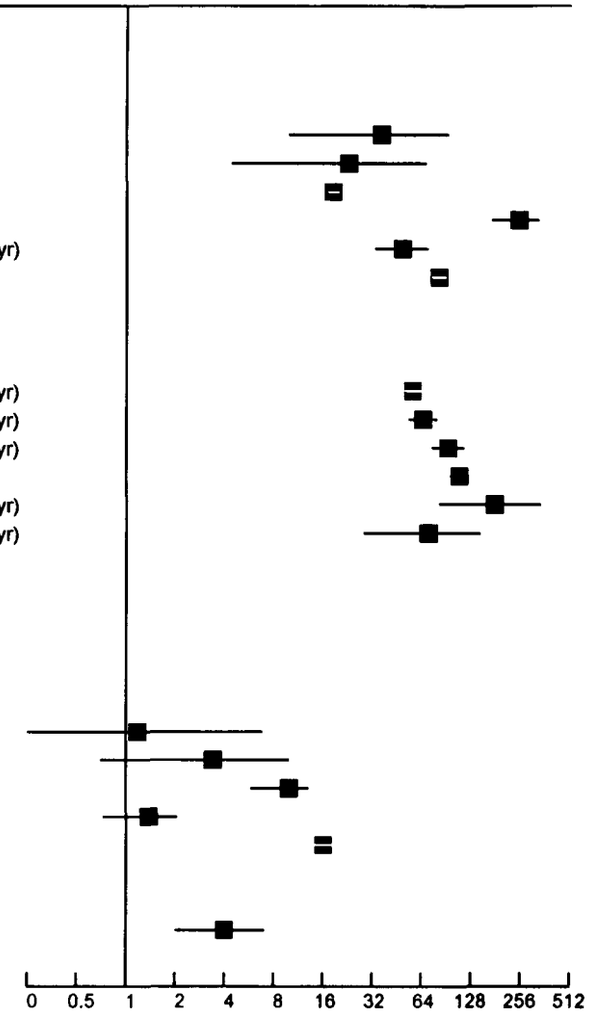
These differences between Cancer Registries imply inaccuracies and incompleteness of data which need to be kept in mind when making comparisons or interpretations. It should also be borne in mind that transplant recipients are more likely to have had a higher dermatological surveillance than the general population and to have had all their lesions recorded in dermatological clinics.

3.3 Standardised Incidence Ratios (SIR)

A systematic review was performed through a Pubmed search using synonyms for relevant words on studies reporting on SIR for SCC and/or BCC, and published up to October 2008 [search terms: basal cell carcinoma, squamous cell carcinoma, standardised incidence ratio, skin cancer, cutaneous, non-melanoma, transplant, general population], supplemented by searches of references in identified papers, by hand searches of relevant journals. No restriction was placed on language of publication. No attempt was made to identify unpublished studies or to obtain unpublished data from published studies. All published studies reporting on SCC and/or BCC were included in this review. Black squares indicate the SIR and horizontal lines represent 95% confidence interval (Figure 3.1).

HISTOLOGY/ORGAN STUDY (YEARS)	COUNT	O/E	SIR (95%CI)	No. patients	Country or city / Comparison group	Standardised by sex/age/reference years
SQUAMOUS CELL CARCINOMA						
KIDNEY TRANSPLANTATIONS						
Hoxtell et al, 1977 (68-74)	First	4/0.11	36 (10-93)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72
Kinlen et al, 1979 (70-78)	First	3/0.13	23 (4-68)	3823	UK/Birmingham CR*	Y/5yrs/74
Gupta et al, 1986 (66-84)	NK	NK	18 (17-20)	523	Toronto / Detroit	Y/1yr/77-78
Hartevelt et al, 1990 (66-88)	All	38/0.15	253 (172-334)	764	Leiden / Eindhoven CR	Y/5yrs/83-85
Gjersvik et al, 2000 (83-92)	NK	NK	49 (33-70)	1020	Norway / national CR	Y/1yr/calendar(1yr)
Moloney et al, 2006 (94-01)	All	282/3.4	82 (73-91)	1558	Ireland / national CR	Y/1yr/94-2001
OTHER ORGANS +/- KIDNEY TRANSPLANTATIONS						
Adami et al, 2003 (70-97)	First	278/4.95	56 (50-63)	5931	Sweden / National CR	Y/1yr/calendar(1yr)
Jensen et al, 1999 (63-92)	First	97/1.49	65 (53-79)	2561	Oslo / National CR	Y/1yr/calendar(1yr)
Lindelof et al, 2000 (W) (70-94)	All	77/0.8	93 (73-116)	5356	Sweden / National CR	Y/1yr/calendar(1yr)
(M)		248/2.3	109 (95-123)			
Gjersvik et al, 2000 (83-92)	NK	NK	177 (81-336)	148	Norway / national CR	Y/1yr/calendar(1yr)
Haagsma et al, 2001 (79-96)	First	7/0.1	70 (28-144)	174	The Netherlands / Mixture**	Y/1yr/calendar(1yr)
BASAL CELL CARCINOMA						
KIDNEY TRANSPLANTATIONS						
Kinlen et al, 1979 (70-78)	First	1/0.85	1.2 (0-7)	3823	UK / Birmingham	Y/5yrs/74
Hoxtell et al, 1977 (68-74)	First	3/0.88	3 (0.7-10)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72
Hartevelt et al, 1990 (66-88)	All	18/1.81	10 (6-15)	764	Leiden / Eindhoven CR	Y/5yrs/83-85
Gupta et al, 1986 (66-84)	NK	NK	1.4 (0.7-2)	523	Toronto / Detroit	Y/1yr/77-78
Moloney et al, 2006 (94-01)	All	143/8.9	16 (14-18)	1558	Ireland / national CR	Y/1yr/94-2001
LIVER TRANSPLANTATIONS						
Aberg et al, 2008 (82-05)	NK	11/2.97	4 (2-7)	540	Finland / national CR	Y/5yr/82-2005

SIR & 95% CI



* CR= Cancer Registry; ** Mixture= Eindhoven before 1989 and National Cancer Registry after 1989
Y= yes; NK: not known; O= observed; E= expected; yr= year; No= number; CI= confidence interval

Figure 3.1: Standardised Incidence Ratios (SIR)

Eleven studies have compared SCC and BCC incidence rates among transplant recipients with those from the general population [89, 23, 28, 39, 17, 32, 34, 37, 20, 43, 44]. Studies have been undertaken in Europe, the USA and Canada. All of them have found that transplant recipients are at increased risk of developing NMSC compared to the general population. This result is mainly driven by a strong and statistically significant increased risk of SCC. Rate ratios for SCC and BCC lie respectively between 18 and 250 and between 1.2 and 16.

Several dissimilarities due to study design and methods used might possibly explain these variations.

- **Counting lesions**

  Highest SIRs have been found in two studies which counted each new tumour in an individual separately, as compared to other studies which only counted the first tumour of each type [32, 37]. They found respectively a 250-fold and 100-fold increased risk in transplant recipients for SCC lesions. The highest risk for BCC tumours was also found by the Dutch study with a 10-fold increased risk in transplant recipients compared with the general population. Some studies do not specify how lesions were recorded in their comparison group.

- **Organ type**

  SIRs appear higher in studies which looked at organ types other than kidney alone [23, 28, 37, 20]. Risk ratios for SCC lie between 56 and 177. However, 95% confidence intervals are wide and overlap with those from studies based on kidney transplants only. None of these studies collected information on BCC lesions.

- **Choice of the "standard population"**

  Expected rates of NMSC are estimated from a variety of difference sources, such

as:

- **National cancer registries**

  Of the six studies which used their national cancer registry data, five are from Scandinavian countries [89, 23, 37, 20, 44]. This predominance is explained by their longer history of cancer registration. In Finland, for instance, this started in 1952 [2]. Scandinavian studies compared the observed number of cases with expected numbers based on national calendar year, age-, and sex-specific incidences.

- **Combination of national and city cancer registries**

  One study has been undertaken in the Netherlands where National Cancer Registration started only in 1989. Haagsma *et al.* (2001) used the Eindhoven Cancer Registry as a comparison group for the period before 1989 [28].

- **City or regional cancer registries**

  Kinlen *et al.* (1979) used the Birmingham Cancer Registry as a good representation of the UK population [17]. Two other studies used cancer registries with the same latitude: Detroit Cancer Registry for the study undertaken in Toronto and Eindhoven Cancer Registry for the study based in Leiden [34, 32].

- **National Surveys**

  Hoxtell *at al.* (1977) used a cancer survey to derive their ratios [39].

- **Inclusion of lips cancers**

  One study grouped NMSC and lips cancers [28] and 2 other studies did not specify clearly if lips cancers were included in their calculations [20, 23].

[2] http://www.cancerregistry.fi/v2001/v2001introduction.html

Excess risks of other cancer sites has also been shown among transplant recipients. For example, increased SIRs have been found for NHL and KS. NHL is 5 to 49 times more common in transplant recipients than in the general population and Kaposi sarcoma is between 150 to 500 times more frequent [16, 17, 18, 26, 30, 20, 27, 29, 36, 31, 25, 42, 41, 40, 43].

3.4 Ratio SCC/BCC

In Australia, the ratio of SCC to BCC was estimated to be approximately 1:4 in the general population in 1985 [57], although Staples *et al.* (1998) indicates a possible shift towards SCC with a ratio of 2.5:1 in 1998 secondary to a reduction in BCC occurrence in younger people [56]. Studies around the world have reported a wide range of estimates. For instance, a ratio of 1:3.7 was obtained among patients from Hong Kong and 1:6.8 in southern European countries [90, 91]. SCC skin lesions are predominant in organ transplant recipients. As a result, a reversed BCC to SCC ratio is often observed in immunosuppressed people compared with the general population. Figures have to be compared across studies with caution, as the way of counting lesions and the length of follow-up vary from study to study. For instance, Mithoefer et al. (2002) examined 151 liver transplant patients who developed 56 squamous cell (in 23 patients), 23 basal cell (16 patients) and 7 melanomas (in 6 patients). Ratio SCC/BCC could be reported as 2.4 (56/23) or as 1.4 (23/16) [92]. Ratios (SCC/BCC) range between 0.3 and 16 when multiple lesions in one person are counted separately and from 0.6 to 3.5 using when only the first lesion per person is considered. Transplant recipients with skin cancers have often already developed multiple skin lesions at the time of their first skin examination. Hardie *et al.* (1980) found that half of their patients who were examined every 3 months had multiple skin lesions at first diagnosis [22].

Two outstanding results arise from an Australian and a Scottish study where SCC was respectively 16 and 15 times more frequent than BCC [14, 93]. These two studies considered the total number of lesions in respectively 7 and 10 patients only. Curiously, some Spanish and Italian studies did not find the expected reversed ratio although there was still a higher proportion of SCC than in the general population [94, 95, 96, 97, 98, 99].

In summary, the SCC to BCC ratio appears difficult to quantify precisely due to the occurrence of multiple lesions, difference in follow-up time and mean age of the cohort. Nevertheless, the prevalence of SCC appears exceptionally elevated when compared to the general population and exceeds the number of BCC.

3.5 Cumulative incidence

The cumulative incidence of SCC and BCC in transplant recipients increases sharply with the time since transplantation [100, 101, 102, 103, 23, 33, 32, 97]. However, dissimilarities between these tumour types are observable (Figure 3.2). Cumulative incidence for SCC increases more sharply with time than for BCC.

Hartevelt *et al.* (1990) found that the cumulative risk at 20 years after transplantation for SCC and BCC were respectively 35% and 10% [32]. This result is supported by a British study which found that the risk of developing SCC post transplantation appears to increase exponentially whereas the risk of developing BCC seems to increase linearly with increasing years of immunosuppression [100]. Jensen *et al.* (1999) reported a more rapid rise in the cumulative incidence for SCC in heart rather than in kidney transplant recipients. At ten years after transplantation, the cumulative incidence for SCC in heart and kidney transplant recipients was respectively 21% and 7% [23]. No studies relating to transplantation of other organs (such as liver) are available for comparison.

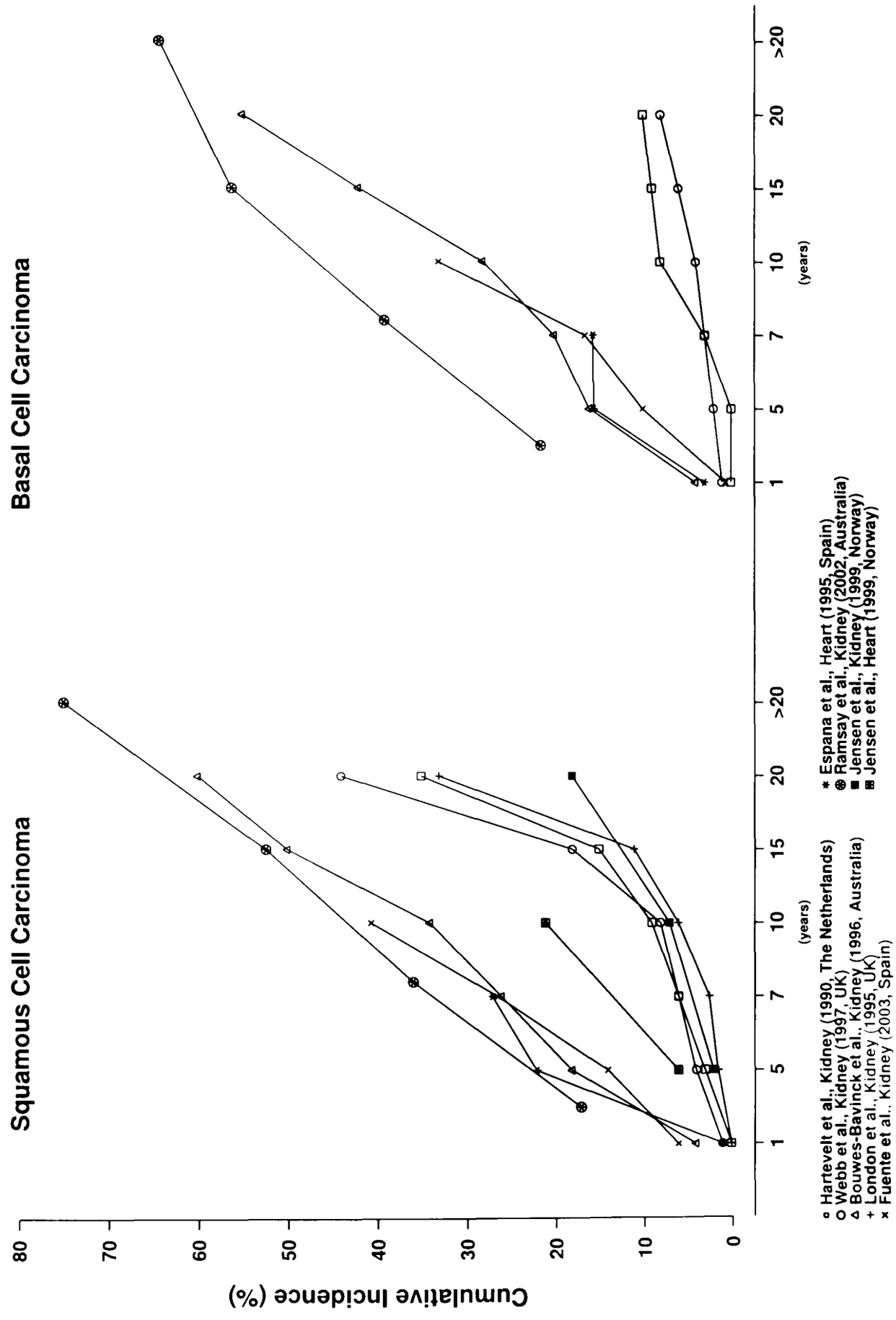


Figure 3.2: Cumulative incidence rates after transplantation for SCC and BCC

3.6 Multiplicity, recurrence and aggressiveness

Almost all studies of NMSC among transplant recipients, show that patients tend to have multiple tumour lesions, with a higher recurrence rate than in the general population. In addition, SCC in particular, tend to behave more aggressively. For instance, Hoxtell *et al.* (1977), Lindelof *et al.* (2000) and Mc Gregor *et al.* (1995) found respectively on an average of 1.9, 2.4 and 3.8 skin malignancies per patients [39, 37, 104]. In Blohme *et al.* (1984) two patients develop several hundred lesions each over a period of several years [36]. In Australia, Bouwes-Bavinck *et al.* (1996) found 2751 NMSC in 271 patients [103]. The prevalence of patients with multiple skin lesions varies from study to study due to differences in length of follow-up and age of patients. It ranges between 26% and 73% [92, 22, 32, 34, 36, 37, 105, 106]. Euvrard *et al.* (2007), who followed their patients for 5 years, reported that 71% of them developed at least two lesions with an overall mean number of SCC higher in kidney than in heart transplant patients [107]. Some studies also found that patients had multiple skin lesions at first presentation [22, 34]. It is also very common to find several skin cancers with different histological types in the same patient. Bouwes-Bavinck *et al.* (1996) found in their study that 83 patients had SCC, 52 patients had BCC and 136 had both types [103]. Transplant-associated SCC are more frequently recurrent and/or aggressive in behaviour than SCC in the general population [108, 109, 100, 110, 111, 112, 113]; similarly, patients with multiple lesions appear to get more aggressive SCC [114].

3.7 Mortality and survival

The principle causes of mortality among transplant patients are cardiovascular diseases (40-55% of all death causes) followed by malignancies and infections (15-20% each)

[115]. As described earlier, SCC are more likely to progress to metastasis among transplant recipients compared with the general population and hence, mortality rates from SCC are higher in this population. Ong *et al.* (1999) reported that 11 patients out of 152 died from skin cancers (6 SCC, 4 MM, 1 Merkel Cell carcinoma) representing 27% of all deaths after 4 years (15% for SCC) [116]. McGregor *et al.* (1995) found that 3% of patients diagnosed with skin cancer, subsequently died of metastatic SCC (2 out of 59) [104]. In an Australian study, of the 19 patients diagnosed with NMSC, 8 died from the disease and 5 others relapsed, but were alive at the end of follow-up [117]. Jain *et al.* (1998) found one and three years survival of 91% and 86% for liver transplant recipients with skin cancer [118]. Adamson *et al.* (1998) classified BCC and SCC as aggressive and non-aggressive tumours. Survival figures were 100% at 1 year, 88% at 3 years, 75% at 5 years and 53% at 7 years for aggressive skin tumours and respectively 100%, 100%, 82% and 82% for so-called non-aggressive tumours [110].

3.8 Age at transplant and time from transplant in relation to the first appearance of NMSCs

In immunocompetent people, NMSC occur most frequently in people in their 60s and 70s or older whereas in transplant recipients lesions develop on average 15-20 years earlier [46, 119, 113]. This trend is observed in all studies.

As might be expected, there is a correlation between the age of the patient at transplantation and the time from transplantation to the time to development of the first skin tumour - a coefficient of correlation of 0.4 and 0.3 was found [99, 105]. There is some evidence that BCC occur on average between one and two years earlier than SCC [116, 102, 98, 120, 121] but, only for the study by Ong *et al.* (1999) this difference

was statistically significant [116]. Mihalov *et al.* (1996) found the same result in heart transplant recipients (about 2 years earlier) but not in kidney transplant recipients where the opposite trend was noticed (about 1 year later) [121]. No difference was found between SCC and BCC onset in a Canadian study (median: 4 years for both type of lesions) [122]. Post transplant lymphoproliferative diseases (PTLD) and KS occur on average earlier than skin cancers after transplantation. KS appears the earliest on average between 9 and 27 months after transplantation followed by PTLD which occurs on average between 33 and 78 months [123, 124, 125, 126, 127, 128, 129, 130, 131]. Non-Hodgkin's Lymphoma (NHL) occurs most frequently in the first year after transplantation and its incidence then falls and remains constant thereafter [17, 132, 127]. KS is a rare neoplasm in western populations found in HIV infected homosexual men and among individuals of Mediterranean or African origin [133]. Webb *et al.* (1997) reported KS cases only in Afro-Caribbean and Mediterranean transplant patients [100]. Highest prevalence of KS in transplant recipients is found mainly in the Middle-East or regions where the underlying viral cause - HHV8 - is endemic [134, 135, 124, 125, 136, 21]. The highest prevalence has been observed in Saudi Arabia, where it comprises 88% of the total number (14/16) of post transplant tumours [137]. High prevalence is also seen in Iran, Pakistan, South Korea and Russia with KS representing around 45% of all post-transplant tumours [135, 126, 138, 139, 140].

In summary, PTLD and KS appear earlier than NMSC in transplant recipients and the older the patient the earlier the first skin cancer occurs with BCC probably appearing on average earlier than SCC.

3.9 Risk factors (excluding human papillomaviruses)

3.9.1 Design of studies

Most studies that looked at risk factors associated with the development of post-transplant SCC and BCC used retrospective cohort designs. Eligible patients contributed person years from the date of transplantation until the date of registration of skin cancers, or the end of follow-up. However, for many studies the date of the end of follow-up is not clearly stated [120, 101, 89, 121]. Few studies were based on case-control design [141, 142, 143, 101].

3.9.2 Solar and ultraviolet (UV) exposure

Sun exposure

Almost all studies have reported a significant positive association between skin cancers and exposure to UV radiation in transplant recipients [99, 105, 19, 101, 92, 142, 144, 145, 91, 146, 147, 81, 143, 148]. These studies looked at different variables to quantify sun exposure such as sunbathing, episodes of childhood sunburn, outdoor occupation, holidays abroad, sunscreen use, number of sunburns, number of painful sunburns or use of sunbeds. Outdoor occupations were associated with development of skin cancers in six out of eight studies [141, 19, 101, 92, 111, 95, 36, 81]. Patients with SCC have reported heavier cumulative sun exposure than those without [114, 105, 149, 91].

Bouwes-Bavinck *et al.* (1993) looked at the risk of developing NMSC in three different levels of sunlight exposure ($\leq$10,000 hours, 10,000-20,000 hours, 20,000+ hours). Patients in the highest sun exposure group apparently had a greater risk of SCC than BCC but 95% confidence intervals were very wide and overlapping. This result was stronger in multivariate analysis after adjusting for sex, age at examination, skin type and number

of keratotic lesions. The adjusted odds ratios comparing the highest sun exposed group versus the lowest one was 97.5 (only 14 cases and 59 controls; 95%CI: 6.6-1444) for SCC and 49.3 (95%CI: 2.8-878) for BCC [142]. This result was corroborated by Ramsay *et al.* (2000) and Rosso *et al.* (1996) where SCC showed a significant positive association with increased cumulative sun exposure whereas BCC did not reach statistical significance [101, 91]. One study found that in univariate analysis, patients with BCC and patients without skin cancers had the same cumulative exposure to sunlight [105].

Mithoefer *et al.* (2002) and Bouwes Bavinck *et al.* (1993) found that, the number of painful or second degree sunburns increases the risk of developing both SCC and BCC [92, 142]. This result was stronger when sunburns occurred between the age of 12 and 29 but histology types were not examined separately [142]. This result was corroborated by 2 other studies; a multicentre study where the risk of developing SCC and BCC was double in patients with 5 or more sunburns before the age of 20 compared with those who never burn [143] and in an Australian study where a 3-fold increase risk for SCC and BCC was reported between patients who frequently burned during their childhood and those who never or rarely burned [143]. Mithoefer *et al.* (2002) did not find any association with childhood sun exposure [92]. Only one study has not reported an association between skin cancers and sun exposure [150].

Location of skin malignancies

Most studies found that skin lesions in transplant recipients are more frequent on sun-exposed areas of the body [105, 98, 151, 101, 92, 22, 95, 116, 34, 39, 14, 120, 111, 114, 152, 32, 36, 37, 142, 93, 70, 153, 119]. The distribution of skin malignancies differs by histology types. SCC appear predominant on the head, neck and dorsum of hands whereas BCC are also frequent on the trunk and legs [111, 114, 101, 152, 32, 37, 120,

142, 119, 106, 154, 122]. Gupta *et al.* (1986) did not find any difference between the distribution of the body location of skin cancers in transplant recipients and the general population [34]. Staples *et al.* (1998) in their third Australian National Survey reported that 49% and 40% of BCC and SCC respectively occurring on the head and neck. They also reported a higher proportion of BCC on the trunk (21%) than SCC (8%) [56].

The location of NMSC appears different between men and women. Male patients tend to have more lesions on the head and neck whereas women are more susceptible to have lesions on their trunk [119]. In transplant recipients, aggressive tumours were also more frequent on the head than other body sites [110, 114]. Euvrard *et al.* (1995) found that for kidney transplant recipients who were less than 40 years old at transplantation skin cancers on the head represent 19% of the total number of lesions (181/225 lesions in 45 patients) whereas the older patients had 67% of their skin lesions (120/179 lesions in 43 patients) located on the head ($P \leq 0.001$). This group also found 70% of malignant skin lesions on the head of heart transplant recipients and 59% of skin lesions on other body locations in kidney recipients ($P \leq 0.001$) [111]. This may reflect the older age of heart compared with renal transplant recipients. The location of skin malignancies reflects the importance of sun exposure and highlights the aetiological differences between SCC and BCC.

Skin, hair and eye colour

The Fitzpatrick classification is the most commonly used to identify the skin type and is based on pigmentation and tanning ability. It can be described as follow:

- Skin type I - Very fair skin or freckled, always burns, never tans
- Skin type II - White, usually burns easily, tans with difficulty

- Skin type III - White to medium skin tone, sometimes burns, tans gradually
- Skin type IV - Medium skin tone, rarely burns, always tans well
- Skin type V - Olive to dark skin tone, very rarely burns, tans very easily
- Skin type VI - Black, never burns

In most studies, but not in all [122], skin type VI and sometimes type V were excluded as no skin cancers occurred in these groups [19, 141, 100, 155]. Euvrard *et al.* (1995) used eye colour as a better marker of skin type since transplant recipients' skin colour might become darker after immunosuppressive treatments [111, 156]. Many studies examined the relationship between skin type, hair and eye colour and risk of developing skin tumours in transplant recipients [99, 105, 141, 101, 92, 111, 102, 116, 95, 142, 144, 81, 143, 122]. As expected, patients with skin types I and II have a statistically significant greater risk of NMSC than types III and IV [99, 105, 116, 147, 143]. All 14 cases in Espana *et al.* (1995) were type II or III but no comparison with the skin type distribution in the control population was reported [102]. Bouwes Bavinck *et al.* (1993) found that patients with skin type I and II have a higher risk of SCC, but not BCC, in comparison with patients with skin types III or IV [142].

Transplant patients with light-coloured eyes are also at higher risk for skin cancers [111, 101, 92, 143] especially of developing SCC rather than BCC or premalignant lesions [111, 101, 19]. Regarding hair colour, Lindelof *et al.* (2003) found people with light blond or red hair were at greater risk of developing skin cancer than people with dark hair [141]. Having red hair was also associated with increased risk of skin cancers in transplant recipients in another study but the sample size was very small [92], but not all studies report such an association [122]. Results from transplant recipients are in accordance with those from the general population.

3.9.3 Age at transplantation

All studies but one found that patients who develop NMSC are significantly older at the time of transplantation than recipients without NMSC [99, 105, 98, 19, 101, 92, 116, 95, 94, 32, 36, 29, 103, 120, 142, 157, 143, 148]. These results seem independent of the type of the organ transplanted and the histological type of NMSC [98, 101]. Naldi *et al.* (2000) found a nine fold increased risk in patients of 50 years or more compared with those less than 30 years old at the age of transplantation (95%CI: 4.3-20.5). This result was adjusted for age, sex, treatment types and graft organs [98]. Another study based on multivariate analyses adjusted for sex, skin type, eye colour, treatment type and primary sclerosing cholangitis found a 15% increased risk of developing skin cancer for each year of age older patients were at time of liver transplantation (P=0.0003) [92]. Surprisingly, Adami *et al.* (2003) did not support this finding their results were adjusted for follow-up time, gender and type of organ. Patients less than 40 years old were 4 times (95% CI: 2.7 to 5.4) more likely to develop NMSC than people older than 60 years and patients between 40 and 60 years old were 1.5 (95% CI: 1.0 to 1.9) more likely to develop NMSC than the same reference group [20].

3.9.4 Sex

Of the 21 studies which looked at the association between sex and skin cancers in transplant recipients, nine did not report any association [105, 141, 152, 95, 94, 20, 142, 144, 157]. Ong *et al.* (1999) found a significant higher risk in males in univariate analysis it was no longer statistically significant after adjustment for age at transplantation, follow-up time, skin type, occurrence of HLA-mismatching and residence [116]. Six studies found a significantly higher risk of skin cancer in men in univariate analysis and did not examine multivariate analysis [111, 101, 35, 158, 146, 147]. Four studies found a significant higher

risk in males in univariate and multivariate analysis. Their analyses were adjusted for age at transplantation and:

-follow-up time and organ type [103]

-eye colour, hair colour, primary sclerosing cholangitis and use of ciclosporin [92]

-organ type and different type of treatments [98]

-creatinine at 1 year, donor type and length of immunosuppression [120]

-not specified [122]

Very few studies have reported on the risk of BCC and SCC for men and women separately [99, 141, 95, 143, 101, 98, 103]. None of the studies controlled for sun exposure.

3.9.5 Type of transplantation

Six studies have examined the association between the risk of developing BCC and/or SCC and the type of transplanted organs [105, 98, 89, 111, 20, 143]. Three of them did not find any association between NMSC and type of organ after controlling for different confounding variables. Adami *et al.* (2003) looked at kidney versus all other organ types and adjusted their analyses for age at transplantation and follow-up time [20]. Naldi *et al.* (2000) and Fortina *et al.* (2000) looked at kidney versus heart transplant recipients in multivariate analysis adjusted respectively for age, sex and type of treatment and for age, sex, type of treatment, type of skin, sun exposure, presence of keratosis and warts [98, 105]. A recent multicentre case-control study reported no association between the type of organ and the development of either BCC or SCC [148].

Two Norwegian studies based on the same data found respectively a 2.8 (95% CI: 1.2-6.7) and a 2.9 (95%CI: 1.3 - 6.2) fold increased risk of SCC in heart transplant recipients compared with kidney transplant recipients after controlling for age at transplantation and type of treatment [23, 89]. Euvrard *et al.* (1995) found a higher frequency of tumours in

heart than kidney transplant recipients without controlling for any variables [111].

3.9.6 Number of rejections and number of transplantations

- *Number of rejections*

  All studies but one did not find any association between the number of rejections and the development of NMSC in transplant recipients [110, 98, 19, 101, 92, 116, 120, 94, 159].

- *Number of transplantations*

  All studies that examined the risk of developing NMSC with increased number of transplantations did not report any association [101, 94, 70].

Patients with higher number of rejections or transplantations might actually have spent less time on immunosuppressive therapy and might therefore be at lower risk of skin cancer.

3.9.7 Actinic keratoses (AK)

Actinic keratoses are pre-malignant skin lesions which are caused by long-term sun exposure and are considered to be a precursor lesion of SCC. They consist of epidermal dysplasia affecting one to two thirds of the epidermis and are regarded by some as in situ SCC [93]. In one Australian study, fewer than 1/1000 AKs progressed to SCC [71]. Studies on transplant recipients reported similar findings for AKs to the general population. Thus, AKs are more common on sun-exposed areas [101, 142, 95] and their number increases with time since transplantation [145]. A significantly higher number of AKs are found in skin cancer patients in particular those with SCC [19, 149, 145, 160, 161, 81, 99, 142]. In the Netherlands, Bouwes-Bavinck *et al.* (1993) found a strong significant association with number of keratoses (AK and other warty keratoses) and the presence of SCC

after controlling for sex, age at physical examination and skin type. Patients with more than 100 keratotic skin lesions were 21 times more likely to develop skin cancers than patients with fewer than 50 keratoses (95%CI: 5.3-81.7). Odds ratios for patients with SCC and BCC were respectively 54 (95% CI: 8.2-351) and 9 (95% CI: 2.0-43.8). The analysis was based on 29 patients with SCC, 16 with BCC and 96 controls. This study looked also at risk factors associated with development of keratoses in transplant recipients and did not find any association with cumulative exposure to sunlight and with the number of episodes of painful sunburns [142]. An Australian study found that the number of AKs was clearly associated with outdoor occupations and fair skin colour [160]. A recent multicentre case-control study based in the Netherlands, United Kingdom, Germany, France and Italy confirmed the strong association between the presence and number of keratoses (AKs and other warty keratoses) and the development of SCC and BCC in transplants patients [148]. The development of keratoses (AK and other warty keratoses) was clearly associated with time since transplantation, age and fair skin.

3.9.8 Viral warts and non-HPV cutaneous infections

- *Viral warts*

  Viral warts are benign hyperproliferative epithelial skin lesions induced by human papillomavirus (HPV) and, are particularly common among transplant recipients. Their prevalence is very high and increases with time since transplantation [101, 95, 35, 156, 149, 93, 151, 162]. Barr *et al.* (1989) found that 20% of patients with 5 or less years of follow-up developed viral warts and 77% of patients with more than 5 years of follow-up ($P \leq 0.001$) [93]. In a French study looking at 152 patients, the prevalence of viral warts was 16% at the time of transplant, 23% at one year, 35% at 3 years, 45% at 5 years and 54% at 7 years [162]. Viral warts are more

frequent on sun-exposed areas of skin and on patients who had a higher lifetime exposure to sunlight [102, 149]. Skin warts are often multiple, resistant to treatment and recurrent [35, 93, 162, 144, 149] and are sometimes difficult to distinguish from other keratotic lesions such as actinic keratoses and seborrhoeic keratoses [93, 148]. Koranda *et al.* (1974) noticed that nearly all patients with warts report a history of having had them in childhood [156]. Most studies found an association between the presence of warts and the development of skin cancer in transplant recipients [19, 151, 141, 101, 148, 122]. However, the evidence suggests that warts are associated with the development of SCC but not with BCC [148, 105, 36].

- *Non-HPV infections*

Immunosuppressed patients are susceptible to many infections such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis B and C viruses (HBV, HCV), herpes simplex virus (HSV) and varicella-zoster virus (VZV) [163]. Very few studies have reported results on infections other than HPV and their association with the development of skin cancers [164]. Kelly *et al.* (1985) tested sera for antibody against HSV1, CMV, EBV, VZV, Adenovirus, Influenza A and Influenza B. Mean log-titres were compared between 6 groups: controls patients matched for age with transplant recipients, haemodialysis patients, renal transplant recipients without cancer up to one year post-transplant, renal transplant recipients without cancer one to two years post-transplant, renal transplant recipients with NMSC three to eight years post-transplant. Differences in means were found for CMV and EBV with higher mean log titre in patients who underwent renal transplantation ($P \leq 0.01$), interpreted as reflecting viral reactivation leading to a rise in antibody titres. No association with any infections and development of NMSC was found [164]. Boyle *et al.* (1984) found that renal transplant recipients have significantly more herpes zoster infections and

fungal infections than a control group matched for age and sex. They did not find any difference for HSV. However, no analysis of patients who developed skin cancers was performed [149]. Koranda *et al.* (1974) observed a higher prevalence of skin infections in transplant recipients: 43% had verrucae, 35% herpes simplex, 13% herpes zoster and 18% tinea versicolor [156].

Following a recent report on a possible association between polyomavirus and Merkel cell carcinoma [165], Ridd *et al* (2008) examined 85 SCC and 37 KA for the presence of polyomvirus DNA and found that it was rare in both groups, with no difference between them [166].

3.9.9 Immunosuppressive drug treatment

It is extremely difficult to identify which drug or combination of drugs, if any, is associated with the development of skin cancers. This is due to the high variation in doses and types of immunosuppressive drugs and to the introduction of new treatments over time. It is also problematic to assess the total intensity of immunosuppression in a given individual for a specific drug regimen, as this may not depend exclusively on dose. Furthermore, improvement in techniques over time such as HLA matching and awareness of the danger of sun exposure complicates the task of evaluating the impact of immunosuppressive drugs on skin cancer risk, and the interpretation of results presented in the literature has to be made with caution [103].

The Introduction of ciclosporin (1981)

Before the introduction of ciclosporin, the conventional immunosuppressive regimen was azathioprine and steroids (CONV). Ciclosporin was then either used alone or in combination with CONV. From 1986, triple therapy based on azathioprine, ciclosporin and steroids

was the preferred choice (TRIPLE). Most studies have therefore looked at the introduction of ciclosporin as a risk factor for the development of skin cancers in transplant recipients. These studies can be classified into 3 groups:

those which compared-

- Ciclosporin +/-steroids *versus* CONV [103, 150, 167, 168, 23, 169, 170, 112, 171]
- Ciclosporin +/-steroids +/-azathioprine *versus* CONV [157, 103, 172, 171, 168, 23, 169, 173, 174]
- Ciclosporin +/-steroids *versus* TRIPLE [171, 168, 23, 169]

There is no evidence of an increased risk of NMSC due to ciclosporin (+/- steroids) treatments compared with CONV [103, 167, 23, 168, 169, 170, 112, 171]. Bunney *et al.* (1990) took follow-up time into account as patients on azathioprine have been followed longer than ciclosporin users and found the same prevalence of skin malignancies in both groups [170]. The only significant positive association was from a study based only on 63 patients taking ciclosporin and 33 patients taking azathioprine. Furthermore, the association was reported only for a specific time period after transplantation (37-48 months) [150].

A randomised trial of 231 patients comparing low and normal doses of ciclosporin found a higher number of skin cancers ($p \leq 0.05$) in the group with higher doses after controlling for azathioprine dose. This study also found an increased risk for KS in normal dose of ciclosporin users compared with those on lower doses [175]. A Turkish study reported an increased incidence rate for KS with ciclosporin users compared with non-users but not for other cancers [21]. Penn *et al.* (1991) reported also a higher incidence of NHL and KS in ciclosporin users [176]. In several studies, an earlier development of skin cancers or other cancers in ciclosporin users has also been reported [177, 157, 30, 174]. However,

this result might be confounded by age [177].

Patients on TRIPLE appear at higher risk of developing NMSC than patients using ciclosporin either alone or with steroids or those using CONV [169, 23, 168, 174, 173]. This result seems stronger in patients with SCC than BCC [173, 169]. Jensen *et al.* (1999) found a 3-fold increased risk of skin cancers in TRIPLE versus CONV users (95%CI: 1.4-5.3) after controlling for age at transplantation and type of transplantation [168]. Glover *et al.* (1997) supported this result with a 3-fold increased risk for NMSC. After stratifying by histological types, they found that TRIPLE users where eight times more likely to develop SCC than patients under CONV (95%CI: 1.3-54.8) and found a non significant higher risk for BCC. No adjustment variables were listed (e.g. age at transplantation, sunlight exposure, fair complexion) [173]. In contrast, three studies did not report increased incidence rates of NMSC in TRIPLE users versus bitherapy users [105, 103, 171]. Blohme *et al.* (1992) compared crude prevalence at 5 years post transplantation and did not find any differences [171]. In addition, Bouwes-Bavinck *et al.* (1996) did not find also any increased risk of skin cancers in TRIPLE versus CONV users after controlling for age and sex of the patients [103].

Sirolimus (Rapamycin) and tacrolimus (FK 506)

At one year after transplantation, tacrolimus is prescribed in 59% and 29% of new kidney tranplant patients in the USA and in Australia respectively [178, 179]. The Cochrane review comparing "tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant" includes 123 reports of 30 trials (4102 patients). No difference in the incidence of new malignancies was found between the two groups up to five years after transplantation. The follow-up time was probably too short to detect any differences in skin cancer occurrences [180]. To my knowledge, there is no meta-analysis of the im-

pact on therapy on skin cancer among OTR. A recent randomized controlled trial based on 121 liver transplant recipients receiving either tacrolimus and steroids or a quadruple regimen (ciclosporin, azathioprine, steroids and antithymocyte globulin) reported an increased number of malignancies in ciclosporin users after a 12 year follow up. However, results on NMSC were not detailed. A better graft survival was clearly found for patients under tacrolimus, but the use of antithymocyte globulin at the start of the ciclosporin regimen was thought to be the main reason for the higher occurrence of malignancies in this group [9]. Several studies have suggested a decreased risk of malignancy in patients using treatment based on sirolimus [181, 182, 183]. However, the short follow-up time does not allow reliable conclusions [183].

Mycophenolate mofetil

To my knowledge there is no study that has looked at the association of mycophenolate mofetil and the development of NMSC.

Other medications

The monoclonal antibodies OKT3 used to treat acute rejection in heart transplant recipients has been associated with an increased risk of lymphomas [184, 185, 186] but results vary [187, 188]. Two studies have reported an increased risk of skin malignancy in OKT3 users compared with never users [188, 44]. Another study in immunocompetent people found a higher risk of SCC and BCC in corticosteroid users compared with their age and sex matched controls [189].

Summary

There is not enough evidence that ciclosporin increased the incidence of skin cancers in transplant recipients compared with the conventional therapy. However, the addition of ciclosporin to CONV may increase the risk of developing NMSC. The overal intensity of immunosuppression rather than the specific drugs used might be the most important

factor for development of skin cancers but it is difficult to quantify [103, 190]. In relation to sirolimus, the short follow up does not allow definite conclusions. Confounding variables such as age, follow-up time and sun exposure would also have to be taken into account to examine associations between medication and skin cancers.

3.9.10 Donor characteristics

Studies examining the association between donor characteristics and development of skin cancers in transplant recipients are sparse. This might be due in part to publication biases i.e negative results were not published, but also to issues relating to confidentiality and consent.

ABO blood group

Three studies have reported results on the risk of skin cancer according to donor ABO blood group in renal transplant recipients, but no associations were identified [120, 96, 17]

Donor age

The use of elderly donors is becoming more frequent [191]. In renal transplant recipients, age of donor has been associated with a greater incidence of delayed graft functions, chronic allograft nephropathy, increase of cardiovascular disease and therefore worse survival [191, 192, 193]. Two studies mentioned age of donor but did not report any association with development of skin malignancies [120, 96].

Donor sex

No association has been reported with skin malignancies [120].

Living or cadaveric donor

Controversial results have been reported. Gruber *et al.* (1994), Ramsay *et al.* (2000) and Roeger *et al.* (1992) did not find any increased risk associated with the donor/recipient relationship [101, 157, 169]. However Bordea *et al.* (2004) found that renal recipients with living donors are at lower risk of developing skin cancers. This result remains statistically significant after controlling for age at transplantation, sex of the recipient, length of immunosuppression and blood creatinine levels (OR=0.34 & 95%CI (0.14-0.82); p=0.02) but this result might be confounded by sun exposure [120]. It could also be argued that a better recipient/donor match implies a lower immunosuppressive treatment and hence a lower risk of skin cancer however living donor are not always well-matched and therefore might require higher immunosuppressive drugs.

Transmission from donor

Infections and malignancies can be transmitted from donor to recipient. Low risk of malignancy transmission has been reported [194, 195]. Birkeland *et al.* (2002) reported 0.2% prevalence of transferring a cancer and 1.3% prevalence of having a donor with undetected malignancy [194]. Transmission of melanoma from a donor has been reported [194, 196, 197] but there is no epidemiological evidence of BCC or SCC transmission from a donor. However, two recent studies suggested that a BCC and some KS-associated HHV8 could have originated from non-malignant cells transmitted from the organ donor [198, 199]. These studies examined tumours in female renal recipients who received a kidney from a male donor.

Human Leukocyte Antigens

Since the discovery of the HLA system in the 1950s, new HLA sequences are continuously being recognised. Consequently, early HLA typing might lack class II antigen results. Human Leukocyte Antigens (HLA) have been examined in two different ways to study their association with NMSC in transplant patients. Some studies have looked at HLA frequencies in patients with NMSC and those without [200, 201, 202, 203, 116, 204, 102, 152, 205, 206]; other studies have looked at the degree of mismatch between donor and recipient as a potential risk factor [101, 102, 152, 203, 169, 116, 23, 17, 116, 207]. Studies are generally quite small and only two enrolled more than 100 cases [116, 203].

- Frequency of HLA sequences

  Conflicting results have been published on the association between HLA frequencies and BCC [200, 201, 202, 203, 116, 204, 102] or SCC [203, 201, 202, 116, 152, 205, 169, 204, 102] or all skin cancers [201, 202, 206, 203, 208, 116, 205, 102] or NMSC specifically [209]. Heterogeneous control groups have also been used (either transplant recipients without skin cancers or healthy donors from the general population). Moreover some studies performed multiple tests for association without taking into account chance findings. A few studies corrected their P-values for the number of performed tests [203, 201, 206, 203, 205] or specified that their HLA choice was prior to analysis [203, 201].

  HLA-A11 frequency: Early and small studies with numbers of cases varying from 14 to 81 tend to show a protective effect of HLA-A11 against NMSC [201, 202, 206, 208, 205]. Bouwes-Bavinck *et al.* (1991) was the only study reporting significant results after correcting the P-value for number of tests. Two recent studies based on 271 and 152 cases have respectively reported a significant positive association [203] and no association [116]. Espana *et al.* (1995), Jensen *et al* (1999) and Bouwes-Bavinck *et al.* found no association either [102, 23, 142]. In 2004, Bock

et al. reported that patients with NMSC were twice (95% CI: 1.1 to 3.5) as likely to be HLA-A11 positive compared to those without NMSCS [209]. No association between HLA-A11 frequency in controls and SCC or BCC can be concluded from the literature.

HLA-DR7: Czarnecki *et al.* (1992) found a statistically significant higher frequency of HLA-DR7 in patients with skin cancers compared to controls, but no P-value correction was reported [208] and Bouwes-Bavinck *et al* (1997) found the same trend, but their result did not reach statistical significance [203]. Another study found a statistically significant negative association [116] and three other studies did not report any associations [201, 202, 23, 209]. There is no evidence either of differences in HLA-DR7 frequency between controls and patients with BCC or SCC.

HLA-B27: Eight studies have reported on HLA-B27 frequency and skin cancers [201, 202, 203, 208, 116, 102, 205, 23, 209]. Only Czarnecki *et al* (1992) reported a significant positive association (not corrected). Bouwes-Bavinck *et al* (1991) found a statistically significant higher frequency in renal transplant recipients compared with two different control groups (healthy donors and transplant recipients). This result did not remain statistically significant after correction when using renal transplant recipients as controls. The five other studies did not report any differences.

- Level of HLA mismatch

  Bouwes-Bavinck *et al.* (1991) reported a positive association between the development of SCC and increasing level of mismatches at HLA B loci [207]. They also found an increased risk of SCC with DR homozygosity whereas Ong *et al.* (1999) reported similar findings in patients with all NMSC [116]. None of these findings were corroborated by other authors and no association between level of HLA mismatches and the development of skin cancers in transplant recipients for class I and

II were reported [101, 102, 152, 203, 169, 116, 23, 17].

3.9.11 Other potential risk factors

Hormones

There is no information on the association of hormones and development of BCC or SCC either in transplant recipients, or in immunocompetent people. To my knowledge there is no study on vitamin D (a steroid hormone) and the development of NMSC in transplant recipients.

Body mass index

Kasiske *et al.* (2004) found a decreased risk of NMSC with increasing body mass index [158]. This result was controlled for donor status (living or cadaveric), hepatitis virus infection, education, employment, donor race, donor age, HLA mismatches, and panel of reactive antibodies.

Tobacco

Studies which looked at all skin cancers did not find any significant association with smoking in transplant recipients [92, 116, 120]. Of the studies that examined the association between smoking and different histology types, five reported an increased risk of developing SCC in transplant recipients who smoked tobacco [19, 101, 146, 82, 147] and one did not find any association [141]. A recent multicentre case-control study did not find consistent associations between smoking and the development of SCC or BCC [148] between the 6 centres (United Kingdom, Australia, the Netherlands, Germany, Italy and France).

Alcohol

Mithoefer *et al.* (2002) and Bouwes-Bavinck *et al.* (2008) did not find any association between skin cancer development and alcohol consumption in transplant recipients [92, 148]. Conversely, Xiol *et al.* (2001) found a significant positive association between skin cancers and alcohol consumption in univariate analysis (p=0.04) [94].

Type and time on dialysis

Three studies which looked at the association between the time spent on dialysis, the type of dialysis and the risk of developing skin cancers after transplantation did not find any association [101, 95, 120]. A recent American study found a significant protective effect with increased time on dialysis before transplantation [158]. This result was controlled for donor status (living or dead), hepatitis virus infection, education, employment, donor race, donor age, HLA mismatches, and panel reactive antibodies but not sun exposure.

Family history of skin cancer

Ramsay *et al.* (2000) and Mithoefer *et al.* (2002) did not find any link between family history of skin cancer and patients who developed skin malignancies [101, 92].

Radiation history (other than exposure to ultraviolet radiation)

Mithoefer *et al.* (2002) did not find any association between skin cancer development in transplant recipients and radiation history [92] and Ramsay *et al.* (2000) did not find any association with arsenic exposure [101].

Diabetes (primary disease)

Gruber *et al.* (1994) reported that non-diabetic patients are more likely to develop skin cancers than diabetics (RR=2.09; P=0.001) [157]. Roeger *et al.* (1992) also reported a lower significant risk of SCC in diabetics [169]. This is in agreement with a recent study in immunocompetent people that reported lower risk of NMSC in patients with type 2 diabetes mellitus using insulin [210]. However, only one study controlled for sun exposure [169] and it should also be borne in mind that non-diabetic patients might also live longer post-transplantation. Transplant patients with primary disease of diabetes were also less likely to develop NMSC than those without diabetes [211].

Creatinine

Bordea *et al.* (2004) found that skin cancers in kidney transplant recipients are associated with high creatinine level at 1 year post transplantation. This result was still significant in multivariate analysis after controlling for age at transplantation, sex, length of immunosuppressive treatment and donor relation (cadaveric or living donor) [120]. However, this finding might be due to the intensity of immunosuppression which is difficult to assess.

Blood transfusion

There is no information on the association of blood transfusion and development of BCC or SCC in transplant patients.

Education

Bouwes-Bavinck *et al.* (2008) in a multicentre case-control study did not find an association between SCC development and education but found higher risk of BCC with higher education after adjustment for age, sex, years after transplantation and study center. [148].

3.10 Summary

Transplant patients are at increased risk of skin cancers, in particular SCC, in comparison with the general population (SIR from 18 [95% CI: 17 to 20] to 253 [95% CI: 172 to 334]). SCC occurs significantly more frequently than BCC and consequently a reversed SCC to BCC ratio is often reported in the transplant population compared with the general population. NMSC are often multiple and recurrent and SCC behave more aggressively. NHL and KS occur on average earlier than NMSC. The cumulative incidence of skin lesions especially SCC increases sharply after transplantation. The time from transplantation to development of first cutaneous lesions varies from study to study due to differences in patients' age at transplantation. The distribution of the lesions on the body is similar in the general and transplanted population. A higher proportion of SCCs occur on sun exposed areas, whereas BCC is also more common on the trunk and the limbs. UV radiation is the principle agent responsible for the development of skin lesions and it is therefore crucial to collect information on it since it might confound the association between skin cancer and HPV. It is however not clear if sun exposure before and/or after transplantation or early and/or later in life is associated with an increased risk. Tobacco might also increase the risk of developing SCC. Patients with skin cancers are more likely to have pre-malignant lesions, such as actinic keratoses and carcinoma in situ, or viral warts. There is no clear evidence on the oncogenic effect of a specific immunosuppressive regimen. The level of immunosuppression and not a specific agent might be the most important factor for the development of skin cancers. However, the addition of ciclosporin to CONV may increase the risk of developing NMSC and sirolimus might be protective. It is not clear if males are at higher risk of skin cancers than females. Lower risk of skin cancer in diabetic patients has also to be taken with caution since the role of UV exposure has not been examined in some of these studies. Publications on donor characteristics (gender, age, blood type

and rhesus status) and skin cancers are sparse and there is so far limited evidence of any associations. There is also not enough evidence of an association between HLA mis-matching or any HLA frequency and the development of NMSC in transplant recipients.

CHAPTER 4

Literature review: Epidemiology of human papillomavirus in relation to squamous cell carcinoma and basal cell carcinoma

4.1 Introduction

The aim of this chapter is to describe the papillomavirus family, to describe the serological and genotyping methods for detection of HPV and to review the epidemiological evidence available to date on the association between HPV and the risk of SCC and BCC in both transplant recipients and immunocompetent patients. Case-control studies have been used to examine the relationship between HPV and the development of SCC or BCC.

4.2 Human papillomaviruses (HPV)

In 1933, Shope and Hurst described the first mammalian tumour virus in cottontail rabbits in North America [212] but it was not until the late seventies that the virus was first cloned

and sequenced [213]. Papillomaviruses are small circular double-stranded DNA viruses with sizes around 8kb.

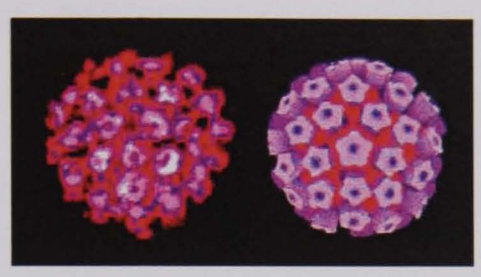


Figure 4.1: A human papillomavirus (from http://www.virology.net)

Their genome can be divided into three components. The early (E) genes code for proteins involved in the regulation of viral transcription and replication (E1 and E2), cell proliferation (E5, E6 and E7), viral life cycle (E4). Two genes with unknown roles (E3 and E8) are not present in human papillomaviruses. The late (L) genes contain two genes coding the capsid proteins L1 and L2. The long control region (LCR) or upstream regulatory region located between the L1 and E6 genes contains response elements and the origin of replication (Figure 4.2).

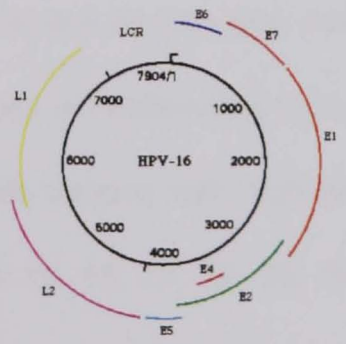


Figure 4.2: Example of HPV genome (HPV16)

The L1 gene of the open reading frame (ORF) is the most comparable gene across all HPVs. This nucleotide sequence is used to identify new HPV types. In 1995, at the International Papillomavirus Workshop in Quebec, participants decided that a new HPV type is identified if its L1 ORF is distinct by more than 10% from any existing HPV type.

At the time of writing, one hundred and eighteen papillomaviruses have been completely described of which 96 are human and 22 are animal types [214]. Classification has been modified over the years. This report uses the recent terminology from de Villiers *et al.* (2004) and focuses on human types only. HPVs can be partitioned into 'genera' which in turn can be split into 'species', and can be further subdivided into 'types' (Figure 4.3). The HPV phylogenetic tree is formed of 5 genera called alpha, beta, gamma, mu and nu papillomaviruses. The DNA sequence of the L1 ORF differs by more than 40% between genera. Within each genus, species have in common between 60% and 70% of their DNA sequence of the L1 ORF. Within species, the L1 ORF of HPV types have in common between 71% and 89% of their DNA sequence. Types are also divided into 'subtypes' and 'variants'. Subtypes and variants have respectively 90% to 98% and more than 98% of their DNA sequences in common with any HPV type of the same species. Alpha-papillomaviruses are the biggest genus and contain mucosal and cutaneous HPV types. The concept of high and low risk HPV types was introduced in 1985 by Zur Hausen [215], based on the strength of association with cervical cancer. In 2003, Munoz *et al.* put together data from 11 case-control studies and investigated in more detail the risks associated with various HPV types in relationship to the development of squamous-cell cervical cancer [216]. As a result, viruses with high and low oncogenic risks of genital HPV were better defined. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are carcinogenic and to some extent types 26, 53 and 66 also. The low risk group includes types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108. The beta-papillomavirus genus is composed mainly of the HPV types with skin cancers occurring in the context of epidermodysplasia verruciformis (EV), a rare inherited skin disease. The next largest genus, gamma-papillomaviruses, contains 7 cutaneous HPV types and the two other genera, mu- and nu-papillomavirus, are small and contain respectively 2 and 1

cutaneous HPV.

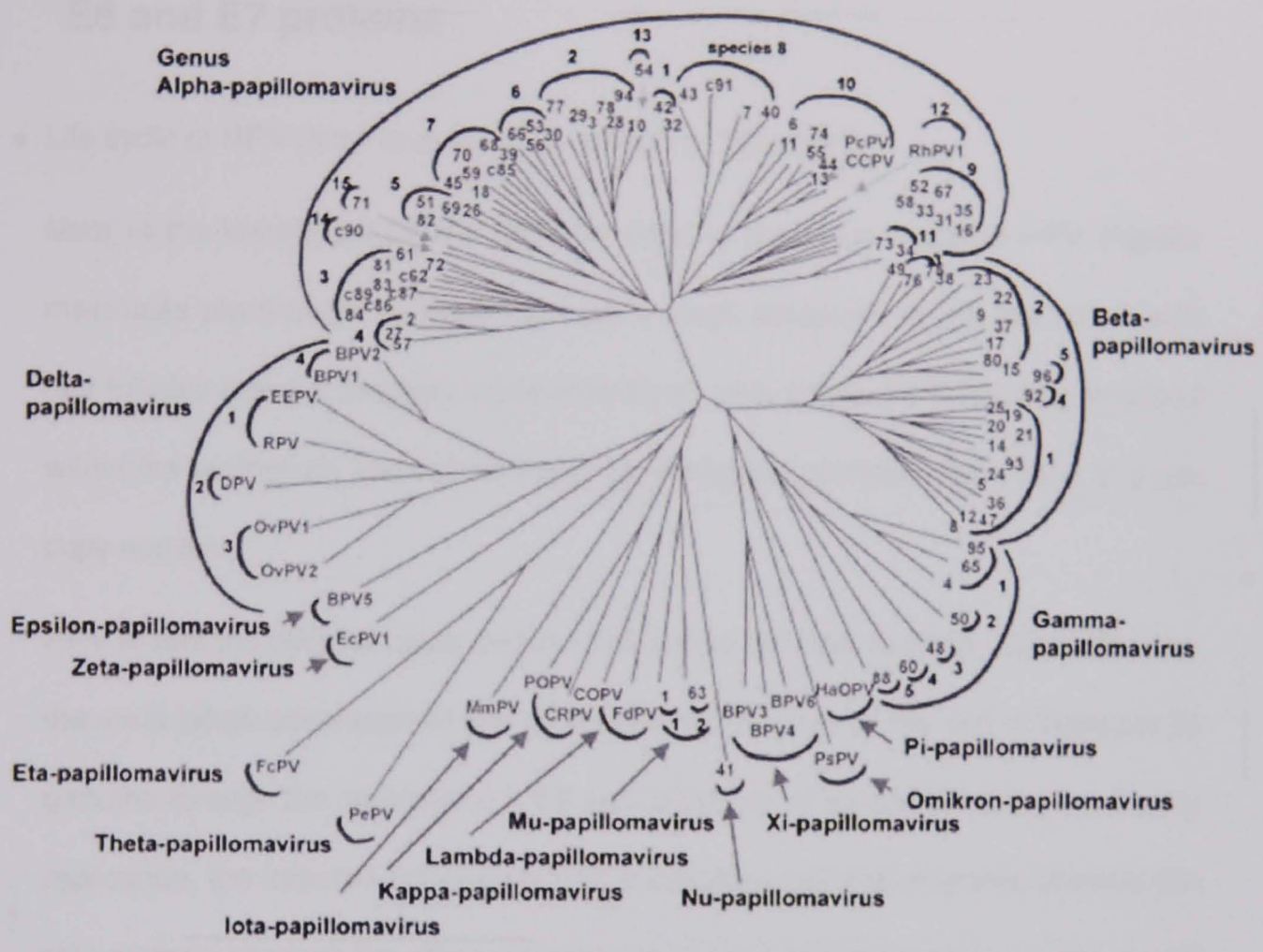


Figure 4.3: The HPV phylogenetic tree

High risk mucosal HPVs cause cervical cancer but infection with some other types is also responsible for benign lesions of cutaneous and mucosal epithelia [217]. For instance, common warts seen on the skin of arm, hand and leg are associated with HPV 1, 2, 4, 7, 27 and 57; flat warts are usually caused by HPV 3, 10 and sometimes 2 and are more common in immunosuppressed patients or patients with EV. Genital warts (condyloma acuminata), oral warts and low-grade cervical squamous intraepithelial lesions are mainly caused by HPV 6 and 11.

4.3 Life cycle of HPV, host immune response and the role of E6 and E7 proteins

- Life cycle of HPV (from reviews [218, 219, 215, 220, 221])

  Most of the knowledge on the HPV life cycle is based on mucosal HPV. Papillomaviruses are thought to infect the host through abrasions in the epithelium with hair follicles being a probably route of entry for beta types. HPV is solely localised within the epithelium and infects basal keratinocytes probably stem cells at a low copy number.

  HPV enters the cells, uncoats and its DNA enters the host nucleus. Upon infection, the virus takes advantage of the replication mechanism of the cell to replicate its genome through the control of E1, E2 and, probably, of E6 and E7 proteins. During replication, the infected cell divides into a daughter cell that migrates towards the skin surface whereas the other cell remains in the basal layer and provides a viral reservoir for future cell division. The infected cells in the basal layer can remain for several years (latent infection).

  In uninfected epithelial, basal cells detach from the basement membrane and stop dividing. HPV is therefore challenged to replicate in nondividing cells approaching terminal differentiation. HPV proteins (E6 and E7) interact with the host cells proteins to delay the death of the cell and to reactive viral DNA replication in the cell. It is however not understood how the virus starts initiating DNA replication.

  In the upper layers of the epithelium, the viral genome is highly amplified. At that stage, the late genes L1 and L2, encoding the viral capsid, are activated when cell approaches terminal differentiation and virions are assembled. The new virus particles are shed with the dead skin cells and can spread to other hosts.

- Host immune response (from reviews [218, 220, 222, 215])

  HPV replication depends on continued cellular division and the virus has therefore to adapt to a milieu where cell division has stopped. The virus stratagem is to replicate its viral genome and to express viral proteins in terminally differentiating cell and to be invisible to the host immune system.

  Under normal circumstances, infected cell should present on their surface special molecules, known as class I and II major histocompatibility complex (MHC) molecules, to initiate an immune response. Class I molecules are found on virtually all cell types of the body and present endogenous antigen to cytotoxic T-cells (CD8). Class II molecules, particularly associated with B-cells, dendritic cells and macrophages, present exogenous antigen to helper T-cells (CD4). HPV evades the host immune mechanism by preventing antigen presentation. As HPV are not lytic and none or little inflammation is present, Langerhans cells, the main antigen presenting cells in the skin, are therefore not activated. Consequently, the innate immunity might not be triggered and the adaptive immunity is delayed.

  Production of cytokines is another mechanism of the host immune system to make virally infected cells more susceptible to be detected by dendritic cells and, therefore, to launch cell-mediated immunity. However, HPV can also downregulate cytokine production and the interaction of E6 and E7 HPV proteins with type 1 interferons seems also to inhibit further cell-mediated immunity.

  Failure in HPV detection may lead to persistent infection and a higher probability of cervical cancer. In most cases of cervical cancer, the viral genome has been integrated to the host cell genome. Despite HPV ability to manipulate the host immune system, around 80% to 90% of genital HPV infections are controlled or cleared by the host immune system. Around 10% to 20% of individuals have persistent infec-

tion.

- E6 and E7 proteins

The mechanism of action of high risk HPV genital types is well understood and seems clearly to differ from cutaneous HPV types. The E6 and E7 proteins of high risk mucosal types have been associated with the degradation of p53 and retinoblastoma (Rb) respectively and their inactivation is necessary for induction of cervical cancer. The interaction of E6 and E7 proteins with the host proteins induce cell abnormalities, continued cell proliferation and immortalisation. This phenomenon has not been observed with low risk mucosal types such as HPV6 or HPV11.

The transforming potential of E2, E6 or E7 proteins has been investigated for few beta HPV types (mainly HPV5, HPV8 and HPV38) [223, 224, 225, 226]. In contrast to high-risk mucosal types, the direct degradation of p53 was not observed with HPV5 or HPV8 E6 proteins [223, 224, 225, 226]. Some transforming potential has been reported for HPV38 in studies on animals or in vitro [227, 228] and particularly in relation with UV irradiation [229]. Jackson *et al.* (2000) reported that HPV5 E6 proteins could also degrade another pro-apoptotic protein called Bak [230] and the transforming potential of diverse E6 HPV proteins following UV damage was also reported [231, 232] (for a review, [233]). Integration into the host DNA has only been reported once in metastastic lesions [234].

4.4 Methods of detection

Papillomaviruses are difficult to culture in vitro but have been characterised by molecular methods based on DNA sequence homology. Most studies of NMSC over the past 15

years have focused on HPV DNA detection and genotyping. Cutaneous HPV type prevalence varies dramatically between such studies and this is mainly due to the detection methods employed. In contrast with genotyping methods which can only examine current infection, serology can detect past and present infection and, in anogenital infections, can be a marker of cumulative HPV 16 exposure [235].

- HPV-DNA detection

  The methods for HPV-DNA detection are divided into two groups based on amplification and non-amplification techniques. In the 1990s, the non-amplification techniques (Southern blot, dot blot hybridation, in situ hybridation, filter in situ hybridation) have been replaced by DNA amplification techniques mainly due to their lack of sensitivity and specificity.

  The amplification techniques are composed of target-amplification (polymerase chain reaction also called PCR), signal-amplification [second or third version of Hybrid Capture (DIGENE, Gaithersburg, USA) also called HC2 and HC3] and of probe-amplification (ligase chain reaction).

  HC tests consist of the denaturation of the specimen, the hybridisation with RNA probes, the capture of hybrids, the reaction with its conjugate and the production of light signals proportional to the amount of HPV-DNA present in the specimen. HC2 can detect HPV types 6, 11, 42, 43, 44 and 59 so-called 'low' risk types and 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, which are 'high' risk types for cervical cancers.

  Performing PCR tests involves denaturing the DNA in vitro by heating, 'annealing' or binding the two primers complementing a specific sequence at one end of the tar-

get sequence, replicating or 'extending' the target sequence and repeating the last three steps. PCR can amplify very small amounts of DNA and hence produces sufficient quantity of DNA to be analysed with conventional laboratory methods. PCR and HC have similar sensitivity and specificity but most studies have used PCR methods. PCR is a highly sensitive technique and contamination is the main issue of concern, which can lead to false positive results. Amplification by PCR methods requires Tag primers and the choice of these primers is essential. Consensus (or general) primers target the conserved sequence in the HPV L1 gene and degenerate primers are a mixture of primers and can detect a wider range of HPV types. New primers have been developed over time and the most commonly used primers from the HPV L1 gene are: MY09/MY11 and GP5+/GP6+. They have been compared in several studies [236, 237]. PCR techniques have permitted the detection of novel HPV types. The frequency and the range of HPV types identified in SCC vary widely between PCR assays [238, 239]. Recently, new methods using broad spectrum PCR (PM-PCR) combined with a reverse hybridation system (RHA) has been developed for betaHPV genotyping [240].

- Serology

  In the early 1990s, studies used Western blots with bacterially expressed major capsid protein L1 and early genes E6 and E7 of HPV8. Due to their lack of specificity this technique has been replaced by enzyme-linked immunosorbent assay (ELISA) techniques using HPV-type specific virus-like particles (VLP). ELISA techniques have to determine a cut-off value for positivity. Studies have employed different methods for calculating this threshold. Calculations are always based on a control group. One method of calculation consists of excluding people with readings higher than the mean plus 3 standard deviations (SD) and repeating this process until no

readings exceed the recalculated mean + 3SD [161]. Another method excludes readings higher than mean plus 3 SD, recalculates the mean of the remaining readings and the cut-off point is chosen at the 90th percentile of this distribution [81]. New technology using an antibody detection method that is based on a glutathione S-transferase capture enzyme-linked immunosorbent assay in combination with fluorescent bead technology has recently been developed. This technology, called Luminex, can detect antibodies of up to 100 HPV types at the same time. More details are given in the following chapter.

4.5 Humoral response to HPV infection

The evaluation of HPV as a risk factor for cervical cancer has been recently reviewed by the Monograph Programme of the International Agency for Research on Cancer (IARC) [215]. Serology is a very useful epidemiological tool for defining past and cumulative exposure to HPV infection and the assays are reasonably type-specific [215].

Serology is less sensitive than genotyping methods and among cases whose tumours contained HPV 16 DNA, seropositivity ranged from 25% to 73% [241]. Hence, serology is not useful for screening but, in epidemiological studies, it has shown to be a good tool to detect an association between HPV infection and cervical cancer [235, 242, 243]. In prospective studies of cervical intra-epithelial neoplasia (CIN) or cancer of the uterine cervix, cases have been found to have a higher prevalence of antibodies against certain HPV types (in particular HPV16), prior to diagnosis, as compared to controls with relative risk from 2.5 to 30. For instance, in one prospective study of antibody levels against HPV-16, women who subsequently developed invasive cervical cancer (28 out of 99 cases [29%]) within 10 years of sample collection were more than twice as likely to be HPV-16 seropositive than controls (43 out of 194 [22%]) [244].

HPV-DNA and serological data in prospective studies of mucosal HPV types and cervical cancer are in agreement [215]. Comparable findings have been reported in prospective studies of other established oncogenic viruses such as human herpesvirus-8 (in relation to Kaposis sarcoma) and hepatitis B (in relation to hepatocellular carcinoma), in which a higher than expected prevalence and titre of antibodies has been identified in blood taken years before diagnosis of cancer [245, 244].

The persistence of HPV infection is necessary for progression to high grade CIN or invasion, but CIN lesions can also spontaneously regress [215]. Ho *et al.* (2004) examined the natural history of HPV16 virus-like particle antibodies in 608 young women and reported that detectable levels of antibodies against HPV16 are associated with persistent high viral load in blood and/or persistent infection [241]. The median duration of antibodies against HPV16 was 3 years [241]. The median time from HPV16 infection to the detection of antibodies has been reported to be between 8 and 12 months [241, 246]. The seroprevalence of HPV tend to be lower in men than women [215].

4.6 Epidermodysplasia verruciformis of Lewandowsky-Lutz (EV) and human papillomaviruses

EV is a rare genodermatosis, characterised by disseminated and persistent warty lesions on the skin and was first described in 1922 by Lewandowsky and Lutz [247]. EV patients develop common and plane warts, depigmented pityriasis versicolor-like lesions and red wart-like lesions and plaques [248, 249, 250, 251]. First lesions appear usually during childhood; between 30% and 50% of EV patients develop skin cancers, essentially localised to sun exposed areas, between 20 and 40 years after the occurrence of their first benign lesions [252, 253, 254].

In the late 1960s and early 1970s small case reports (between 1 and 14 patients) using light and electon microscopy reported a large number of viral particles in EV patients' lesions [255, 256, 257, 258]. With the development of hybridisation methods, it was possible to differentiate between these new HPV types. The first isolated types in EV patients were 3 and 4 which differed clearly from the known HPV1 and HPV2 associated with plantar and common warts [259, 250]. Clinical appearances and tendency to malignancies were soon related to different HPV types. HPV3 was harboured in flat wart lesions with very rare progression to malignancies whereas HPV4 (nowadays called HPV5) was more likely to be present in very flat verrucous reddish lesions or plaques with oncogenic potential [260, 249, 248, 250, 261, 251].

Early detection techniques identified new types only in EV patients and a few immunosuppressed people [214, 262, 263, 264, 265, 266, 267]. As a result, these new HPV types were called "EV-HPV types". With the development of new extremely sensitive techniques, such as PCR and the use of degenerate primers, a wide range of HPV types, included EV-types, are now found not only in EV patients but also in hairs, lesions and normal skin of immunocompetent and immunosuppressed people of patients with psoriasis [268, 145, 269, 270, 271, 272, 273, 274, 275, 276, 277, 117, 278, 279, 280, 239]. Consequently, some authors have questioned the terminology 'EV-HPV types' since their presence is not restricted to EV patients [281].

Today the so-called EV-types are within the beta papillomvairus genus and include: 5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47, 80 and 93 [282, 214, 262, 263, 264, 265, 266]. Multiple HPV infections are extremely common in EV-patients [283, 263, 252] and types 5, 8, 14, 17, 20 and 47 have been suggested to be oncogenic [262, 260, 283, 284, 285]. Recently, Dell'Oste *et al.* (2008) examined 4 EV patients (at least 3 Italian patients) and reported higher titers for 16 betaHPV and to a less extent to 9 gammaHPV compared to

54 sex and age-matched German controls [286]. HPV-DNA of type 5 was found in the skin cancer of one patient and up to 18 beta-HPV genotypes were found in eyebrow hairs and skin samples.

EV-HPV particles have been rarely found in premalignant and malignant lesions [262, 258, 266, 287]. Integration into the host DNA has only been reported once in metastastic lesions [234] and recently, two mutated genes EVER1 and EVER2 have been associated with EV [288, 289].

However, the rarity of EV does not favour epidemiological studies and no definite conclusion on the association between HPV and development of SCC can be assessed.

4.7 Current epidemiological evidence for the risk of SCC and BCC from HPV

4.7.1 Using genotyping methods

HPV-DNA prevalence varies widely from study to study in part due to a number of factors including the immunosuppressed status of the patient, the choice of sample (hairs, skin swabs, peri-lesional samples, biopsy of lesions or normal skin, unique or multiple samples per patient), the use of different PCR methods (nested, specific or degenerate), the choice of primers and the number of HPV types tested. Forslund *et al.* (2004) reported higher HPV positivity in samples from the top of both healthy skin and skin cancer than inside the tumour [290]. It is also important to note that most studies were based on small numbers and there is no study that looked at both immunocompetent and immunosuppressed patients with more than 50 people.

- **Case-control studies (Appendix A)**

  A systematic review was performed through a Pubmed search using synonyms for

relevant words on studies reporting on HPV genotyping and SCC, and published up to October 2008 [search terms: BCC, SCC, DNA, HPV, genotyping, skin cancer, cutaneous, non-melanoma, transplant, general population, immunocompetent], supplemented by searches of references in identified papers, by hand searches of relevant journals. No restriction was placed on language of publication. No attempt was made to identify unpublished studies or to obtain unpublished data from published studies. All published case-controls reporting on the association between SCC and/or BCC and HPV-DNA positivity were included in this review.

Fourteen case-control studies have considered the presence of HPV-DNA in patients with NMSC and patients without. Eleven studies have been undertaken in immunocompetent patients [291, 292, 147, 278, 293, 294, 295, 296, 153, 297, 298] and three in both immunocompetent individuals and renal transplant recipients [268, 145, 299]. The presence of HPV-DNA was examined in plucked hairs from eyebrows, scalp, arm and leg [291, 292, 147, 145, 299, 297], or skin biopsies [278, 268, 276, 153, 298] or skin swabs [295]. Most studies were based on primers from the L1 gene but E7 gene [297, 292, 147] and E1 gene [278, 298] were also used. Two studies using primers from the E7 gene seemed to be based on the same data and found similar results [292, 147].

In 2000, Boxman *et al.* undertook the first case-control study with 51 BCC, 25 SCC and 89 controls and found no statistically significant association between the presence of EV-HPV DNA in plucked hairs from eyebrow, scalp, arm and legs and the development of SCC (unadjusted OR: 2.0 and 95%CI: 0.5 to 80.0) [291]. Cases were matched for age and sunscreen allocation, and primers were from the L1 region. No association was reported between the development of BCC and EV-HPV DNA positivity.

Three and four years later, two studies carried out in Leiden looked at the presence of HPV-DNA (5, 8, 15, 20, 24, 38; plus 2 and 16 in [292]) in plucked hairs in 155 and 156 individuals with SCC and 371 and 320 controls respectively [147, 292]. They used primers from the same region of E7 and got similar results. Individuals who were positive to any beta-HPV DNA were twice as likely to have SCC than the beta-HPV DNA negative people (unadjusted OR and 95% CI: 1.4 to 3.2 and 1.3 to 3.2 respectively). The result was still statistically significant after adjustment for age and sex [292] and age, sex, skin type, sun exposure and painful sunburns [147]. Only HPV 5, 8, 20 were found to be associated with the development of SCC after controlling for these variables. No association between HPV 16 or HPV 2 and SCC was reported [292].

Two case-control studies examined skin biopsies [278, 268] and used primers from E1 and L1 genes respectively. Iftner *et al.* (2003) looked at mucosal types (16, 31, 33, 35 and 51) and beta-types (5, 8, 12, 17, 19, 22, and 36) and Harwood *et al.* (2004) examined skin biopsies for the presence of all HPV-DNA types. Iftner *et al.* (2003) examined the HPV-DNA prevalence in 72 patients with SCC and 106 controls. The risk of SCC was thirty times higher in patients who were positive for any HPV types compared with those who were negative after controlling for age, sex and location of skin lesions (95%CI: 10.9 to 83.0) [278]. Harwood *et al.* (2004) examined 39 immunocompetent individuals (57 samples) and 38 renal transplant patients (67 samples) with and without NMSC and reported a statistically significant association between beta-HPV positive (OR: 6.4; 95%CI: 1.8 to 22.9) and the development of NMSC after controlling for transplant status, sex and either location on the body from which the sample came (sun exposed or not) or number of samples per individuals. A non-statistically significant positive association was

reported between positive for any HPV types and the development of NMSC and a non-statistically significant negative association was noted between cutaneous HPV types specifically, and the development of NMSC [268].

In a case-control study involving 54 patients with xeroderma pigmentosum, skin biopsies of 40 SCC were 3 times more likely to be HPV-DNA positive to any types (95%CI: 0.6 to 37.7) or to beta types (95% CI: 0.5 to 34.2) compared to those from 9 samples from healthy skin [294]. There was no difference between BCC and healthy skin biopsies.

In 2006, Struijk *et al.* looked again at HPV-DNA using primers from the E7 gene for type 5, 8, 15, 16, 20, 24 and 38 using plucked hairs from 64 cases and 58 tumour-free individuals and they reported negative non-significant associations for each of the HPV type examined [297]. Using skin biopsies, Andersson *et al.* (2008) and Forlsund *et al.* (2007) reported higher odd ratios in patients with SCC compared to those without lesions for detection of HPV-DNA of any types (OR: 2.1; 95% CI: 1.0 to 4.2) and in particular for beta types of species 2 (OR: 4.4; 1.9 to 10.1) [293, 153]. No association with HPV-DNA of beta types from species 1 was identified, and BCC was not related to the presence of HPV-DNA. Asgari *et al.* (2008) examined 72 SCC and 121 benign lesions and found no difference in presence of beta HPV-DNA after adjusting for sex, age, location of lesions, previous sunburns and smoking [296]. In a case-control study looking at 101 SCC and 101 BCC, the proportion of SCC with HPV-DNA was compared to BCC [298]. No difference was found for infection to any betaHPV but, SCC were more likely to have HPV-DNA of betaHPV types of species 1.

In summary, very few studies gave informative results with large CI and, overall, there is no consistent association between the presence of HPV DNA and SCC.

- **HPV-DNA prevalence (Appendices B and C)**

- Prevalence of HPV-DNA in skin and hair follicles from patients with SCC or BCC (Appendix B)

Among immunocompetent people with SCC, the prevalence of HPV-DNA positivity varies from 19% to 84% in any samples and from 13% to 83% in patients [300, 301, 277, 238, 117, 276, 302, 303, 304, 160, 278, 297, 153, 296, 293, 298], whereas in renal transplant recipients with SCC the prevalence in samples varies between 50% and 91% and in patients between 50% and 84% [305, 301, 306, 277, 238, 279, 276, 302, 145, 307, 303]. Studies examined mainly skin biopsies and only four used plucked hairs [291, 292, 147, 297]. Only 2 studies had more than 100 cases and reported that 71% and 84% respectively of patients with SCC were HPV-DNA positive [147, 298].

A lower prevalence has generally been reported in patients with BCC. A study using plucked hairs reported that 61% of immunocompetent patients with BCC were beta HPV-DNA positive [291]. Studies using skin biopsies found from 8% to 78% HPV-DNA positive samples in immunocompetent patients [278, 301, 277, 303, 160, 290, 280, 308, 293, 153] and 0% to 83% of samples from renal transplant patients [305, 301, 277, 279, 145, 303, 269, 280, 308, 239]. Between 21% and 78% of immunocompetent patients [291, 277, 117, 280, 298] compared to 75% of renal transplant recipients were found to be HPV-DNA positive [277].

- Presence of HPV-DNA in normal skin, psoriasis, peri-lesional samples, Bowen's disease and actinic keratoses, viral warts and patients with other conditions (Appendix C).

The prevalence of HPV-DNA in normal skin samples of immunocompetent people varies between 13% to 54% [309, 302, 308, 268, 278, 276, 275, 296, 293] and in renal transplant recipients it ranges between 11% and 87% [302, 308, 279, 268, 145, 269, 276]. The prevalence increases when normal skin biopsies come from patients with psoriasis or connective tissue diseases [274, 271, 276, 310, 311]. Highest HPV-DNA prevalence has been reported in viral warts where more than three quarters of patients' tested samples are HPV-DNA positive [302, 279, 276, 278, 270, 269, 239, 308]. All viral warts are expected to be HPV-DNA positive and lower prevalence indicates a lower sensitivity of the methodology used or might be due to misdiagnosis. More than three quarters of samples from patients with psoriasis have also been reported to be HPV-DNA positive [272, 273, 274, 269, 275, 311]. Biopsies from AK and/or BD have also shown a high prevalence of HPV-DNA ranging from 11% to 70% in immunocompetent patients [302, 308, 278, 280, 290, 277, 117, 278, 312, 313, 153, 293, 295] and between 0% and 88% in renal transplant recipients [302, 308, 279, 145, 280, 239, 277]. High proportions have also been reported in peri-lesional samples [309, 290, 30, 280, 296], in skin swabs [281] and hair follicles of patients with or without NMSC [307, 299, 291, 160, 308, 314].

- HPV types

No HPV-DNA from predominant types has been consistently found in samples. Beta types seem however to have a higher HPV-DNA prevalence than other types [239, 238, 308, 314, 310, 293, 296]. PCR is such a sensitive tool that new HPV types are found in many studies [279, 301, 276, 291, 305, 239, 307, 145, 310, 281]. Multiple HPV-DNA types are commonly found in a single sample [280, 277, 291].

- HPV-DNA cutaneous type prevalence from 3 continents

One study has been undertaken in several countries to compare prevalence. In 2000, Antonsson *et al.* studied HPV-DNA in skin swab samples of immunocompetent people from Bangladesh, Japan, Ethiopia, Sweden and Zambia. Respectively 68% (34/50), 54% (26/48), 52% (26/50), 70% (35/50), 42% (21/50) were found positive to any HPV-DNA. Eighty eight HPV types and putative types were found of which 22 were new. Thirty nine percent of the samples (53/137) had multiple infections. Respectively, 12, 9, 9, 18 and 2 types were exclusively found in these countries. HPV-5 was the only single type to be detected in the five countries and was present in 6.5% (16/248) of all tested samples [315].

4.7.2 Using serological methods

A systematic review was performed through a Pubmed search using synonyms for relevant words on studies reporting on HPV genotyping and SCC, and published up to October 2008 [search terms: BCC, SCC, serology, antibodies, HPV,skin cancer, cutaneous, non-melanoma, transplant, general population, immunocompetent, VLP, Luminex, ELISA], supplemented by searches of references in identified papers, by hand searches of relevant journals. No restriction was placed on language of publication. No attempt was made to identify unpublished studies or to obtain unpublished data from published studies. All published case-controls reporting on the association between SCC and/or BCC and antibodies against HPV were included in this review.

Twelve case-control studies have looked at the association between HPV and NMSC using serological methods [316, 317, 318, 161, 271, 306, 146, 81, 147, 153, 297, 319]. All studies but one examined immunocompetent patients only. The common HPV types

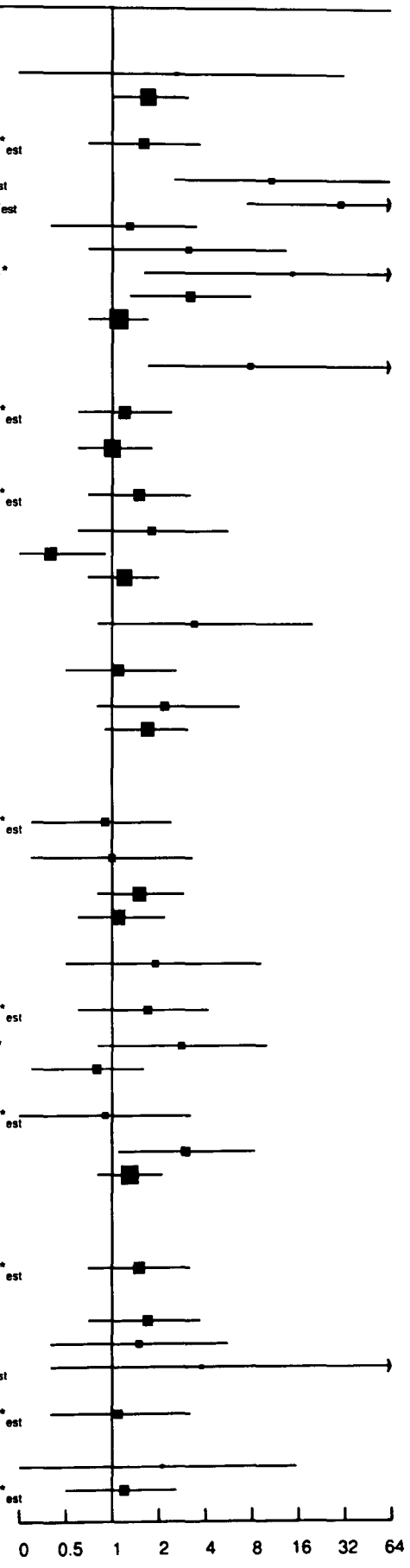
investigated were HPV1, HPV5, HPV8, HPV9, HPV15, HPV16, HPV20, HPV23, HPV24, HPV36 and HPV38. One study also examined more alpha HPV types (HPV6, HPV10, HPV32 and HPV57) [153]. Most studies based on ELISA used IgG-specific ELISA with VLP composed of the major L1 capsid protein of the specific HPV type and most recent studies used the Luminex technology looking at antibodies against L1 capsid protein of HPV types.

Figures 4.4 and 4.5 show results of all case-control studies that used serology for all betaHPV types tested and 2 alpha types HPV1 and HPV16. Figures include results on L1 protein. Cases are respectively patients with SCC (Figure 4.4) or BCC (Figure 4.5) and controls are those without the disease. Heterogeneity was observed between studies and very wide 95% CI were also found. The number of positive sera varies markedly between studies for both cases and controls, suggesting that methods detecting serological response might vary with respect to sensitivity. For example, studies have shown that 4% to 73% of sera from patients with SCC harbour antibodies against HPV8 (Figure 4.4). Only four studies controlled for crucial confounding factors such as UV radiation [161, 146, 81, 153] and these studies suggest an association between HPV5 and SCC. Only one study has examined antibodies against E6 protein of HPV types [297]. They reported non-statistically significant at 5% level negative associations with SCC and all examined HPV types (HPV8, 15, 20, 24, 38 and 16).

From this review there is no consistent evidence that immunocompetent patients with antibodies against any single HPV types examined have a higher risk of developing SCC or BCC than controls. Patients with antibodies against HPV8 were associated with presence of SCC in some studies but this result was not corroborated in a recent study with larger number of patients [319]. This largest study has only find a statistically significant (at 5% level) association between SCC and HPV 5. Significant findings were actually

associated with studies with less power suggesting evidence of publication bias. Overall HPV5 might be associated with the presence of SCC but more studies are needed to clarify the association. A recent study from Andersson (2008) [153] looked also at other alpha types (HPV6, 10, 32 and 57) but did not find any association with SCC.

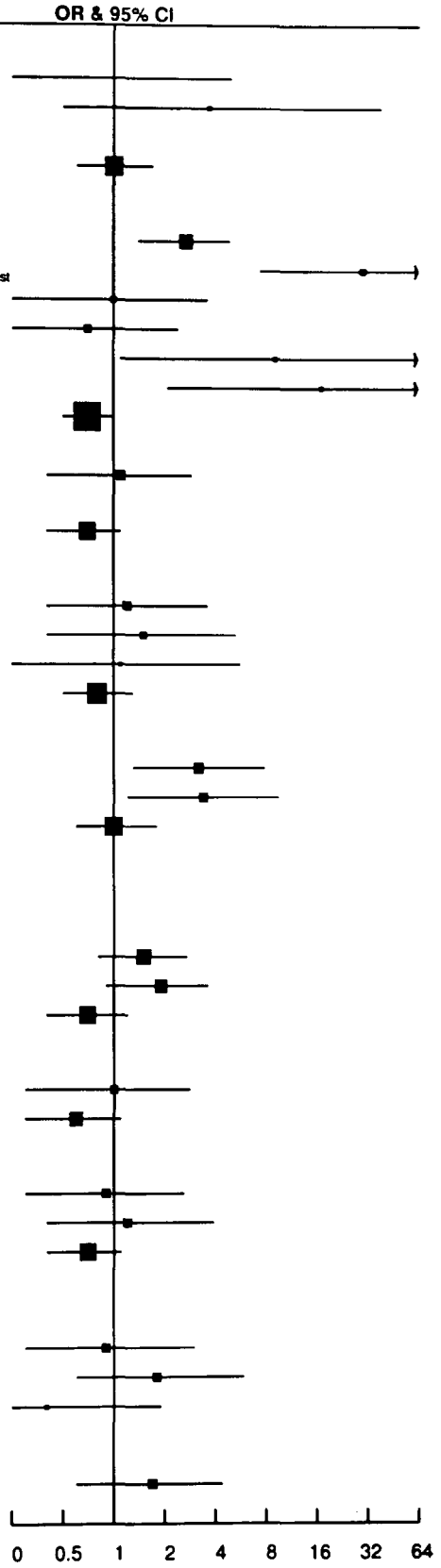
First author, Year	Country	Method	Laboratory technique	Number Cases/ controls	% Positive	OR (95% CI)
BETA GENUS						
HPV-5						
Favre, 2000	France/Poland	A	ELISA	6/119	0/5	NA
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	1/0.3	2.6 (0.2-31.9)*
Karagas, 2006	USA	A	Luminex	252/461	7/11	1.7 (1.0-3.1)$_{est}$
		B				1.8 (1.0-3.1)****
Andersson, 2008	Sweden	A	Luminex	72/121	19/13	1.6 (0.7-3.7)*****$_{est}$
HPV-8						
Steger, 1990	Germany	A	Western blot	11/445	73/20	10.7 (2.5-63.2)$_{est}$
Stark, 1998	Germany	A	ELISA	14/210	71/8	30.3 (7.4-142.5)$_{est}$
Stark, 1998	The Netherlands	A-Tx	ELISA	30/89	27/23	1.3 (0.4-3.5)$_{est}$
Bouwes-Bavinck, 2000	The Netherlands	A	ELISA	13/82	69/45	3.1 (0.7-13.3)**
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	4/0.3	14.7 (1.6-135.0)*
Masini, 2003	Italy	A	ELISA	46/84	57/32	3.2 (1.3-7.9)***
Karagas, 2006	USA	A	Luminex	252/461	16/15	1.1 (0.7-1.7)$_{est}$
		B				1.2 (0.8-1.8)****
Struijk, 2006	Australia	A	ELISA	64/58	22/3	7.8 (1.7-73.4)$_{est}$
		B				9.3 (1.9-45.6)*
Andersson, 2008	Sweden	A	Luminex	72/121	29/26	1.2 (0.6-2.4)*****$_{est}$
HPV-9						
Karagas, 2006	USA	A	Luminex	252/461	9/9	1.0 (0.6-1.8)$_{est}$
		B				1.0 (0.6-1.8)****
Andersson, 2008	Sweden	A	Luminex	72/121	21/12	1.5 (0.7-3.2)*****$_{est}$
HPV-15						
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	4/2	1.8 (0.6-5.6)**
Masini, 2003	Italy	A	ELISA	46/84	35/48	0.4 (0.2-0.9)***
Karagas, 2006	USA	A	Luminex	252/461	10/9	1.2 (0.7-2.0)$_{est}$
		B				1.3 (0.8-2.3)****
Struijk, 2006	Australia	A	ELISA	64/58	16/5	3.4 (0.8-20.0)$_{est}$
		B				3.8 (0.9-15.8)*
Andersson, 2008	Sweden	A	Luminex	72/121	18/17	1.1 (0.5-2.6)$_{est}$
HPV-20						
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	5/2	2.2 (0.8-6.7)**
Karagas, 2006	USA	A	Luminex	252/461	10/6	1.7 (0.9-3.1)$_{est}$
		B				1.7 (0.9-3.0)****
Struijk, 2006	Australia	A	ELISA	64/58	8/0	NA
		B				NA
Andersson, 2008	Sweden	A	Luminex	72/121	13/13	0.9 (0.3-2.4)*****$_{est}$
HPV-23						
Masini, 2003	Italy	A	ELISA	46/84	15/12	1.0 (0.3-3.3)***
HPV-24						
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	13/7	1.5 (0.8-2.9)**
Karagas, 2006	USA	A	Luminex	252/461	7/6	1.1 (0.6-2.2)$_{est}$
		B				1.2 (0.6-2.2)****
Struijk, 2006	Australia	A	ELISA	64/58	13/7	1.9 (0.5-9.2)$_{est}$
		B				2.6 (0.7-9.7)**
Andersson, 2008	Sweden	A	Luminex	72/121	17/11	1.7 (0.6-4.2)*****$_{est}$
HPV-36						
Masini, 2003	Italy	A	ELISA	46/84	20/8	2.8 (0.8-10.0)***
Karagas, 2006	USA	A	Luminex	252/461	4/6	0.8 (0.3-1.6)$_{est}$
		B				0.8 (0.4-1.8)****
Andersson, 2008	Sweden	A	Luminex	72/121	7/7	0.9 (0.2-3.2)*****$_{est}$
HPV-38						
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	6/3	3.0 (1.1-8.4)**
Karagas, 2006	USA	A	Luminex	252/461	13/11	1.3 (0.8-2.1)$_{est}$
		B				1.3 (0.8-2.1)****
Struijk, 2006	Australia	A	ELISA	64/58	2/0	NA
		B				NA
Andersson, 2008	Sweden	A	Luminex	72/121	25/18	1.5 (0.7-3.2)*****$_{est}$
ALPHA GENUS						
HPV-16						
Stark, 1998,	Germany	A	ELISA	14/210	3/2	1.7 (0.7-3.7)
Feltkamp, 2003	The Netherlands	A	ELISA	160/333	17/10	1.5 (0.4-5.6)**
Struijk, 2006	Australia	A	ELISA	64/58	6/2	3.8 (0.4-190.3)$_{est}$
		B				7.9 (0.8-81.7)*
Andersson, 2008	Sweden	A	Luminex	72/121	11/10	1.1 (0.4-3.2)*****$_{est}$
MU GENUS						
HPV-1						
Favre, 2000	France/Poland	A	ELISA	6/119	33/19	2.1 (0.2-15.5)
Andersson, 2008	Sweden	A	Luminex	72/121	21/18	1.2 (0.5-2.6)*****$_{est}$



NA: Not available
Tx: Transplant patients
OR: Odds ratio, CI: confidence interval
A: compared to those who are HPV negative to this HPV type
B: compared to those who are Beta-HPV negative in all analyses
est: unadjusted OR estimated if not provided
*: adjusted for age and sex
**: adjusted for age, sex, hair and eye colour and sun exposure
***: adjusted for age,sex,history of lifetime professional or recreational sun exposure and eye colour
****: adjusted for age,sex and skin sensitivity
*****: adjusted for age,sex, skin sensitivity, eye colour, sunburn history and location of lesion

Figure 4.4: Studies of cutaneous squamous cell carcinoma in relation to the detection of antibodies against L1 protein of some beta, alpha and mu HPV types.

Study. Year Country	Method	Lab technique	Number Cases/ controls	% Positive	OR (95% CI)
BETA GENUS					
HPV-5					
Favre et al. 2000 France/Poland	A	ELISA	34/119	3/5	0.6 (0.01-5.0)
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	1/0.3	3.7 (0.5-38.4)**
			S.:141/333	0/0.3	NA
Karagas et al. 2006 USA	A	Luminex	525/461	7/7	1.0 (0.6-1.7)$_{est}$
	B				1.0 (0.6-1.7)****
HPV-8					
Steger et al. 1990 Germany	A	Western blot	60/445	40/20	2.7 (1.4-4.9)
Stark et al. 1998 Germany	A	ELISA	14/210	35/8	30.3 (7.4-142.5)$_{est}$
Stark et al. 1998 The Netherlands	A-Tx	ELISA	18/89	22/23	1.0 (0.2-3.6)
Bouwes-Bavinck et al. 2000 The Netherlands	A	ELISA	19/82	32/45	0.7 (0.2-2.4)*
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	2/0.3	9.2 (1.1-78.2)**
			S.:141/333	5/0.3	17.3 (2.1-143)**
Karagas et al. 2006 USA	A	Luminex	525/461	11/15	0.7 (0.5-1.0)$_{est}$
	B				0.7 (0.5-1.0)****
Wieland et al. 2000 Germany	A	ELISA	40/200	18/16	1.1 (0.4-2.9)
HPV-9					
Karagas et al. 2006 USA	A	Luminex	525/461	6/9	0.7 (0.4-1.1)$_{est}$
	B				0.7 (0.4-1.2)****
HPV-15					
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	2/2	1.2 (0.4-3.6)**
			S.:141/333	3/2	1.5 (0.4-5.3)**
Wieland et al. 2000 Germany	A	ELISA	40/200	5/5	1.1 (0.1-5.7)
Karagas et al. 2006 USA	A	Luminex	525/461	7/9	0.8 (0.5-1.3)$_{est}$
	B				0.8 (0.5-1.3)****
HPV-20					
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	5/2	3.2 (1.3-7.9)**
			S.:141/333	6/2	3.4 (1.2-9.5)**
Karagas et al. 2006 USA	A	Luminex	525/461	6/6	1.0 (0.6-1.8)$_{est}$
	B				1.0 (0.6-1.7)****
HPV-23	NONE				NA
HPV-24					
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	11/7	1.5 (0.8-2.7)**
			S.:141/333	12/7	1.9 (0.9-3.6)**
Karagas et al. 2006 USA	A	Luminex	525/461	4/6	0.7 (0.4-1.2)$_{est}$
	B				0.7 (0.4-1.3)****
HPV-36					
Wieland et al. 2000 Germany	A	ELISA	40/200	13/13	1.0 (0.3-2.8)
Karagas et al. 2006 USA	A	Luminex	525/461	3/6	0.6 (0.3-1.1)$_{est}$
	B				0.6 (0.3-1.1)****
HPV-38					
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	2/3	0.9 (0.3-2.6)**
			S.:141/333	3/3	1.2 (0.4-3.9)**
Karagas et al. 2006 USA	A	Luminex	525/461	8/11	0.7 (0.4-1.1)$_{est}$
	B				0.7 (0.4-1.1)****
ALPHA GENUS					
HPV-16					
Feltkamp et al. 2003 The Netherlands	A	ELISA	N: 291/333	2/2	0.9 (0.3-3.0)**
			S.:141/333	4/2	1.8 (0.6-5.9)**
Wieland et al. 2000 Germany	A	ELISA	40/200	5/11	0.4 (0.05-1.9)
MU GENUS					
HPV-1					
Favre et al. (2000) France/Poland	A	ELISA	34/119	29/19	1.7 (0.6-4.4)



NA: Not available
Tx: Transplant patients
OR: Odds ratio; CI: confidence interval
A: compared to those who are HPV negative to this HPV type
B: compared to those who are Beta-HPV negative in all analyses
N: nodular basal cell carcinoma
S: superficial multifocall asal cell carcinoma
est: unadjusted OR estimated if not provided
*: adjusted for age, sex, hair and eye colour and sun exposure
**: adjusted for age and sex
***: adjusted for age,sex,history of lifetime professional or recreational sun exposure and eye colour
****: adjusted for age,sex and skin sensitivity

Figure 4.5: Studies of cutaneous basal cell carcinoma in relation to the detection of antibodies against L1 protein of some beta, alpha and mu HPV types.

4.8 Association between HPV prevalence, UV exposure, sex and age

Harwood *et al.* (2004) did not have enough statistical power to draw conclusions on the association of HPV-DNA positivity and sun exposure [268]. Struijk *et al.* (2003) and Forlsund *et al.* (2008) reported a higher EV HPV-DNA prevalence in males than in females and did not find any association with sun exposure [292, 293]. De Jong-Tieben *et al.* (2000), however, noticed a higher EV-HPV DNA prevalence in samples on sun exposed areas compared with those from non exposed locations. This result was only found in patients with skin cancers [160]. Two studies have reported an association between HPV-DNA positivity and sun exposure [147, 160]. Sunburn episodes between 13 and 20 years was associated with higher EV-HPV DNA prevalence compared with people who never had any and lifetime sun exposure was inversely associated with HPV-DNA prevalence [147]. EV-HPV DNA prevalence was also found to be higher in plucked hairs of patients with any outdoor occupation (mainly indoor occupations versus in and outdoor occupation (OR: 1.82; 95%CI: 1.20 to 2.76) versus mainly outdoor occupation (OR: 2.53; 95%CI: 1.52 to 4.23). These results were not controlled for any other factors [160].

HPV-DNA prevalence has been found to increase with increasing age in some studies [281, 160, 292, 320] but not all [147, 319, 153, 316, 318] . The largest previous study of HPV seroprevalence in the immunocompetent population reported on age and sex distributions of alpha, beta, gamma, nu and mu HPV types among 1797 German adults and children [320]. Overall, detection of antibodies against nu and mu types was evident in childhood whereas seroprevalence to alpha types was higher in women after puberty; seroprevalence to beta and gamma types was found to increase with age. Other studies

have reported only on beta HPV seroprevalence and risk factors among immunocompetent individuals [147, 319, 153, 146, 316, 318]. Termorshuizen et al (2004) reported no association in 313 controls patients between seropositivity to any of 6 beta HPV types (HPV5, 8, 15, 20, 24 or 38) and age, sex, skin type, lifetime sun exposure and painful sunburns at different age periods [147]. Karagas et al (2006) also reported that seropositivity to any of 8 beta types (HPV 5, 8, 9, 15, 20, 24, 36 or 38) in 461 immunocompetent patients without skin cancer did not differ in terms of age, level of education, smoking status, skin phototype and number of sunburns but noted higher beta seroprevalence in men compared to women [319]. Andersson et al (2008) looked at 434 immunocompetent patients with and without skin cancer (basal and squamous cell carcinoma) and also found no relationship between age, sex, skin type, smoking and previous sunburn and seropositivity to any beta types (HPV 5, 8, 9, 10, 15, 20, 24, 36 and 38) [153]. Only Feltkamp et al (2003) found a statistically significant (at 5% level) association between unadjusted seroprevalence of HPV24 and increasing age and male sex in immunocompetent patients [146]. Psoriasis patients treated with psoralen and UV-A (PUVA) have also been found to have high seropositivity [318].

4.9 Summary

Case reports on patients with the rare inherited skin disease EV suggested that specific HPV types such as 5 and 8 might be involved in the development of skin cancers in EV patients. As a result, these HPV types were called EV-types. It is however not possible to make definite conclusions on the basis of these earlier reports in such a rare condition. Furthermore, following the improvement of HPV-DNA detection methods such as PCR, EV-HPV DNA (now termed beta HPV) types were not only found in EV patients. Many

HPV types appear to be ubiquitous and persistent in normal skin, hair follicles, psoriasis and other hyperproliferative skin lesions. Higher HPV-DNA prevalence has been suggested in immunosuppressed people compared to immunocompetent ones, but data are sparse and studies are based on small numbers.

The interaction between the immune system and HPV is complex and not fully understood. The oncogenic mechanism of the E6 and E7 proteins of HPV high risk genital types is well known but the same properties have not been established for other HPV types of relevance to skin cancers. Even if EV-associated SCC suggest a role for HPV, there is no integration of viral DNA into the host genome. It would therefore imply that if HPV plays a role in skin carcinogenesis it is via a different and complex mechanism and interaction with the mutagenic effects of UV radiation has been suggested.

Most studies have used HPV-DNA detection methods to examine the association with NMSC. Due to the high sensitivity of PCR methods and the ubiquity of HPV, new types were often detected. The prevalence varied widely between samples within the same patient depending on its type (i.e hair or skin), its layer (before or after tape stripping) or its body location (sun exposed or not). To avoid these limitations, some recent studies have been looking at serology as a different tool to investigate the association between HPV and the development of SCC or BCC. In the earlier studies, only a few HPV types were examined and all but one of the studies was undertaken in immunocompetent people. Patients who are HPV5 and/or HPV8 seropositive might be at higher risk of developing SCC but more studies are needed to be done to confirm this finding and to look at serology for more HPV types. The prevalence of many HPVs seems to increase with age and might be associated with sun exposure. It is therefore very important to collect information on UV exposure and other possible confounding variables to better understand the association between HPV, NMSC and sun exposure.

CHAPTER 5

Methods, subjects and laboratory techniques

5.1 Introduction

This chapter describes the two case-control studies and the cohort study that will form the basis of this thesis. Design of the studies, questionnaires, the definition of case and control status, specimen collection and storage are presented. The last part of this chapter describes multiplex serology.

5.2 A prospective pilot study nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford)

5.2.1 Aim of the study

The aim of this pilot study was to examine the relationship between antibodies against HPV-L1 antigens for 38 HPV types in relation to SCC in apparently immunocompetent patients with plasma collected before or after the diagnosis of their skin tumour. We used biological samples and data from the Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford).

5.2.2 Methods

- **EPIC-Oxford**

Between 1993 and 1999, 65,429 people aged 20 years and above and living in the UK were recruited into the Oxford component of the European Prospective Investigation into Cancer and Nutrition (for further details of the study see [321] and [322]). Recruitment was through collaborating general practitioners, vegetarian and vegan societies, health-food magazines and from friends and relatives of the participants. Data were obtained via questionnaire on demographic, diet and other lifestyle factors, but no information on sun exposure or skin type was available. For about 19,500 volunteers, a blood sample was taken, sent through the mail to the laboratory, where plasma was separated, aliquoted and stored in liquid nitrogen. All participants are followed up for mortality and cancer incidence by record linkage with the National Health Service Central Register. Ethical approval was granted by

the Royal College of General Practitioners Clinical Research Ethics Committee, the Central Oxford Research Ethics Committee and local research ethics committees. All cases and controls for this pilot study were identified from within the EPIC-Oxford cohort.

- **Definition of cases and controls**

All cases and controls were of Caucasian origin. Cases with a diagnosis of cutaneous SCC were identified from within the cohort using codes for cancer site (C44) and morphology (8070/3, 8071/3, 8074/3, 8076/3), based on the 10th revision of the International Classification of Diseases for Oncology (ICD-10). Thirty-nine people who had plasma collected prior to the diagnosis of their first reported SCC were identified (they were not all eligible cases from EPIC). Of these, four had previously had a cutaneous basal cell carcinoma diagnosed and two others had a BCC diagnosed after recruitment and blood collection, but prior to the diagnosis of SCC. These 39 individuals will be referred to as the 'incident' cases. In addition, 15 individuals who had a cutaneous SCC diagnosed prior to recruitment and blood collection were also included. These individuals are referred to as 'prevalent' cases. Of these, one had a BCC diagnosed following recruitment and two had been diagnosed with BCC prior to recruitment. One incident and two prevalent cases had another cancer type diagnosed prior to recruitment (one prostate, one cervix and one thyroid cancer respectively). Four incident cases had other cancers detected following recruitment and the development of their first SCC (two with lung cancer, one with prostate cancer and one with both cancers of the pancreas and digestive organs).

The control group includes 80 persons who had not had a diagnosis of SCC registered with the National Health Service Central Register either before or after re-

cruitment. They were randomly selected from among other study participants for whom a plasma sample was available. Information on medication and medical history available from the questionnaire revealed that none of the participants reported being on immunosuppressive therapy, or having ever received an organ transplant. Three controls developed breast cancer following recruitment.

5.3 The Oxford and London case-control studies

5.3.1 Aim of the studies

Two case-control studies based at Barts and London NHS Trust and at the Oxford Radcliffe Hospitals were carried out to investigate the seroepidemiology of HPV among OTR, to detect potential confounders for the association between SCC and HPV, to examine the HPV seroprevalence by ethnicity and by different immunological status and finally, to examine the role of HPV and other factors in the development of NMSC and particularly SCC. In London, patients have access to a dedicated dermatology clinic at their routine visit at the transplant centre whereas in Oxford patients are referred to a dermatologist if transplant clinicians or GPs detect a suspicious skin lesion. Using questionnaire data, the impact of dedicated skin clinics on the level of awareness of the danger of skin cancers incurred by organ transplant patients and on the use of adequate protections against UV exposure will also be examined.

5.3.2 Retrospective power calculation

Preliminary data on which to base a power calculation were not available when I started the thesis. 119 prevalent SCC and 425 controls (around 1:4 case-control ratio) were actually recruited. The number of cases in an unmatched case-control study with a significant

level of 5% and a power of 95% for 1:4 case-control ratio is (Figure 5.1):

Proportion of exposed in control group	Relative risk 1.5	2	2.5	3	4
5%	1503	467	248	163	93
10%	811	256	138	92	54
15%	585	188	103	69	41
20%	476	155	86	59	36
25%	415	138	77	53	33
30%	378	128	73	50	32

Figure 5.1: Retrospective power calculation

5.3.3 Methods

A flow chart of the recruitment process is shown in Figure 5.2. Case-control studies within two cohorts of organ transplant recipients from London and Oxford were conducted. Transplant recipients from Barts and London NHS Trust were recruited between October 2002 and August 2006. To increase the power of the study, a case-control study from the Oxford Radcliffe Hospitals was set up and conducted between May 2005 and August 2006.

In London, all patients have access to a dedicated dermatology clinic following their usual visit to the transplant centre and undergo routine dermatological examinations, at which all benign and malignant lesions are recorded and treated if necessary. Patients were recruited at routine clinic visits and completed a specialist nurse-led questionnaire and were examined by a dermatologist. In Oxford patients are referred to a dermatologist if a suspicious skin lesion is present, but are not otherwise under routine surveillance. Therefore, transplant recipients attending the Oxford Transplant Centre were invited by mail to take part in the study and to complete a questionnaire. At the next clinic visit, this questionnaire was checked and finalised by a dermatologist who also conducted

an examination of the participants' skin, recording all benign and malignant cutaneous lesions. Treatments were initiated where indicated and educational information relating to the risks of skin cancer in transplant recipients was also provided. In both centres, a blood sample was taken and serum, buffy coat and red blood cells were separated, aliquoted and frozen at -80 °C.

5.3.4 Questionnaire, data entering and storage

- **Questionnaires**

A copy of the questionnaire is included in Appendix D. The same questionnaire was used in both centres to collect information on (i) social and demographic details (age, sex, height, weight, ethnicity, marital status, educational level, area of residence, country of birth) (ii) smoking and alcohol history; (iii) medical history (skin and/or other cancers, psoriasis); (iv) exposure to ultraviolet (UV) radiation (outdoor occupation and hobbies, sun exposure before and after transplantation, sun exposure currently, number of moles and freckles before and after transplantation, history of sunburn in childhood, protective measures against UV radiation, time spent abroad); (v) history of HPV-related viral infection (cutaneous and genital warts and history of abnormal cervical smear in women); (vi) transplantation and dialysis (number of transplantations, dates, type of dialysis, time spent on dialysis before and after transplantation, primary diagnosis); (vii) gynaecological and reproductive history for women (age at menopause, number of pregnancies, use of hormonal contraception, hormone replacement therapy, surgical removal of uterus). Since it is not clear how cutaneous HPV are transmitted, the questionnaire also included some questions on possible risk factors for infection (e.g. shared bedroom

or bed as a child, number of siblings and number in household, as surrogates for crowding and proximity). All information on transplantation, medications and skin cancers was cross-checked against information held in the renal-centre database and medical records.

- **Data entering and storage**

  All data were entered using the database *FoxPro version 2.6* and a quality control for data was performed on 30% of the dataset. Less than 1% of errors was found. Personal identifying information was kept separately from the database and an identifier number was given to each patient, so that anonymity was maintained throughout.

5.3.5 Specimen and storage

Transplant patients were asked to donate 8ml of venous blood at their routine blood test and plucked hairs were also collected. A blood specimen was collected into EDTA tubes, centrifuged and the serum, buffy coat and red blood cells were separated and frozen at -80° C. Hair samples for HPV DNA testing were stored in envelopes and frozen at -80 ° C. Anonymous samples (sera for the case-control studies and plasma for the EPIC-Oxford) were shipped on dry ice to the laboratory of Infection and Cancer Program (F020, DKFZ) in Heidelberg, Germany for HPV testing. All assays were performed by a single person (KMM). All laboratory investigators were blind to the case or control status of the patients.

5.3.6 Definition of cases and controls

Only malignant lesions with confirmed pathological verification of diagnosis were included. Patients were classifed as cases if review of histological records revealed evidence of SCC with or without other non-melanoma skin cancers. Of the 145 patients with

SCC, 70 (48%) had SCC with or without in-situ carcinoma of the skin (CIS), 66 (46%) had SCC with a history of BCC and 9 (6%) had SCC with a history of other non-melanoma skin cancers (porocarcinoma, Merkel cell carcinoma, eccrine nodular carcinoma or tricholemmal carcinoma) and with or without BCC. Controls were patients without confirmed diagnosis of skin cancer or CIS. In the course of the study, 20 patients from the London group developed their first SCC and were excluded from the control group.

Patients with blood taken prior to development of their first lesions were classified as 'incident' cases and those with sera taken after the development of their first lesion were classified as 'prevalent' cases.

The different case groups examined are:

- **Case 1**: Any squamous cell carcinoma
- **Case 2**: Basal cell carcinoma only
- **Case 3**: Prevalent squamous cell carcinoma
- **Case 4**: Incident squamous cell carcinoma
- **Case 5**: Prevalent basal cell carcinoma only
- **Case 6**: Incident basal cell carcinoma only

Patient cases 1 and 2 were selected to examine the risk factors associated with the development of SCC or BCC only based on the questionnaire data. Patient cases 1 to 5 were used to investigate the association between squamous cell carcinoma, basal cell carcinoma and HPV seropositivity. The case group 6 which includes only 5 patients was not analysed thoroughly. Final numbers of cases and controls by centre, ethnicity, availability of blood specimen and/or completed questionnaire are summarised in Figure 5.2.

5.4 Immunocompetent (IC) and dialysis patients from London

Patients with end-stage renal disease on dialysis, at increased risk of infections and cancers probably due to abnormalities of the immune system, and immunocompetent patients were also included to compare seroprevalence across different immune status (OTR, IC and dialysis patients). Caucasians and non-Caucasians immunocompetent patients without a history of skin cancer were enrolled from ophthalmology, plastic surgery or phlebotomy departments. A short questionnaire on basic socio-demographic details (sex, date of birth and ethnicity) and skin cancer history was completed and a blood sample was obtained and frozen at -80 ° C. No information on refusal rates for IC was available. In Oxford, data from EPIC-Oxford that used the same laboratory methodology to assess HPV serostatus were used to compare seroprevalence between OTR and IC individuals (Section 5.2). In order to evaluate the influence of renal failure pre-transplantation on HPV seroprevalence, stored sera from Caucasian and non-Caucasian dialysis patients from London with no history of transplantation, were also included. Detailed skin cancer history was not available for these patients, although it was known that none had previously attended the dermatology department for treatment of skin cancer. Basic socio-demographic details (sex, date of birth and ethnicity) were provided from the hospital renal database. Table 5.1 shows the number of patients that are included in each analysis.

5.5 Multiplex serology

HPV antibody detection was by multiplex serology, an antibody detection method that is based on a glutathione S-transferase capture enzyme-linked immunosorbent assay, as previously described [323, 324], in combination with fluorescent bead technology [325,

326]. All antigens were expressed in E.coli as double fusion of full-length viral proteins with a N-terminal glutathione S-transferase domain and a C-terminal peptide consisting of the last 11 amino acids from the large T antigen of simian virus 40 [323].

The expression constructs for the L1 proteins of HPV types 16 and 18 have been described elsewhere [324]. Expression constructs for the full length L1 proteins of HPV types 1a, 2a, 3, 4, 5b, 8, 9, 10, 11, 15, 17, 20, 23, 24, 36, 38, 41, 48, 49, 50, 57, 60, 63, 65, 75, 76, 77, 92, 93, and 95 were generated using the same methods and are described in detail elsewhere [324, 320]. Finally, expression constructs for L1 of HPV types 7 (amplified HPV nucleotides 5798-7312 in GenBank accession number X74463), 13 (5742-7238, X62843), 27b (5704-7191, AB211993), 96 (5856-7391, AY382779), 101 (5034-6578, DQ080081), and 103 (4945-6489, DQ080078) were newly generated all using EcoRI before the start and SalI at the end of the L1 open reading frame as cloning enzymes. Sequence variations to the published sequences present already in the parental clones were found for HPV-27b (T6230C (F to S); T6465C; A6765G; T6894C) and HPV-103 (A5774T (E to V); A6293G (D to G)). Genomic clones for HPV types 93 and 96 have not been published yet. HPV-93 (FAIMVS6) (O. Forslund, unpublished data) was recently isolated from an actinic keratosis on the dorsum of the hand of an 82-year-old male and HPV-96 (FA47) (O. Forslund, unpublished data) from an SCC in situ on the upper chest of a 75 year old male [280]. HPV101 and HPV103 were recently isolated from cervico-vaginal cells of two women in their 30s [327]. Surprisingly, phylogenetic analyses revealed that these two types cluster together with the gamma- and pi-PV groups usually associated with skin HPV.

Glutathione-casein was coupled to internally fluorescence-labelled polystyrene beads (Luminex, Austin, TX), and fusion proteins were affinity-purified on the beads directly in a one-step procedure. Beads with glutathione S-transferase alone were prepared for

background determination. Binding of the antigens (i.e. the glutathione S-transferase fusion proteins) to various bead sets was verified with a monoclonal antibody against the common C-terminal peptide [323]. The differently labelled bead sets carrying different antigens were then mixed and incubated in 96-well plates with human plasma diluted 1:100 in blocking buffer, as described previously [326]. The analyses were performed blinded with respect to the case or control status of the samples. Antibodies bound to the beads via the viral antigens were then stained with biotinylated anti-human immunoglobulin and fluorescent reporter conjugate streptavidinR-phycoerythrin (Molecular Probes, Eugene, OR). Antibodies bound to antigens on beads were quantified in the Luminex analyzer, which also identified the internal bead colour and thus the antigen carried by the bead. Antibody quantity was determined as the median R-phycoerythrin fluorescence intensity (MFI) from at least 100 beads of the same internal colour after subtraction of background reactivity (glutathione S-transferase alone).

5.6 Sensitivity and specificity of the assay

HPV type cut-off values were based on a reference panel of 164 sera with antibody prevalence defined in a previous study [320] and reanalyzed in this study. The cut-offs were chosen in a way that produces high specificity. For all HPV types but HPV6 analyzed here, MFI cut-offs to define seropositivity for all antigens were set to 200 MFI [320]. To reduce the influence of borderline seropositive sera, a stringent (doubled) cut-off of 400 MFI was applied to HPV6. Data analysis using geometric mean MFI values instead of cut-off values did not materially change the results.

For assays of antibodies to sexually transmitted genital HPV cut-off definition can be based on the seroreactivity in groups of virgins [328] while for the cutaneous types, which is the vast majority of HPV types analysed here the definition of a mostly uninfected and

thus mostly seronegative group is not possible. In the absence of defined reference sera that could be used as international standards, any cut-off definition has an arbitrary component and seroprevalence values obtained by different laboratories in general should only be compared with caution. Here, all antigens were identically constructed L1 fusion proteins expressed in the same bacterial expression system. Thus, given the uniformity of the antigens the use of a uniform cut-off appears justified. To avoid false-positivity by low-level cross-reactivity and thus to increase type specificity of the seroprevalence values a cut-off well above background levels was chosen. Thus, the chosen cut-off is rather stringent and probably underestimates the true seroprevalence of cutaneous HPV infections [320].

One of the limitations of the evaluation of the performance of the assay is that it is currently the 'gold standard' for cutaneous HPV types. Direct comparison of GST L1 fusion protein- and VLP-based ELISA (VLP: virus-like particle) with 105 human sera has been performed for HPV 16 and 18, and showed a good correlation of 0.7 [324]. Of 46 monospecific monoclonal antibodies detecting conformational epitopes on VLP of 9 mucosal types all reacted with the GST-L1 fusion protein of the same type [329] demonstrating the ability of GST-L1 to bind type-specific antibodies. Based on these observations, it is reasonable to assume similar properties for the other HPV types investigated here. Cross-reactivity cannot be entirely ruled out and thus unspecific antibody reactions. Like VLP, GST-fusion proteins present conformational and neutralising epitopes but display a higher amount of linear/cross-reacting epitopes than VLP [329]. Thus, under the condition that only highly purified, intact VLP preparations are used as antigens, VLP-based assays may yield a higher ratio of specific to unspecific reactions than with GST-L1-based serology.

It should also be born in mind that this HPV test is not used as a screening tool but only as a measure of an exposure for epidemiological studies. Despite a lower sensitivity,

serology has shown to be a good tool in epidemiologic studies to detect an association between HPV infection and cervical cancer [235, 242, 243] but not for screening.

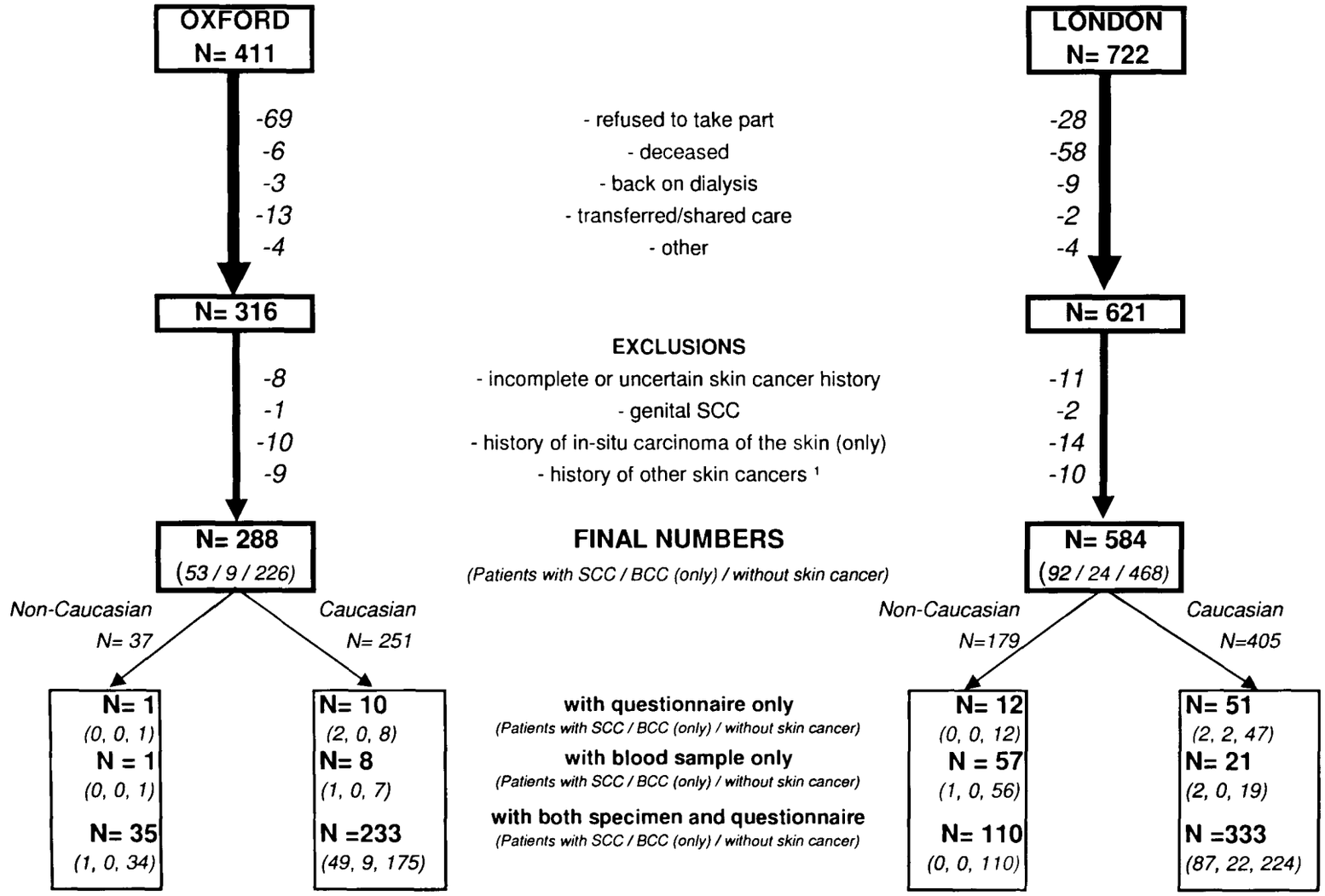


Figure 5.2: Flow chart of data collection of the Oxford and London case-control studies.

N: number; SCC: Squamous cell carcinoma; BCC: Basal cel carcinoma

[1] Exclusions of 19 patients with other skin cancers (9 patients with a history of malignant melanoma (with or without SCC), 8 patients with a history of Kaposi's Sarcoma (with or without SCC), 1 patient with poro carcinoma and BCC, and 1 patient with lentigo maligna)

Table 5.1: Summary of number of patients for each analysis.

Study	Analysis	Results	Immune status	Questionnaire	Blood sample	SCC cases (with or without BCC)	BCC cases	Without skin cancer
London and Oxford studies	**HPV, immune status and ethnicity**	Chapter 6	OTR, IC, dialysis	basic	yes	none	none	**Organ transplant recipients:** **N= 201** non-Caucasians (Oxford: 35; London: 166) **N[2]= 425** Caucasians (Oxford: 182; London: 243) **Immunocompetent:** **N= 50** non-Caucasians (Oxford: 0; London: 50) **N= 182** Caucasians (Oxford[3]: 80; London: 102) **Dialysis:** **N= 145** non-Caucasians (Oxford: 0; London: 145) **N= 222** Caucasians (Oxford: 0; London: 222)
	HPV and risk factors from the questionnaire[1]	Chapter 6	OTR	yes[1]	yes	none	none	**N[2]= 425** Caucasians [Oxford: 182; London: 243]
	Skin cancers and HPV	Chapter 7	OTR	yes	yes/no	prevalent and incident **N= 140** [Oxford: 51; London: 89]	prevalent and incident **N= 33** [Oxford: 9; London: 24]	**N= 454** Caucasians [Oxford: 183; London: 271]
EPIC-Oxford	**SCC and HPV**	Chapter 7	IC	basic	yes	**N= 39** incident **N= 15** prevalent	none	**N[3]= 80** Caucasians

N: number; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; OTR: organ transplant recipients; IC: immunocompetent

[1]: includes 7 patients from Oxford and 19 patients from London with blood only and basic information (age, sex, time since transplantation and skin type).

[2] same patients; [3]: same patients

CHAPTER 6

Risk factors associated with the presence of antibodies against human papillomavirus

6.1 Introduction

Transmission and prevalence of high risk mucosal HPV types associated with genital warts, abnormal smears or cervical cancer have been studied intensively. It is, however, unknown how most other human papillomaviruses (HPV) are transmitted and distributed in the population and what factors are associated with HPV seropositivity - all important factors in understanding and interpreting the significance of the association between HPV seroepidemiology and NMSC risk. This chapter reports on HPV seroprevalence among Caucasian control OTR of the London and Oxford case-control studies and on the association between human papillomavirus and risk factors established from the questionnaire. Finally, an examination of the sero-epidemiology of HPV among different ethnic groups and among people of different immunological status is presented.

6.2 Statistical methods

All analyses considering HPV and the association with various risk factors from the questionnaire were restricted to Caucasian patients without skin cancers (NMSC are rare in non-Caucasian population and our ultimate aim is to examine the association between SCC and HPV and, therefore, to detect potential confounding factors). The association between HPV, ethnicity and immunological status was examined separately.

To assess the relationship between seropositivity to a single HPV type and various risk factors measured by questionnaire, conditional (on centre) logistic regression adjusted for sex, age at recruitment (less than 44, 45-59, 60 or more) and time since transplantation (less than 5 years, 5 to 9 years, 10 or more years) was applied. Conditional logistic regression on centre was used to deal with the potential for bias due to confounding by center. Age and sex were included in the model due to their strong established association with mucosal types. The adjustment for time since transplantation was decided following results on univariate analysis showing a possible decline in antibodies production within the first 5 years of transplantation (Table 6.5 for crude proportions). Seropositivity to multiple HPV types can be looked at a count data (seronegative to all HPV types, seropositive to one HPV type, seropositive to two HPV types...). The association between multiple HPV seropositivity and risk factors can therefore be analysed using negative binomial regression adjusted for the same factors (over-dispersion was observed when Poisson models were fitted).

Skin type was defined using Fitzpatrick classification scale as follows (I) never tans, always burns, (II) rarely tans, usually burns, (III) usually tans, can burn, (IV) always tans, rarely burns, (V) Asian, Middle Eastern and (VI) African/Afro-Caribbean. Ethnicity was defined as Caucasian (those who identified themselves as 'White and were usually individuals of European descent) and non-Caucasian (those who identified themselves as

'Asian, 'Far Eastern, 'Black or 'other and were usually individuals of non-European descent).

To compare single HPV seroprevalence between immunocompetent, dialysis and transplant patients from London and between OTR and IC patients from Oxford, logistic regression adjusted for age (<45, 45 to 59, ≥60 for London and <55, 55 to 64, ≥65 for Oxford) and sex was used. To assess the association between multiple HPV seropositivity and immunological status, negative binomial regression adjusted for age and sex was used with further adjusment for centre where appropriate. Sensitivity analysis was performed to compare HPV seroprevalence among IC, dialysis and transplant patients with kidney graft only.

Where results are presented in the form of plots, black squares indicate the point estimates and horizontal lines represent 95% confidence intervals (CI). To deal with multiple significant tests the level of statistical significance was set to 1% and when a sufficient number of patients was available, agreement of results across centre or population was used to detect genuine associations. Missing value categories were added to adjustment variables with incomplete information in order to retain all the observations in the analyses. Likelihood ratio tests were used to assess heterogeneity and trend tests. All P-values are two-sided. Statistical analyses were carried out using STATA 9 (StataCorp, 2005).

6.3 Descriptive statistics

In total, seroprevalence data from 1225 individuals were available. They comprised 425 Caucasian and 201 non-Caucasian OTR (5 patients [0.8%] from London with a solid organ graft other than kidney) with a blood sample and a completed questionnaire, but without skin cancer. Information from 182 Caucasian and 50 non-Caucasian IC patients

without skin cancer, and 222 Caucasian and 145 non-Caucasian dialysis patients were also included.

Table 6.1 shows the distribution of OTR by sex, time since transplantation, age at recruitment and ethnicity for each centre. There was no statistically significant difference at 5% level in distribution between the two centres in terms of sex, age at recruitment and time since transplantation, but, 35 patients (16%) in Oxford were non-Caucasians compared to 166 (41%) in London. Of the 201 non-Caucasian patients, 53% identified themselves as Asian (54% versus 46% in London and Oxford respectively), 31% as Black (31% versus 31% respectively), 9% as Far Eastern (8% versus 14% respectively) and 7% as other ethnic group (7% versus 9% respectively). In terms of country of birth, 97% of Caucasian OTR were born in Europe and 80% of non-Caucasians were born outside Europe (42% Indian subcontinent, 29% Africa, 13% Caribbean, 10% Far East, 4% Middle East and 2% Other). Caucasian patients tended to have been transplanted for longer than non-Caucasians (P=0.002).

6.4 Multiple HPV seropositivity among Caucasian OTR

Figure 6.1 shows the count ratio of seropositivity to multiple types of one genus by seropositivity to multiple types of another genus. Those individuals seroreactive to multiple types of one genus were more likely to be seroreactive to multiple types of another genus. There was no difference in terms of age at recruitment, time since transplantation, sex or skin type between patients HPV seronegative to any types and those being seropositive to at least one HPV type (Table 6.2).

To explore further the relationship between each of the HPV types, double seropositivity was examined i.e the proportion of patients who were seropositive to one HPV type and also to another type based on the laboratory cut-off definitions. Table 6.3 shows the

results. For instance of the 39 patients who were seropositive to HPV5 79% (31 patients) were also seropositive to HPV8.

Clear patterns were observed within and between genera:

- alpha types:

  The proportion of people with double HPV seropositivity was lower between alpha types and any other types compared to other genera. Patients who were seropositive to HPV6 had lower seroprevalence for other alpha types. Higher double seropositivity (64%) was observed between HPV 13 and HPV6, which are both members of the same species (10).

- beta types:

  A high proportion of patients who were seropositive to a single beta HPV type (other than HPV 93) were also seropositive to HPV 8, 9, 15, 17, 38 or 49 (dotted area on Table 6.3). Patients who were seropositive to HPV24 or HPV36 from species 1 or to HPV75 or HPV76 from species 3 were also seropositive to most beta HPV types but also gamma and mu types. Between half and a third of patients seropositive to gamma types (not HPV4) or to HPV63 (mu genus) or to HPV101 or HPV103 (other types) were also more likely to be seropositive to HPV15, 17, 38 or 49. Seropositivity to HPV93 was distinct from the other types with very low seroprevalence.

- gamma types:

  Between 46% and 79% of patients seropositive to a single beta or gamma HPV type were also seropositive to HPV 65 or HPV 95. In particular patients seropositive to gamma HPV types were more likely to be seropositive to gamma HPV types from the species 1 (HPV4, HPV65 or HPV95). These results are highlighted in the dotted area on Table 6.3.

- other types:

  Around three quarters of patients seropositive to HPV 103 were also seropositive to gamma HPV types from species 1 and between half and a third to HPV9, 15, 17, 38, 49, 75, 76 and 92.

These findings are summarised in the Table 6.4. Seropositivity to beta types was mainly explained by seropositivity to HPV8, 9, 15, 17, 38 and/or 49 and for gamma types by species 1. Of the 425 Caucasian OTR, 237 (56%) were seropositive to any beta types, 205 patients (48%) were seropositive to HPV8, 9, 15, 17, 38 and/or 49 and only 32 patients (8%) were seropositive to any of the other 10 betaHPV types. Regarding the gamma types, 200 patients (47%) were seropositive to any gammaHPV types, 178 (41%) were seropositive to HPV4, 65 and/or 95 (species 1) and 22 patients (5%) were seropositive to any of the three other types.

6.5 Risk factors associated with HPV based on questionnaire data among Caucasian OTR

- *Seropositivity to individual HPV type*

  Table 6.5 summarises results on seropositivity for a single HPV type among Caucasian control OTR by centre, sex, time since transplantation, age at recruitment and skin type. The prevalence of 8/34 HPV types differed significantly ($P \leq 0.05$) between centres. In both centres, higher seroprevalence was observed for HPV6 (33% and 26% for London and Oxford respectively), HPV8 (24% and 18%), HPV15 (26% and 29%), HPV17 (24% and 21%), HPV38 (23% and 21%), HPV49 (19% and 21%), HPV4 (27% and 23%), HPV65 (30% and 25%), HPV95 (22% and 20%), HPV1 (33% and 24%) and HPV63 (28% and 17%). Seroprevalence was statistically

significantly higher at the 1% level in London compared to Oxford for 3 cutaneous types; HPV 27 (21% versus 12% respectively), HPV 63 (28% versus 17%) and HPV 101 (10% versus 3%), and for 1 mucosal type, HPV 13 (13% versus 5%). As expected, higher HPV seroprevalence was observed in women for HPV16 (25% in female versus 10% in male) and seroprevalence for mucosal HPV types (16, 6 and 13) decreased with increasing age (P-trend<0.01). Seropositivity to types betaHPV15, 38 and to a less extent betaHPV5, 8, gamma75 and 76 seemed to drop after transplantation and then increased and stabilised. Apart from an increase in seroprevalence of HPV4 with time since transplantation (P-trend= 0.01) and a decrease in seroprevalence of HPV65 with increasing age (P-trend<0.001), no clear association was found with time since transplantation for any of the other HPV types examined. Similarly, no association was found between HPV seroprevalence and skin phototype.

Table 6.6 summarises associations for all other factors that were found to be statistically significant at least at the 1% level between risk factors from the questionnaire and single HPV seropositivity. Analyses were not performed by centre as figures were too small. Overall, given the large amount of available data from the questionnaire, very few variables were associated with single HPV seropositivity. Seropositivity to HPV6 was highly associated with a history of genital warts, increased with number of children (P-trend= 0.001), was higher in current users of oral contraceptive and was higher in past and current smokers as compared to never-smokers (P-value never versus ever smokers= 0.004). Self reported abnormal smear test was statistically significantly associated with mucosal HPV types 6 and 16. Mucosal type HPV13 seropositivity was also associated with a history of genital warts ($P \leq 0.001$). A self-report of history of abnormal smears was also associated with

seropositivity to betaHPV 15, 17, 38, 76 and 92. A history of herpes zoster was inversely associated to seropositivity to betaHPV 24 and 96. Variables related to UV exposure did not show any links with seropositivity except for HPV16 which was associated with sunbathing and outdoor hobbies. Patients with self-reported history of warts were 3 times more likely to be seropositive to alphaHPV 13 (95%CI: 1.2 to 9.2; P=0.01) and betaHPV 5 (95%CI: 1.1 to 9.8; P=0.02) with a large number of transplant patients reporting such history (68%). A history of psoriasis (n= 23 patients) was not associated with any of the 34 HPV types examined. No other distinguishing epidemiological features of transplant recipients with antibodies against any of the 34 HPV types examined were identified. All results are available in Appendix E1.

- *Multiple seropositivity*

Figure 6.2 is a graphical representation of selected risk factors associated with the presence of antibodies against multiple HPV types by genus. Multiple HPV seropositivity was more frequent in patients from London as compared to Oxford for alpha mucosal types and for other types (nu, mu and 2 undefined types). Young women, current smokers and female patients reporting a history of having had an abnormal cervical smear test and/or genital warts were more likely to be seroreactive to multiple mucosal HPV types. Women with a self-reported history of abnormal cervical smear were also more likely to be seropositive to more beta HPV types than those without such a history (CR: 2.3; 95% CI: 1.2 to 4.5; P=0.01) and multiple seropositivity to beta types seemed to be higher in patients with longer time since transplantation (P-trend=0.02). Ultraviolet radiation exposure history was not associated with seropositivity to a single HPV type but patients who reported using a sunbed and sunbathing were twice as likely to have more gamma serotypes

than those who did not (P-trend=0.007). No other distinguishing epidemiological features of OTR with antibodies against multiple HPV seropositivity were evident. All results are available in Appendices E2, E3 and E4.

6.6 Human papillomavirus and ethnicity among OTR

Table 6.7 shows the odds ratios for being seropositive to each single HPV type in non-Caucasian versus Caucasian OTR after controlling for age at recruitment, sex, time since transplantation and centre. The prevalence of L1 antibodies against HPV 5 (Odd ratios [OR]: 2.0 and 95% CI: 1.2 to 3.4; P=0.01), HPV 93 (OR: 2.6; 95% CI: 1.2 to 5.6; P=0.01) and HPV 101 (OR: 1.9; 95% CI: 1.1 to 3.3; P=0.02) were higher among non-Caucasian than Caucasian patients whereas for HPV 1 seroprevalence was lower in non-Caucasian patients compared to Caucasians (OR: 0.5; 95% CI: 0.3 to 0.7; P<0.001). Although the low seroprevalence did not allow for separate examination of results by centre, HPV1 seroprevalence was similar between Caucasian and non-Caucasian OTR from Oxford (24% versus 26%), but only 35 non-Caucasian OTR were recruited in Oxford.

Table 6.9 (part F) summarises results on seropositivity to multiple HPV types by ethnicity and centre. There was no difference at the 1% level of significance between Caucasian and non-Caucasian OTR regarding multiple HPV seropositivity for any types, alpha, beta, gamma or other types (nu, mu and 2 not defined types).

Among non-Caucasian patients, there was no statistically significant difference for any of the 34 HPV types, nor with antibodies against multiple seropositivity, between the 117 OTR with skin type V (Asian/Middle Eastern) and the 62 patients with skin type VI (Africa/ Afro-Caribbean) at the 1% level of significance (Appendix E5). Restricting analysis among the non-Caucasians to 106 Asians versus 62 Black patients showed higher seropositivity for mucosal types (especially HPV7, HPV16 and HPV13) and also

for betaHPV types of the species 2 among Black patients compared to Asians (Appendix E6).

6.7 Human papillomavirus by immunological status and comparison with the published literature

In Table 6.8, seroprevalence of the 34 HPV types examined in the OTR are compared to those from IC patients and dialysis patients recruited for comparison and with those from the published literature. In order to exclude possible methodological variables, only studies using an identical methodology and performed in the same laboratory as our own study were included [320, 1, 330]. The mean age at recruitment varied widely between these studies with older patients from Italy and EPIC-Oxford study [1, 330]. There were also differences in the sex ratio (number of male/number of female) between studies ranging from 0.6 to 1.6. Across countries, the most notable finding was the higher seroprevalence of many HPV types detected in Italy as compared to either Germany or the UK. The only consistent finding across ethnicity groups and across centres was a lower HPV4 seroprevalence among OTR compared to IC or dialysis patients. Otherwise, HPV seroprevalence differed little for most types across the different studies and centres, after adjustment for age and sex. For multiple HPV seropositivity, there was also no statistical difference at the 1% level of significance between ethnic groups or by immunological status (Table 6.9). Seropositivity to any HPV types ranged between 81% and 94%. Differences identified among OTR relating to ethnicity were corroborated for HPV93 (dialysis patients - OR: 3.4; 95% CI: 1.4 to 8.5; IC: OR not available and 0% in Caucasians versus 10% in non-Caucasians) and HPV1 (dialysis OR: 0.5; 95%CI: 0.3 to 0.8; IC: OR: 0.5; 95% CI: 0.2 to 1.0), but not for HPV5, HPV101 or other HPV types among dialysis and IC

patients from London. Exclusion of non-renal transplant patients (n= 5) did not materially change results.

As for transplant patients, those individuals seropositive to multiple types of one genus were more likely to be seroreactive to multiple types of another genus independently of immunological status or ethnicity (Appendices E7 and E8).

6.8 Discussion

Little is known of the seroepidemiology of HPV, with the exception of those mucosal types associated with cancer of the uterine cervix [215]. We report here on risk factors associated with HPV seropositivity for 3 mucosal and 31 cutaneous HPV types across 5 genera among 425 Caucasian and 201 non-Caucasian OTR without skin cancer and we compare HPV seroprevalence between populations with different immunological status and consider ethnic variations.

Eighty six percent of Caucasian transplant recipients without skin cancers in this study were seroreactive to at least one HPV type, identical to previous results from a UK study among Caucasian immunocompetent people (86%) [1] (Chapter 7), illustrating the ubiquity of HPV [281]. Findings for mucosal HPV types were in line with results from previous studies [215, 320] and provide internal validation for the multiplex technology used in this study.

We observed differences in HPV seroprevalence between OTR from 2 geographically close centres (Oxford and London) perhaps reflecting the importance of variations in environmental exposure or even clinical practice such as close proximity in clinics. Individuals seropositive to multiple types of one genus were also more likely to be seroreactive to multiple types of another genus, possibly suggesting similar modes of transmission or cross-reactivity of the assay. This result was not explained by a higher susceptibility of

OTR to get multiple seropositivity since the same pattern was observed among IC and dialysis patients.

Associations with time since transplantation and seropositivity to certain beta or gamma types were not linear but might reflect a decline in antibody production following intensive immunosuppressive treatments in the first years following transplantation. However, this remains speculative and would require prospective HPV serology studies for confirmation. In contrast, in Caucasians, we observed that most HPV seroprevalence did not differ substantially, after controlling for age and sex, between IC individuals, dialysis patients without a history of transplantation and OTR from London or between IC and transplanted individuals from Oxford suggesting apparently low disturbance in production of antibodies by immunological status. The only notable exception was a lower HPV4 seroprevalence among OTR compared to dialysis or IC patients independent of centre or ethnicity. Unfortunately, we do not have complete information on the type of dialysis (continuous ambulatory peritoneal dialysis or haemodialysis) to explore whether this specifically affected seroprevalence. No association was observed between the presence of psoriasis and any of the 34 HPV types examined. Despite the small number of patients, this result is consistent with a recent study on beta HPV-DNA and psoriasis [331]. There is limited statistical power to examine associations with all of the HPV types, in part because the prevalence of some is low. Further limitations may arise because details of risk factors were examined using self-reported information.

The largest previous study of HPV seroprevalence in the immunocompetent population reported on age and sex distributions of alpha, beta, gamma, nu and mu HPV types among 1797 German adults and children [320]. Overall, detection of antibodies against nu and mu types was evident in childhood whereas seroprevalence to alpha types was higher in women after puberty; seroprevalence to beta and gamma types was found to in-

crease with age. We did not find an association between age and presence of antibodies against gamma and beta types in either the transplant population or the immunocompetent group. Other studies have reported only on beta HPV seroprevalence and risk factors among immunocompetent individuals [147, 319, 153, 146, 316, 318]. Termorshuizen et al (2004) reported no association in 313 controls patients between seropositivity to any of 6 beta HPV types (HPV5, 8, 15, 20, 24 or 38) and age, sex, skin type, lifetime sun exposure and painful sunburns at different age periods [147]. Karagas et al (2006) also reported that seropositivity to any of 8 beta types (HPV 5, 8, 9, 15, 20, 24, 36 or 38) in 461 immunocompetent patients without skin cancer did not differ in terms of age, level of education, smoking status, skin phototype and number of sunburns but noted higher beta seroprevalence in men compared to women [319]. Andersson et al (2008) looked at 434 immunocompetent patients with and without skin cancer (basal and squamous cell carcinoma) and also found no relationship between age, sex, skin type, smoking and previous sunburn and seropositivity to any beta types (HPV 5, 8, 9, 10, 15, 20, 24, 36 and 38) [153]. Only Feltkamp et al (2003) found a statistical significant association between unadjusted seroprevalence of HPV24 and increasing age and male sex in immunocompetent patients [146].

No previously reported studies have investigated the association between the prevalence of HPV antibodies by ethnicity. Consistently across immunological status, we observed statistically significant differences between Caucasians and non-Caucasians: betaHPV 93 was higher in non-Caucasians, whereas muHPV type 1 seroprevalence was lower. As most Caucasians were born in Europe and non-Caucasians outside Europe we were not able to distinguish if the observed differences were confounded by birth country. There were some limitations to our analyses on ethnicity as diverse groups (Asian, Black [African and Afro-Caribbean], Far Eastern or other) with different birth country were

all pooled in the 'non-Caucasian' category. Although this essentially equates to non-European, this is inevitably somewhat crude grouping and more detailed geographical studies are essential to examine further the HPV seroprevalence among different ethnicities.

In summary, findings on the association between various risk factors and mucosal HPV types were an internal validation of the methodological approach used in this study. Significant differences in HPV seroprevalence were identified between study centres and according to ethnicity, but no other distinguishing epidemiological feature of transplant patients with antibodies against any of the 34 individuals or multiple HPV types examined were identified. Interpretation of future HPV and cancer association studies will require a better understanding of HPV seroepidemiology and further research is now needed to clarify the risk factors and the natural history of these viruses.

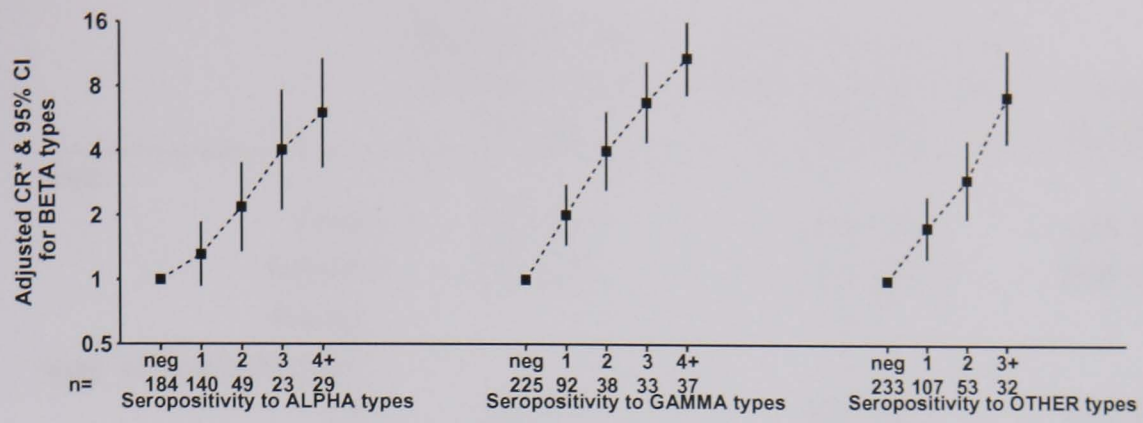
Table 6.1: Descriptive statistics for age at recruitment, sex and time since transplantation among transplant patients by centre and ethnicity

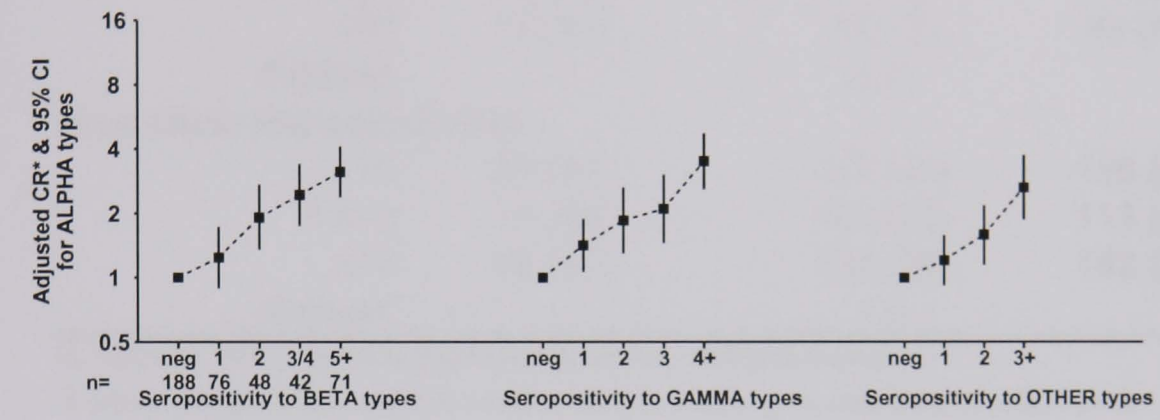
	Caucasian patients		Non-Caucasian patients		Both centres	
	Oxford N=182 no (%)	London N=243 no (%)	Oxford N=35 no (%)	London N=166 no (%)	Caucasian N=425 no (%)	Non-Caucasian N=201 no (%)
sex						
male	110 (60)	150 (62)	19 (54)	96 (58)	260 (61)	115 (57)
female	72 (40)	93 (38)	16 (46)	70 (42)	165 (39)	86 (43)
P-het.		***0.8*** [1]		***0.6*** [1]		***0.6*** [2]
age at recruitment (years)						
<45	80 (44)	107 (44)	16 (46)	69 (46)	187 (44)	85 (42)
45-59	64 (35)	94 (39)	11 (31)	70 (31)	158 (37)	81 (40)
60 or more	38 (21)	42 (17)	8 (23)	27 (23)	80 (19)	35 (17)
P-trend		***0.6*** [1]		***0.8*** [1]		***0.8*** [2]
time since transplantation (years)						
<5	70 (38)	80 (33)	14 (40)	82 (49)	150 (35)	96 (48)
5 to 9	52 (29)	61 (25)	12 (34)	35 (21)	113 (27)	47 (23)
10 or more	60 (33)	102 (42)	9 (26)	49 (30)	162 (38)	58 (29)
P-trend		***0.08*** [1]		***0.7*** [1]		***0.002*** [2]

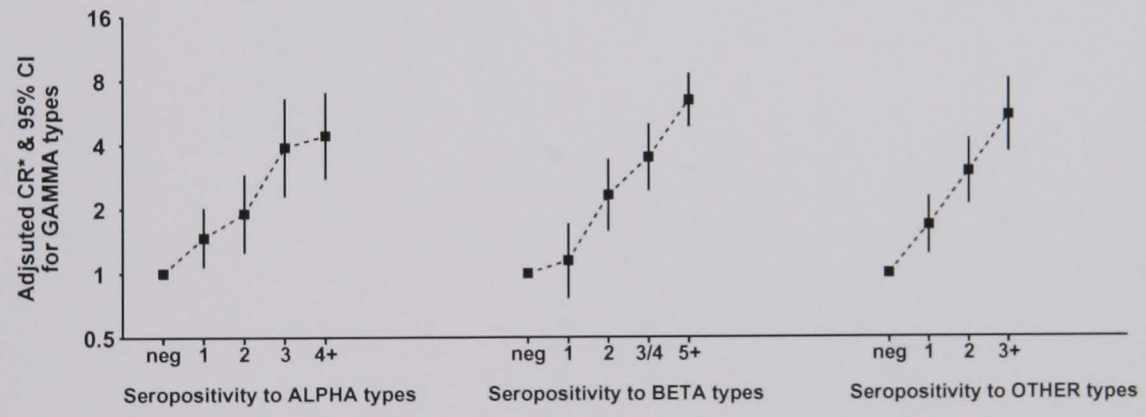
N: Total number; no: number

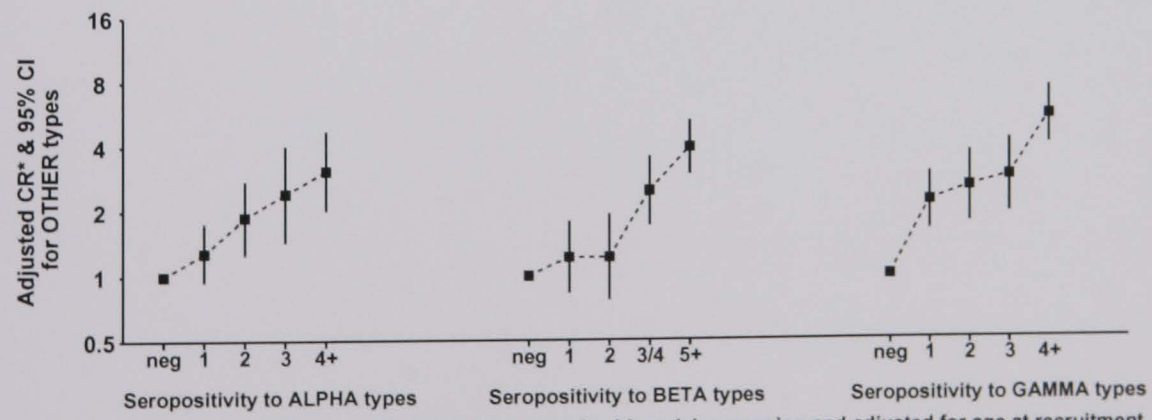
[1] P-values between Oxford and London are based on logistic regression adjusted for each other

[2] P-values between Caucasian and non-Caucasian are based on conditional (on centre) logistic regression adjusted for each other









* Count ratios (CR) were calculated using negative binomial regression and adjusted for age at recruitment, time since transplantation, sex and centre
CI: confidence interval; n: number
Other types: HPV types from nu, mu genera and HPV101 and 103
All P-values for trend <0.001

Figure 6.1: Count ratios (CR) (and 95%CI) of the expected number of seropositivity from one genus by the number of seropositivity of another genus among Caucasian control transplant patients (n=425)

	Seronegative to all HPV types **N= 59**	Seropositive to at least one HPV type **N= 366**	total **N= 425**
sex			
male	25 (42)	140 (38)	**165 (39)**
female	34 (58)	226 (62)	**260 (61)**
P-het.		*0.5*	
age at recruitment			
<45	24 (41)	163 (45)	**187 (44)**
45 to 59	20 (34)	138 (38)	**158 (37)**
≥60	15 (25)	65 (18)	**80 (19)**
P-trend		*0.3*	
time since transplantation			
<5	24 (41)	126 (34)	**150 (35)**
5 to 9	11 (19)	102 (28)	**113 (27)**
≥10	24 (41)	138 (38)	**162 (38)**
P-trend		*1.0*	

N: number; HPV: Human papillomavirus; Het.: heterogeneity

P-value using conditional (on centre) logistic regression adjusted for each other

Table 6.2: Age, sex and time since transplantation distribution in seropositive compared to seronegative to any HPV types among Oxford and London Caucasian without skin cancer

genus	species	type	ALPHA							BETA																GAMMA						NU	MU		ND	
		species	1	4		8	9	10		1						2					3			4	5	1			2	3	4	1	1	2		
		type	3	2	27	7	16	6	13	5	8	20	24	36	93	9	15	17	23	38	49	75	76	92	96	4	65	95	48	50	60	41	1	63	101	103
		no POS (% tot)	36 (8%)	61 (14%)	73 (17%)	36 (8%)	67 (16%)	128 (30%)	42 (10%)	39 (9%)	91 (21%)	61 (14%)	47 (11%)	50 (12%)	12 (3%)	65 (15%)	116 (27%)	100 (24%)	41 (10%)	95 (22%)	84 (20%)	50 (12%)	42 (10%)	52 (12%)	62 (15%)	108 (28%)	119 (28%)	91 (21%)	66 (16%)	32 (8%)	19 (4%)	46 (11%)	124 (29%)	98 (23%)	30 (7%)	26 (6%)
ALPHA	2	3	100	15 (8-26)	11 (6-20)	19 (10-36)	15 (8-26)	8 (4-14) 7	21 (12-36)	21 (11-36)	11 (6-19)	11 (6-22)	9 (3-21)	22 (13-36)	8 (1-41)	20 (12-31)	11 (6-18)	15 (9-23)	17 (8-32)	15 (9-23)	12 (7-21)	10 (4-22)	7 (2-20)	17 (9-30)	8 (3-18)	14 (9-22)	14 (9-22)	16 (10-26)	12 (6-22)	25 (13-43)	26 (11-50)	9 (3-21) 9	15 (9-22)	13 (8-22)	10 (3-27)	23 (11-43)
	4	2	25 (14-41)	100	52 (41-63)	36 (22-53)	28 (19-40)	18 (12-26)	45 (31-60)	41 (27-57)	31 (22-41)	31 (21-44)	36 (24-51)	52 (38-65)	42 (18-69)	38 (27-51)	29 (21-37)	31 (23-41)	49 (34-64)	34 (25-44)	35 (25-45)	40 (27-54)	48 (33-62)	37 (25-50)	31 (20-43)	23 (16-32)	28 (20-36	34 (25-44)	38 (27-50)	28 (15-46)	32 (15-55)	30 (19-45)	15 (10-23)	32 (23-41)	37 (22-55)	50 (32-68)
		27	22 (12-39)	62 (50-73)	100	33 (20-50)	39 (28-51)	22 (16-30)	55 (40-69)	38 (25-54)	30 (21-40)	36 (25-49)	34 (22-49)	42 (29-56)	58 (31-82)	35 (25-48)	28 (20-36)	27 (19-37)	44 (30-59)	35 (26-45)	35 (25-45)	34 (22-48)	36 (23-51)	33 (21-46)	32 (22-45)	27 (19-36)	32 (24-41	35 (26-45)	45 (34-57)	31 (18-49)	32 (15-55)	33 (21-47)	22 (15-30)	33 (24-43)	53 (36-70)	31 (16-51)
	8	7	19 (10-36)	21 (13-33)	16 (10-27)	100	24 (15-35)	14 (9-21)	36 (23-51)	28 (16-44)	18 (11-27)	21 (13-33)	30 (19-44)	28 (17-42)	17 (4-48)	25 (16-36)	18 (12-26)	21 (14-30)	34 (21-50)	23 (16-33)	24 (16-34)	26 (16-40)	29 (17-44)	27 (17-40)	24 (15-36)	17 (11-25)	19 (13-27	24 (16-34)	23 (14-34)	31 (18-49)	16 (5-39)	20 (11-34)	15 (9-22)	17 (11-26)	27 (14-45)	35 (19-54)
	9	16	28 (16-44)	31 (21-44)	36 (26-47)	44 (29-61)	100	23 (17-32)	45 (31-60)	26 (14-41)	26 (18-36)	25 (15-37)	32 (20-46)	30 (19-44)	17 (4-48)	28 (18-40)	24 (17-32)	23 (16-32)	22 (12-37)	27 (19-37)	26 (18-37)	30 (19-44)	26 (15-41)	25 (15-38)	27 (18-40)	25 (18-34)	25 (18-34	33 (24-43)	29 (19-41)	38 (23-55)	42 (23-64)	24 (14-38)	22 (15-30)	22 (15-32)	27 (14-45)	23 (11-43)
	10	6	28 (16-44)	38 (27-50)	38 (28-50)	50 (34-66)	45 (33-57)	100	64 (49-77)	51 (36-66)	41 (31-51)	34 (24-47)	34 (22-49)	42 (29-56)	25 (8-55)	38 (27-51)	34 (26-43)	39 (30-49)	44 (30-59)	36 (27-46)	37 (27-48)	36 (24-50)	36 (23-51)	38 (26-52)	35 (25-48)	32 (24-42)	34 (26-43	37 (28-48)	33 (23-45)	31 (18-49)	42 (23-64)	48 (34-62)	29 (22-38)	34 (25-44)	43 (27-61)	38 (22-58)
		13	25 (14-41)	31 (21-44)	32 (22-43)	42 (27-58)	28 (19-40)	21 (15-29)	100	23 (12-39)	20 (13-29)	18 (10-30)	26 (15-40)	28 (17-42)	33 (13-62)	26 (17-38)	18 (12-26)	18 (12-27)	37 (23-52)	23 (16-33)	21 (14-31)	20 (11-33)	26 (15-41)	29 (18-42)	23 (14-35)	13 (8-21)	18 (12-26	22 (15-32)	24 (15-36)	28 (15-46)	21 (8-45)	24 (14-38)	15 (9-22)	20 (14-30)	27 (14-45)	23 (11-43)
BETA	1	5	22 (12-39)	26 (17-39)	21 (13-31)	31 (18-47)	15 (8-26)	16 (10-23)	21 (12-36)	100	34 (25-44)	31 (21-44)	34 (22-49)	44 (31-58)	42 (18-69)	28 (18-40)	18 (12-26)	21 (14-30)	37 (23-52)	21 (14-30)	19 (12-29)	30 (19-44)	36 (23-51)	29 (18-42)	31 (20-43)	15 (9-23)	18 (12-26	23 (16-33)	21 (13-33)	28 (15-46)	26 (11-50)	20 (11-34)	10 (6-16)	18 (12-27)	33 (19-52)	35 (19-54)
		8	28 (16-44)	46 (34-58)	37 (27-49)	44 (29-61)	36 (25-48)	29 (22-37)	43 (29-58)	79 (64-89)	100	67 (55-78)	85 (72-93)	76 (62-86)	83 (52-96)	69 (57-79)	45 (36-54)	46 (37-56)	61 (45-75)	58 (48-67)	63 (52-73)	64 (50-76)	76 (61-87)	63 (50-75)	68 (55-78)	37 (28-47)	45 (37-54	49 (39-60)	50 (38-62)	59 (42-75)	47 (27-69)	50 (36-64)	31 (24-40)	43 (33-53)	73 (55-86)	46 (28-65)
		20	19 (10-36)	31 (21-44)	30 (21-42)	36 (22-53)	22 (14-34)	16 (11-24)	26 (15-41)	49 (34-64)	45 (35-55)	100	66 (51-78)	64 (50-76)	25 (8-55)	54 (42-66)	36 (28-45)	41 (32-51)	41 (28-57)	44 (35-54	48 (37-58)	52 (38-65)	57 (42-71)	48 (35-61)	52 (39-64)	32 (24-42)	34 (26-43)	42 (32-52)	42 (31-55)	47 (31-64)	37 (19-60)	37 (24-52)	16 (11-24)	31 (22-40)	43 (27-61)	38 (22-58)
		24	11 (4-26)	28 (18-40)	22 (14-33)	39 (25-55)	22 (14-34)	13 (8-19)	29 (17-44)	41 (27-57)	44 (34-54)	51 (38-63	100	54 (40-67)	25 (8-55)	55 (43-67)	34 (26-43)	38 (29-48)	44 (30-59)	41 (32-51	48 (37-58)	60 (46-73)	67 (51-79)	48 (35-61)	61 (49-73)	26 (19-35)	29 (22-38)	34 (25-44)	41 (30-53)	50 (33-67)	42 (23-64)	28 (17-43)	20 (14-28)	28 (20-37)	47 (30-64)	35 (19-54)
		36	31 (18-47)	43 (31-55)	29 (20-40)	39 (25-55)	22 (14-34)	16 (11-24)	33 (21-49)	56 (41-71)	42 (32-52)	52 (40-65	57 (43-71)	100	33 (13-62)	51 (39-63)	31 (23-40)	36 (27-46)	49 (34-64)	39 (30-49	40 (31-51)	56 (42-69)	60 (44-73)	48 (35-61)	45 (33-58)	24 (17-33)	30 (23-39)	36 (27-47)	35 (24-47)	38 (23-55)	53 (31-73)	30 (19-45)	22 (15-30)	33 (24-43)	40 (24-58)	46 (28-65)
		93	3 (0-17)	8 (3-18)	10 (5-19)	6 (1-20)	3 (1-11)	2 (1-7)	10 (4-23)	13 (5-27)	11 (6-19)	5 (2-14)	6 (2-18)	8 (3-19)	100	6 (2-15)	4 (2-10)	5 (2-11)	12 (5-26)	6 (3-13)	5 (2-12)	10 (4-22)	12 (5-26)	10 (4-21)	8 (3-18)	5 (2-11)	5 (2-11)	7 (3-14)	12 (6-22)	13 (5-29)0	16 (5-39)	11 (5-24)	2 (1-7)	4 (2-10)	33 (19-52)	12 (4-30)
	2	9	36 (22-53)	41 (29-54)	32 (22-43)	44 (29-61)	27 (18-39)	20 (14-27)	40 (27-56)	46 (31-62)	49 (39-60)	57 (45-69)	77 (62-87)	66 (52-78)	33 (13-62)	100	48 (39-57)	51 (41-61)	54 (39-68)	55 (45-64	61 (50-71)	68 (54-79)	69 (54-81)	65 (52-77)	68 (55-78)	35 (27-45)	41 (33-50)	49 (39-60)	45 (34-57)	59 (42-75)	68 (45-85)	35 (23-49)	28 (21-37)	42 (33-52)	50 (33-67)	54 (35-72)
		15	36 (22-53)	56 (43-68)	44 (33-55)	61 (45-75)	42 (31-54)	31 (24-40)	50 (35-65)	56 (41-71)	58 (48-68)	69 (56-79)	87 (74-94)	74 (60-84)	42 (18-69)	88 (77-94	100	65 (55-74)	68 (53-81)	78 (68-85	80 (70-87)	84 (71-92)	79 (64-88)	75 (62-85)	81 (69-89)	46 (37-56)	55 (46-63)	58 (48-68)	59 (47-70)	63 (45-77)	74 (50-89)	48 (34-62)	41 (33-50)	55 (45-65)	60 (42-76)	62 (42-78)
		17	42 (27-58)	51 (38-63)	37 (27-49)	58 (42-73)	34 (24-46)	30 (23-39)	43 (29-58)	54 (38-69)	51 (40-61)	67 (55-78)	81 (67-90)	72 (58-83)	42 (18-69)	78 (67-87	56 (47-65)	100	66 (50-79)	67 (57-76	64 (54-74)	72 (58-83)	69 (54-81)	69 (56-80)	69 (57-80)	37 (28-47)	45 (37-54)	59 (49-69)	48 (37-60)	66 (48-80)	63 (40-81)	48 (34-62)	28 (21-37)	43 (33-53)	60 (42-76)	58 (39-75)
		23	19 (10-36)	33 (22-45)	25 (16-36)	39 (25-55)	13 (7-24)	14 (9-21)	36 (23-51)	38 (25-54)	27 (19-38)	28 (18-40)	38 (26-53)	40 (27-54)	42 (18-69)	34 (23-46	24 (17-33)	27 (19-37)	100	31 (22-40	30 (21-40)	38 (26-52)	43 (29-58)	42 (30-56)	34 (23-46)	17 (11-25)	19 (13-27)	22 (15-32)	26 (17-38)	34 (20-52)	21 (8-45)	15 (7-29)	13 (8-20)	21 (14-31)	37 (22-55)	38 (22-58)
		38	39 (25-55)	52 (40-66)	45 (34-57)	61 (45-75)	39 (28-51)	27 (20-35)	52 (38-67)	51 (36-66)	60 (50-70)	69 (56-79)	83 (69-91)	74 (60-84)	50 (24-76)	80 (69-88	64 (55-72)	64 (54-73)	71 (55-83)	100	76 (66-84)	78 (64-87)	76 (61-87)	69 (56-80)	77 (65-86)	42 (33-51)	51 (42-60)	58 (48-68)	56 (44-67)	69 (51-82)	74 (50-89)	46 (32-60)	36 (28-45)	53 (43-63)	67 (48-81)	65 (46-81)
	3	49	28 (16-44)	48 (35-60)	40 (29-51)	56 (39-71)	33 (23-45)	24 (18-32)	43 (29-58)	41 (27-57)	58 (48-68)	66 (53-76)	85 (72-93)	68 (54-79)	33 (13-62)	78 (67-87	58 (49-66)	54 (44-63)	61 (45-75)	67 (57-76	100	84 (71-92)	81 (66-90)	67 (54-79)	77 (65-86)	41 (32-50)	53 (44-62)	60 (50-70)	59 (47-70)	66 (48-80)	58 (36-77)	39 (26-54)	36 (28-45)	51 (41-61)	60 (42-76)	65 (46-81)
		75	14 (6-29)	33 (22-45)	23 (15-34)	36 (22-53)	22 (14-34)	14 (9-21))	24 (13-39)	38 (25-54)	35 (26-45)	43 (31-55)	64 (49-76)	56 (42-69)	42 (18-69)	52 (40-64	36 (28-45)	36 (27-46)	46 (32-61)	41 (32-51	50 (39-61)	100	79 (64-88)	50 (37-63)	55 (42-67)	27 (19-36)	31 (23-40)	36 (27-47)	44 (33-56)	44 (28-61)	58 (36-77)	22 (12-36)	22 (15-30)	32 (23-41)	50 (33-67)	54 (35-72)
		76	8 (3-23)	33 (22-45)	21 (13-31)	33 (20-50)	16 (9-27)	12 (7-19)	26 (15-41)	38 (25-54)	35 (26-45)	39 (28-52)	60 (45-73)	50 (36-64)	42 (18-69)	45 (33-57	28 (21-37)	29 (21-39)	44 (30-59)	34 (25-44	40 (31-51)	66 (52-78)	100	38 (26-52)	47 (35-59)	21 (15-30)	28 (20-36)	31 (22-41)	39 (28-52)	31 (18-49)	32 (15-55)	24 (14-38)	18 (12-25)	27 (19-36)	47 (30-64)	50 (32-68)
	4	92	25 (14-41)	31 (21-44)	23 (15-34)	39 (25-55)	19 (12-31)	16 (10-23)	36 (23-51)	38 (25-54)	36 (27-47)	41 (29-54)	53 (39-67)	50 (36-64)	42 (18-69)	52 (40-64	34 (26-43)	36 (27-46)	54 (39-68)	38 (29-48	42 (32-52)	52 (38-65)	48 (33-62)	100	45 (33-58)	28 (20-37)	32 (24-41)	40 (30-50)	38 (27-50)	44 (28-61)	47 (27-69)	28 (17-43)	19 (13-27)	32 (23-41)	43 (27-61)	50 (32-68)
	5	96	14 (6-29)	31 (21-44)	27 (18-39)	42 (27-58)	25 (16-37)	17 (12-25)	33 (21-49)	49 (34-64)	46 (36-56)	52 (40-65)	81 (67-90)	56 (42-69)	42 (18-69)	65 (52-75	43 (34-52)	43 (34-53)	51 (36-66)	51 (41-60	57 (46-67)	68 (54-79)	69 (54-81)	54 (40-67)	100	29 (21-38)	36 (28-45)	44 (34-54)	44 (33-56)	53 (36-69)	58 (36-77)	33 (21-47)	27 (20-35)	37 (28-47)	50 (33-67)	46 (28-65)
GAMMA	1	4	42 (27-58)	41 (29-54)	40 (29-51)	50 (34-66)	40 (29-52)	27 (20-36)	33 (21-49)	41 (27-57)	44 (34-54)	57 (45-69)	60 (45-73)	52 (38-65)	42 (18-69)	58 (46-70	43 (34-52)	40 (31-50)	44 (30-59)	47 (38-57	52 (42-63)	58 (44-71)	55 (40-69)	58 (44-70)	50 (38-62)	100	52 (43-61)	60 (50-70)	59 (47-70)	63 (45-77)	63 (40-81)	39 (26-54)	35 (27-43)	38 (29-48)	47 (30-64)	77 (67-89)
		65	47 (32-63)	54 (42-66)	52 (41-63)	64 (47-78)	45 (33-57)	31 (24-40)	50 (35-65)	54 (38-69)	59 (49-69)	67 (55-78)	74 (60-85)	72 (58-83)	50 (24-76)	75 (64-84	56 (47-65)	54 (44-63)	56 (41-70)	64 (54-73	75 (65-83)	74 (60-84)	79 (64-88)	73 (60-83)	69 (57-80)	57 (48-66)	100	78 (68-85)	67 (55-77)	56 (39-72)	74 (50-89)	54 (40-68)	46 (37-55)	56 (46-66)	53 (36-70)	81 (61-92)
		95	42 (27-58)	51 (38-63)	44 (33-55)	61 (45-75)	45 (33-57)	27 (20-35)	48 (33-62)	54 (38-69)	49 (39-60)	62 (50-73)	66 (51-78)	66 (52-78)	50 (24-76)	69 (57-79	46 (37-55)	54 (44-63)	49 (34-64)	56 (46-65	65 (55-75)	66 (52-78)	67 (51-79)	69 (56-80)	65 (52-75)	51 (42-60)	60 (51-68)	100	62 (50-73)	69 (51-82)	63 (40-81)	46 (32-60)	34 (26-43)	48 (38-58)	43 (27-61)	73 (53-87)
	2	48	22 (12-39)	41 (29-54)	41 (30-53)	42 (27-58)	28 (19-40)	17 (12-25)	38 (25-53)	36 (23-52)	36 (27-47)	46 (34-58)	57 (43-71)	46 (33-60)	67 (38-87)	46 (34-58	34 (26-43)	32 (24-42)	41 (28-57)	39 (30-49	46 (36-57)	58 (44-71)	62 (47-75)	48 (35-61)	47 (35-59)	36 (28-46)	37 (29-46)	45 (35-55)	100	53 (36-69)	53 (31-73)	37 (24-52)	22 (15-30)	39 (30-49)	63 (45-78)	58 (39-75)
	3	50	22 (12-39)	15 (8-26)	14 (8-24)	28 (16-44)	18 (10-29)	8 (4-14)	21 (12-36)	23 (12-39)	21 (14-30)	25 (15-37)	34 (22-49)	24 (14-38)	33 (13-62)	29 (19-41	17 (11-25)	21 (14-30)	27 (16-42)	23 (16-33	25 (17-35)	28 (17-42)	24 (13-39)	27 (17-40)	27 (18-40)	19 (12-27)	15 (10-23)	24 (16-34)	26 (17-38)	100	37 (19-60)	17 (9-31)	11 (7-18)	16 (10-25)	27 (14-45)	31 (16-51)
	4	60	14 (6-29)	10 (4-20)	8 (4-17)	8 (3-23) 2	12 (6-22)	6 (3-12)	10 (4-23)	13 (5-27)	10 (5-18)	11 (6-22)	17 (9-31)	20 (11-33)	25 (8-55)	20 (12-31	12 (7-19)	12 (7-20)	10 (4-23)	15 (9-23)	13 (7-22)	22 (13-36)	14 (7-28)	17 (9-30)	18 (10-29)	11 (6-19)	12 (7-19)	13 (8-22)	15 (8-26)	22 (11-39)	100	7 (2-18)	8 (4-14)	12 (7-20)0	23 (12-41)	19 (8-39)0
NU	1	41	11 (4-26)	23 (14-35)	21 (13-31)	25 (14-41)	16 (9-27)	17 (12-25)	26 (15-41)	23 (12-39)	25 (17-35)	28 (18-40)	28 (17-42)	28 (17-42)	42 (18-69)	25 (16-36	19 (13-27)	22 (15-31)	17 (8-32)	22 (15-32	21 (14-31)	20 (11-33)	26 (15-41)	25 (15-38)	24 (15-36)	17 (11-25)	21 (15-29)	23 (16-33)	26 (17-38)	25 (13-43)	16 (5-39)	100	18 (12-25)	23 (16-33)	43 (27-61)	23 (11-43)
MU	1	1	50 (34-66)	31 (21-44)	37 (27-49)	50 (34-66)	40 (29-52)	8 28 (21-37	43 (29-58)	31 (18-47)	43 (33-53)	33 (22-45)	53 (39-67)	54 (40-67)	25 (8-55)	54 (42-66	44 (35-53)	35 (26-45)	39 (25-55)	47 (38-57	54 (43-64)	54 (40-67)	52 (38-67)	46 (33-60)	53 (41-65)	40 (31-49)	48 (39-57)	46 (36-56)	41 (30-53)	44 (28-61)	53 (31-73)	48 (34-62)	100	62 (52-71)	47 (30-64)	62 (42-78)
	2	63	36 (22-53)	51 (38-63)	44 (33-55)	47 (32-63)	33 (23-45)	6 26 (19-34	48 (33-62)	46 (31-62)	46 (36-56)	49 (37-62)	57 (43-71)	64 (50-76)	33 (13-62)	63 (51-74	47 (38-56)	42 (33-52)	51 (36-66)	55 (45-64	60 (49-69)	62 (48-74)	62 (47-75)	60 (46-72)	58 (46-70)	34 (26-44)	46 (37-55)	52 (41-62)	58 (45-69)	50 (33-67)	63 (40-81)	50 (36-64)	49 (41-58)	100	57 (39-73)	62 (42-78)
ND		101	8 (3-23)	18 (10-30)	22 (14-33)	22 (12-39)	12 (6-22)	10 (6-17)	19 (10-34)	26 (14-41)	24 (16-34)	21 (13-33)	30 (19-44)	24 (14-38)	83 (52-96)	23 (14-35	16 (10-23)	18 (12-27)	27 (16-42)	21 (14-30	21 (14-31)	30 (19-44)	33 (21-49)	25 (15-38)	24 (15-36)	13 (8-21)	13 (8-21)	14 (8-23)	29 (19-41)	25 (13-43)	37 (19-60)	28 (17-43)	11 (7-18)	17 (11-26)	100	35 (19-54)
		103	17 (8-32)	21 (13-33)	11 (6-20)	25 (14-41)	9 (4-19)	8 (4-14)	14 (7-28)	23 (12-39)	13 (8-22)	16 (9-28)	19 (10-33)	24 (14-38)	25 (8-55)	22 (13-33	14 (9-21)	15 (9-23)	24 (14-40)	18 (11-27	20 (13-30)	28 (17-42)	31 (19-46)	25 (15-38)	19 (11-31	19 (12-27)	18 (12-26)	21 (14-30)	23 (14-34)	25 (13-43)	26 (11-50)	13 (6-26)	13 (8-20)	16 (10-25)	30 (16-48)	100

CI: confidence interval; no POS: number of positive specimen; HPV: human papillomavirus; ND: Not defined. Dotted areas for seroprevalence to HPV8, HPV9, HPV15, HPV17, HPV38, HPV49 and HPV4, HPV65 and HPV95

≥ 25% ≥ 50% ≥ 75%

Table 6.3: Proportions (95%CI) of patients seropositive to one HPV type who are seropostive to another HPV type, among Caucasian transplant patients without skin cancer (N=425)

Seropositivity to	All N=425	Oxford N=182	London N=243
None beta type	188 (44%)	85 (47%)	103 (42%)
HPV 8, 9, 15, 17, 38 and/or 49	205 (48%)	87 (35%)	118 (28%)
Any of 10 other beta types	32 (8%)	10 (5%)	22 (9%)
None gamma type	225 (53%)	114 (63%)	135 (55%)
HPV 4, 65 and/or 95 (gamma, species 1)	178 (41%)	68 (37%)	110 (45%)
Any of 3 other gamma types (HPV 48, 50 and/or 60)	22 (5%)	9 (5%)	13 (5%)

Table 6.4: HPV seroprevalence by most frequent beta and gamma HPV types.

Table 6.5: Human papillomavirus seroprevalence by centre, sex, age at recruitment, time since transplantation and skin type by each type, among Caucasian transplant patients without skin cancer from London and Oxford (N=425)

genus	species	type	All	CENTRE			SEX			AGE AT RECRUITMENT (years)				TIME SINCE TRANSPLANTATION (years)				SKIN TYPE		
				London	Oxford		Male	Female		<45	45-59	≥ 60		<5	5 to 9	≥10		I/II	III/IV	
			N=425 % POS	N=243 % POS	N=182 % POS	*P-value* [1]	N=260 % POS	N=165 % POS	*P-value* [2]	N=187 % POS	N=158 % POS	N=80 % POS	*P-value trend* [2]	N=150 % POS	N=113 % POS	N=162 % POS	*P-value trend* [2]	N=133 % POS	N=273 % POS	*P-value* [2]
alpha	2	3	8%	9%	8%	0.9	8%	9%	0.7	9%	8%	8%	0.6	10%	7%	8%	0.5	6%	10%	0.2
	4	2	14%	17%	11%	0.09	13%	16%	0.5	18%	12%	11%	0.1	11%	18%	15%	1.5	14%	14%	1.0
		27	17%	21%	12%	0.01	15%	21%	0.1	24%	12%	13%	0.01	14%	21%	17%	2.5	20%	16%	0.6
	8	7	8%	9%	7%	0.4	7%	12%	0.1	11%	6%	6%	0.09	6%	11%	9%	3.5	8%	8%	0.7
	9	16	16%	16%	15%	0.6	10%	25%	<0.001	22%	11%	10%	<0.001	16%	15%	16%	4.5	14%	16%	0.3
	10	6	30%	33%	26%	0.1	29%	32%	0.5	36%	28%	20%	0.01	31%	24%	33%	5.5	29%	31%	0.4
		13	10%	13%	5%	0.01	8%	13%	0.04	16%	4%	8%	<0.001	9%	10%	11%	6.5	13%	9%	0.6
	1	5	9%	11%	7%	0.2	10%	7%	0.3	10%	9%	8%	0.5	5%	11%	12%	0.06	9%	9%	0.9
		8	21%	24%	18%	0.2	21%	22%	0.9	26%	18%	18%	0.06	16%	24%	25%	0.1	21%	21%	0.9
		20	14%	16%	12%	0.3	15%	13%	0.4	15%	12%	18%	0.8	11%	14%	18%	0.1	12%	15%	0.4
		24	11%	11%	12%	0.8	11%	12%	0.9	13%	8%	13%	0.7	8%	16%	10%	0.5	9%	11%	0.5
		36	12%	15%	8%	0.03	11%	13%	0.7	13%	10%	13%	0.8	8%	12%	15%	0.1	10%	11%	0.5
		93	3%	5%	1%	0.0	2%	4%	0.5	2%	3%	4%	0.5	2%	3%	4%	0.5	3%	3%	0.8
beta		9	15%	19%	11%	0.03	15%	15%	1.0	17%	15%	14%	0.6	13%	19%	15%	0.7	14%	15%	0.5
		15	27%	26%	29%	0.5	27%	28%	0.9	27%	31%	20%	0.5	18%	40%	27%	0.07	23%	29%	0.4
	2	17	24%	25%	21%	0.3	21%	27%	0.2	24%	23%	24%	0.9	19%	34%	21%	0.8	21%	25%	0.3
		23	10%	12%	7%	0.07	9%	10%	0.8	9%	9%	11%	0.6	5%	13%	11%	0.1	8%	11%	0.3
		38	22%	23%	21%	0.8	22%	23%	0.9	22%	23%	21%	0.9	14%	30%	25%	0.03	19%	24%	0.3
		49	20%	19%	21%	0.5	18%	22%	0.4	22%	18%	18%	0.2	14%	21%	24%	0.02	20%	19%	1.0
	3	75	12%	14%	9%	0.2	12%	12%	0.9	12%	12%	11%	1.0	7%	14%	15%	0.03	9%	13%	0.2
		76	10%	12%	7%	0.1	9%	11%	0.6	9%	11%	9%	0.9	5%	12%	13%	0.03	8%	11%	0.3
	4	92	12%	15%	8%	0.04	12%	13%	0.8	12%	13%	11%	1.0	8%	13%	15%	0.07	14%	12%	0.8
	5	96	15%	15%	14%	0.9	13%	16%	0.5	13%	16%	15%	0.6	9%	20%	16%	0.1	12%	16%	0.4
gamma		4	25%	27%	23%	0.5	27%	23%	0.4	28%	27%	16%	0.09	17%	30%	30%	0.01	23%	26%	0.6
	1	65	28%	30%	25%	0.2	27%	30%	0.4	35%	25%	19%	<0.001	22%	34%	30%	0.2	25%	28%	0.3
		95	21%	22%	20%	0.6	19%	25%	0.2	24%	16%	25%	0.7	17%	25%	23%	0.3	20%	22%	0.6
	2	48	16%	16%	15%	0.7	15%	16%	0.7	19%	13%	14%	0.2	13%	21%	14%	1.0	15%	15%	0.9
	3	50	8%	8%	7%	0.8	6%	10%	0.07	8%	9%	4%	0.3	7%	7%	8%	0.8	5%	9%	0.1
	4	60	4%	5%	4%	0.6	3%	7%	0.08	4%	6%	4%	0.8	4%	5%	4%	1.0	4%	4%	0.7
nu	1	41	11%	13%	8%	0.06	11%	11%	1.0	11%	11%	11%	0.9	11%	11%	10%	0.7	12%	10%	0.8
mu	1	1	29%	33%	24%	0.04	26%	34%	0.1	31%	30%	23%	0.2	25%	27%	35%	0.1	31%	29%	0.9
	2	63	23%	28%	17%	0.01	21%	27%	0.2	27%	20%	20%	0.1	16%	28%	26%	0.07	24%	23%	0.8
ND		101	7%	10%	3%	<0.001	6%	9%	0.2	6%	6%	10%	0.4	6%	7%	8%	0.8	7%	8%	0.4
		103	6%	7%	4%	0.2	6%	6%	1.0	6%	8%	4%	0.8	4%	8%	7%	0.3	7%	6%	0.8

HPV: Human papillomavirus; POS: number seropositive patients; N: number; ND: Not defined. Skin type was defined as: (I) 'rarely tans, usually burns' (II) 'usually tans, can burn' (III) 'always tans, rarely burns' (IV) 'always tans, never burns'. Het.: heterogeneity test.

[1] Analyses were based on unconditional logistic regression adjusted for sex, time since transplantation and age at recruitment. [2] Analyses were based on conditional on centre logistic regression adjusted for sex, time since transplantation and age at recruitment. P-values are based on tests for heterogeneity calculated by likelihood ratio tests and tests for trend were obtained by treating categorical variable as a continuous variable in the model.

Some figures do not add up to 425 due to missing data. P-values were highlighted if less or equal than 0.01.

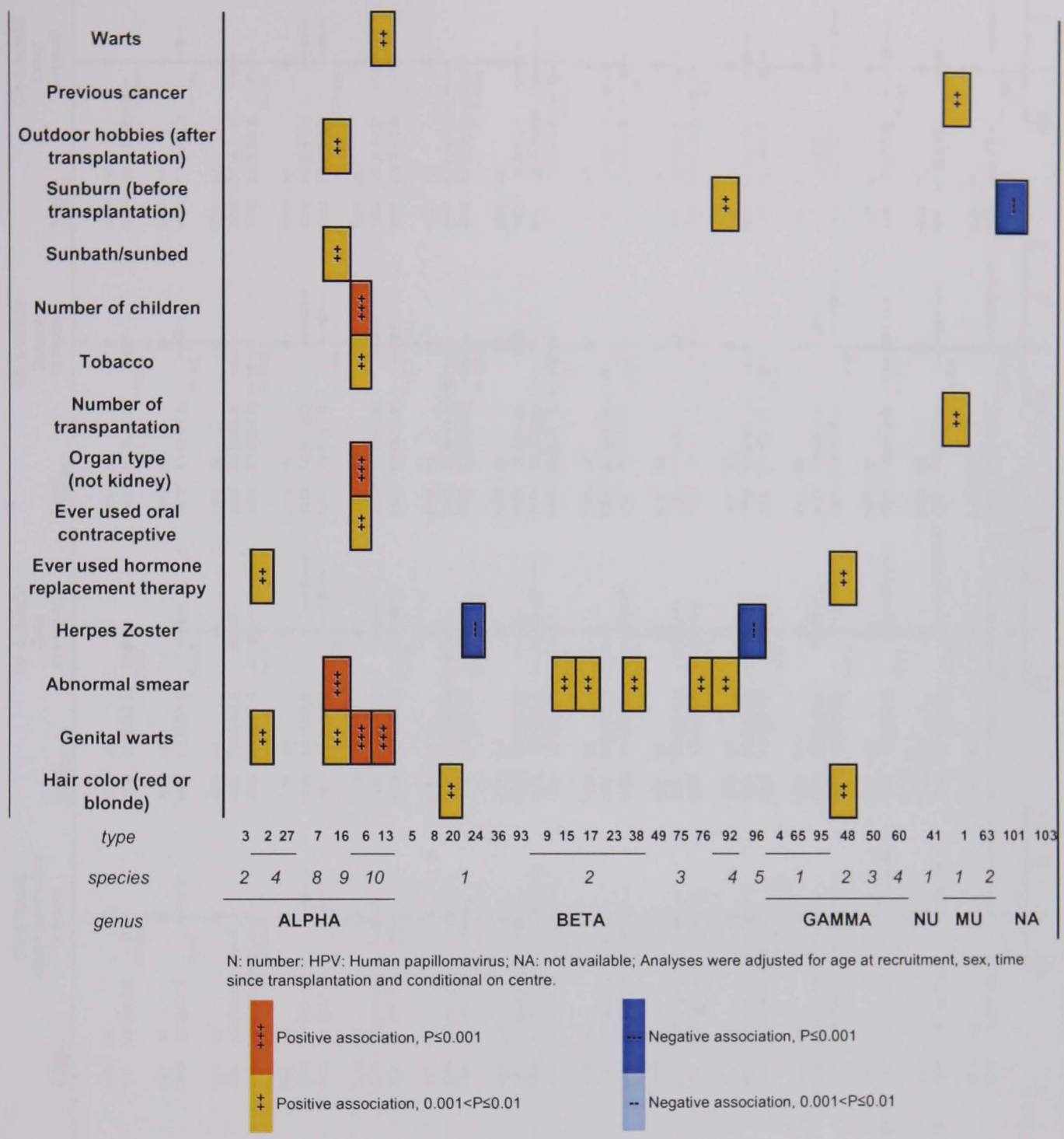
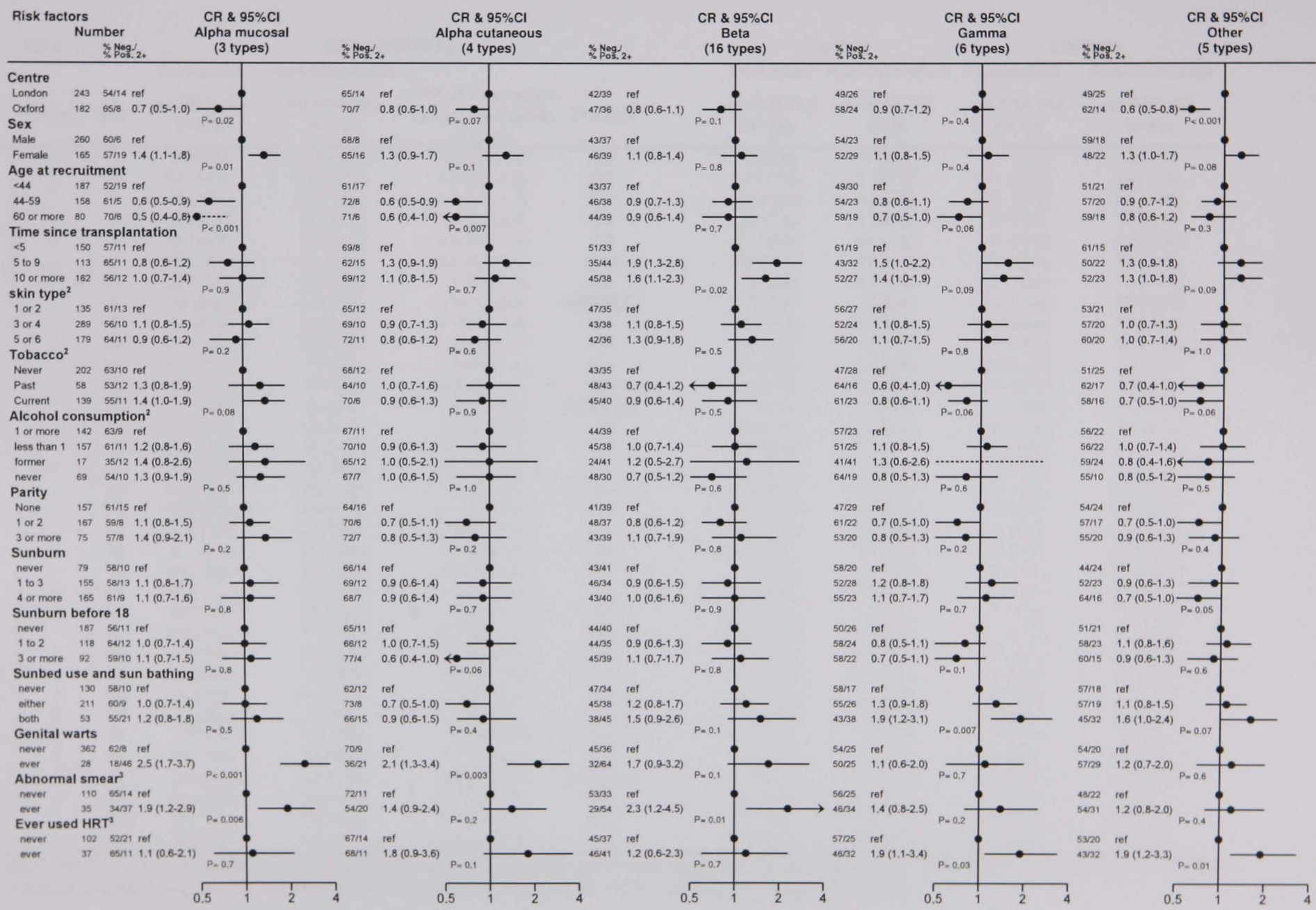


Table 6.6: Summary of risk factors from the questionnaire associated with single HPV seroposi-tivity among Caucasian control transplant patients (N=425)



[1] Using negative binomial regression adjusted for age at recruitment, sex, time since transplantation and centre (where appropriate).
All P-values for exposure with 3 categories are trend-test unless indicated with *
CR: Count ratio; CI: confidence interval; % Neg./ % Pos. 2+: Percentage being seronegative and percentage being seropositive to 2 or more HPV types of the genus
HRT: Hormones replacement therapy; Ref: reference category. [3] Restricted to women only

Figure 6.2: Risk factors associated with multiple HPV seropositivity among Caucasian transplant recipients without skin cancer from Oxford and London

Table 6.7: HPV seroprevalence by ethnicity (Caucasian versus non-Caucasian transplant patients without skin cancer) across centre

HPV			BOTH CENTRES				OXFORD		LONDON	
			Caucasian	Non-Caucasian			Caucasian	Non-Caucasian	Caucasian	Non-Caucasian
genus	species	type	no POS (%) N=425	no POS (%) N=201	OR* of Caucasian vs non-Caucasian (95% CI)	P*-value	no POS (%) N=182	no POS (%) N=35	no POS (%) N=243	no POS (%) N=166
alpha	2	3	36 (8%)	13 (6%)	0.7 (0.4-1.4)	*0.3*	15 (8%)	1 (3%)	21 (9%)	12 (7%)
	4	2	61 (14%)	25 (12%)	0.8 (0.5-1.3)	*0.4*	20 (11%)	6 (17%)	41 (17%)	19 (11%)
		27	73 (17%)	29 (14%)	0.7 (0.5-1.2)	*0.2*	22 (12%)	6 (17%)	51 (21%)	23 (14%)
	8	7	36 (8%)	22 (11%)	1.3 (0.7-2.4)	*0.3*	13 (7%)	7 (20%)	23 (9%)	15 (9%)
	9	16	67 (16%)	30 (15%)	0.9 (0.6-1.5)	*0.7*	27 (15%)	6 (17%)	40 (16%)	24 (14%)
	10	6	128 (30%)	50 (25%)	0.7 (0.5-1.1)	*0.08*	47 (26%)	7 (20%)	81 (33%)	43 (26%)
		13	42 (10%)	24 (12%)	1.1 (0.6-1.9)	*0.8*	10 (5%)	6 (17%)	32 (13%)	18 (11%)
	1	5	39 (9%)	34 (17%)	2.0 (1.2-3.4)	*0.01*	12 (7%)	7 (20%)	27 (11%)	27 (16%)
		8	91 (21%)	45 (22%)	1.0 (0.7-1.6)	*0.9*	33 (18%)	7 (20%)	58 (24%)	38 (23%)
		20	61 (14%)	24 (12%)	0.8 (0.5-1.4)	*0.4*	22 (12%)	6 (17%)	39 (16%)	18 (11%)
		24	47 (11%)	16 (8%)	0.8 (0.4-1.4)	*0.4*	21 (12%)	5 (14%)	26 (11%)	11 (7%)
		36	50 (12%)	15 (7%)	0.6 (0.3-1.1)	*0.07*	14 (8%)	5 (14%)	36 (15%)	10 (6%)
		93	12 (3%)	19 (9%)	2.6 (1.2-5.6)	*0.01*	1 (1%)	2 (6%)	11 (5%)	17 (10%)
beta	2	9	65 (15%)	32 (16%)	1.0 (0.6-1.5)	*0.9*	20 (11%)	7 (20%)	45 (19%)	25 (15%)
		15	116 (27%)	47 (23%)	0.9 (0.6-1.4)	*0.7*	52 (29%)	13 (37%)	64 (26%)	34 (20%)
		17	100 (24%)	46 (23%)	0.9 (0.6-1.4)	*0.7*	39 (21%)	8 (23%)	61 (25%)	38 (23%)
		23	41 (10%)	25 (12%)	1.2 (0.7-2.1)	*0.5*	12 (7%)	4 (11%)	29 (12%)	21 (13%)
		38	95 (22%)	46 (23%)	1.1 (0.7-1.6)	*0.8*	39 (21%)	10 (29%)	56 (23%)	36 (22%)
		49	84 (20%)	33 (16%)	0.9 (0.6-1.4)	*0.7*	38 (21%)	10 (29%)	46 (19%)	23 (14%)
	3	75	50 (12%)	26 (13%)	1.1 (0.6-1.8)	*0.8*	17 (9%)	6 (17%)	33 (14%)	20 (12%)
		76	42 (10%)	27 (13%)	1.4 (0.8-2.3)	*0.3*	13 (7%)	6 (17%)	29 (12%)	21 (13%)
	4	92	52 (12%)	30 (15%)	1.2 (0.7-2.0)	*0.5*	15 (8%)	8 (23%)	37 (15%)	22 (13%)
	5	96	62 (15%)	29 (14%)	1.0 (0.6-1.7)	*0.9*	26 (14%)	8 (23%)	36 (15%)	21 (13%)
gamma		4	108 (25%)	37 (18%)	0.7 (0.4-1.1)	*0.08*	42 (23%)	10 (29%)	66 (27%)	27 (16%)
	1	65	119 (28%)	51 (25%)	0.8 (0.6-1.3)	*0.4*	45 (25%)	10 (29%)	74 (30%)	41 (25%)
		95	91 (21%)	36 (18%)	0.8 (0.5-1.3)	*0.3*	37 (20%)	6 (17%)	54 (22%)	30 (18%)
	2	48	66 (16%)	34 (17%)	1.1 (0.7-1.7)	*0.8*	27 (15%)	5 (14%)	39 (16%)	29 (17%)
	3	50	32 (8%)	18 (9%)	1.1 (0.6-2.2)	*0.7*	13 (7%)	4 (11%)	19 (8%)	14 (8%)
	4	60	19 (4%)	16 (8%)	1.7 (0.8-3.4)	*0.2*	7 (4%)	3 (9%)	12 (5%)	13 (8%)
other mu nu	1	41	46 (11%)	33 (16%)	1.4 (0.8-2.3)	*0.2*	14 (8%)	6 (17%)	32 (13%)	27 (16%)
	1	1	124 (29%)	34 (17%)	0.5 (0.3-0.7)	*<0.001*	43 (24%)	9 (26%)	81 (33%)	25 (15%)
	2	63	98 (23%)	38 (19%)	0.7 (0.5-1.1)	*0.1*	31 (17%)	6 (17%)	67 (28%)	32 (19%)
		101	30 (7%)	30 (15%)	1.9 (1.1-3.3)	*0.02*	5 (3%)	5 (14%)	25 (10%)	25 (15%)
		103	26 (6%)	12 (6%)	0.9 (0.4-1.9)	*0.8*	8 (4%)	4 (11%)	18 (7%)	8 (5%)

* P-values were calculated using conditional (on centre) logistic regression were adjusted for sex, time since transplantation and age at recruitment.
HPV: Human papillomavirus; OR: Odds ratio; CI: confidence interval; no POS: number seropositive patients

Table 6.8: Seroprevalence for single HPV types among transplant, dialysis and immunocompetent individuals of London and Oxford, and by published study using the same Luminex methodology

			Michael *et al.* (2008)	Waterboer *et al.* (2008)	Casabonne *et al.* (2007) & present study			Casabonne *et al.* (2007)			Present study										
country			Germany	Italy	UK			UK													
city			All	Rome	Oxford & London			Oxford			London										
ethnicity			NA	NA	Caucasian			Caucasian			Caucasian			Non-Caucasian			Non-Caucasian versus Caucasian				
number			1610	77 (73)	182	425		80	182		222	102	243	145	50	166					
Subject			IC (all adults)	IC (without skin cancer)	IC (without skin cancer)	Tx (without skin cancer)		IC (without skin cancer)	Tx (without skin cancer)		dialysis	IC (without skin cancer)	Tx (without skin cancer)	dialysis	IC (without skin cancer)	Tx (without skin cancer)			dialysis	IC	
Age at recruitmen			NA	75% older than 64	57 [15] 23 to 91	47 [13] 19 to 83		62 [7.2] 42 to 76	48 [13] 19 to 78		54 [14] 19 to 85	53 [18] 23 to 91	47 [13] 21 to 83	48 [13] 18 to 80	41 [16] 17 to 73	47 [12] 20 to 78					
Ratio sex			NA	1.5 (51/33)	1.0 (92/90)	1.6 (261/165)		1.1 (42/38)	1.5 (110/72)		1.5 (132/90)	1.0 (50/52)	1.6 (150/93)	1.3 (83/62)	0.6 (18/32)	1.4 (96/70)					
genus	species	types	% positive	% positive	% positive	% positive	P-het (a)	% positive	% positive	P-het (b)	% positive	% positive	% positive	P-het (c)	% positive	% positive	% positive	P-het (d)	P-het (e)	P-het (f)	
alpha	2	3	6	12	10	8	0.4	9	8	0.5	10	11	9	0.7	16	22	7	0.008	0.2	0.2	
	4	2	5	16	10	14	0.3	10	11	0.9	11	10	17	0.07	13	12	11	0.9	0.4	1.0	
		27	-	-	11	17	0.08	13	12	1.0	14	10	21	0.02	20	16	14	0.4	0.08	0.4	
	8	7	-	-	7	8	0.6	6	7	0.8	6	7	9	0.5	14	16	9	0.3	0.04	0.2	
	9	16	8	18	19	16	0.1	10	15	0.6	18	26	16	0.1	19	16	14	0.4	0.9	0.06	
	10	6	-	-	-	30	-	-	26	-	36	41	33	0.4	39	40	26	0.02	0.7	0.8	
		13	-	-	8	10	0.7	9	5	0.4	11	8	13	0.2	19	14	11	0.1	0.01	0.6	
beta		5	6	29	16	9	0.03	20	7	0.004	15	13	11	0.6	19	24	16	0.6	0.2	0.1	
		8	12	43	15	21	0.09	20	18	0.5	15	11	24	0.008	18	26	23	0.3	0.3	0.02	
	1	20	6	30	18	14	0.4	21	12	0.2	20	15	16	0.3	22	14	11	0.03	0.6	0.8	
		24	5	27	16	11	0.08	20	12	0.4	17	14	11	0.1	14	12	7	0.1	0.5	0.8	
		36	4	25	12	12	0.9	16	8	0.3	17	8	15	0.09	10	16	6	0.2	0.2	0.5	
		93	1	21	2	3	0.2	4	1	0.2	4	0	5	0.4	11	10	10	1.0	0.006	0.003 (g)	
	2	9	8	27	18	15	0.4	20	11	0.3	22	16	19	0.4	26	16	15	0.05	0.2	1.0	
		15	8	25	19	27	0.2	19	29	0.3	22	20	26	0.5	23	26	20	0.7	0.8	0.3	
		17	8	27	18	24	0.2	15	21	0.1	22	21	25	0.7	27	28	23	0.7	0.3	0.3	
		23	4	23	7	10	0.2	11	7	1.0	10	4	12	0.03	15	20	13	0.3	0.07	0.004	
		38	8	27	19	22	0.6	18	21	0.5	19	21	23	0.7	20	34	22	0.1	0.6	0.1	
		49	10	38	21	20	0.6	20	21	0.6	20	22	19	0.7	22	14	14	0.1	0.8	0.2	
	3	75	6	31	12	12	0.6	13	9	0.8	14	12	14	0.9	16	14	12	0.6	0.7	0.8	
		76	4	39	18	10	0.01	18	7	0.1	15	19	12	0.4	17	26	13	0.1	0.7	0.1	
	4	92	6	42	13	12	0.5	11	8	0.6	18	15	15	0.7	21	20	13	0.1	0.3	0.6	
	5	96	-	-	20	15	0.2	18	14	0.9	19	23	15	0.3	14	18	13	0.8	0.2	0.7	
gamma		4	18	34	40	25	<0.001	31	23	0.1	36	46	27	0.001	31	32	16	0.004	0.4	0.1	
	1	65	11	35	21	28	0.2	23	25	0.7	20	20	30	0.03	17	16	25	0.1	0.5	0.5	
		95	8	38	22	21	0.6	20	20	0.9	23	24	22	0.9	28	22	18	0.1	0.2	0.5	
	2	48	8	27	23	16	0.03	19	15	0.3	23	26	16	0.05	31	28	17	0.01	0.09	0.9	
	3	50	4	17	12	8	0.06	9	7	0.4	13	15	8	0.1	17	14	8	0.05	0.3	0.6	
	4	60	1	9	4	4	0.9	4	4	0.7	4	4	5	0.9	10	4	8	0.4	0.06	0.9	
nu		41	8	27	12	11	0.9	14	8	0.4	7	10	13	0.2	10	20	16	0.1	0.5	0.1	
mu	1	1	18	14	34	29	0.2	23	24	0.9	34	43	33	0.2	23	16	15	0.09	0.007	0.04	
	2	63	12	30	23	23	0.9	15	17	0.8	20	28	28	0.3	23	20	19	0.7	0.5	0.1	
ND		101	-	-	5	7	0.3	3	3	0.6	8	7	10	0.6	14	16	15	0.9	0.1	0.3	
		103	-	-	4	6	0.5	6	4	0.5	5	3	7	0.3	4	8	5	0.7	0.7	0.2	
Seropositivity to any HPV types			NA	NA	90	86	0.2	84	81	0.8	91	94	90	0.3	92	92	87	0.3	0.8	0.9	

IC: Immunocompetent; Tx: transplant patients; NA: not available; ND: Not defined; M: Male; F:Female; SD: Standard deviation

P-value were calculated using logistic regression or conditional logistic regression. Highlighted P-values if less or equal than 0.01.

(a) Heterogeneity test for a difference between immunocompetent and transplant Caucasian patients from both centres, adjusted for age (<45, 45-59, 60 or more) and sex, and stratified by centre.

(b) Heterogeneity test for a difference between immunocompetent and transplant Caucasian patients from Oxford, adjusted for age (<55, 55-64,65 or more) and sex.

(c) Heterogeneity test for a difference between immunocompetent, dialysis and transplant Caucasian patients from London, adjusted for age (<45, 45-59, 60 or more) and sex.

(d) Heterogeneity test for a difference between immunocompetent, dialysis and transplant non-Caucasians patients from London, adjusted for age (<45, 45-59, 60 or more) and sex.

(e) Heterogeneity test for a difference between Caucasian and non-Caucasian among dialsyis patients from London adjusted, adjusted for age (<45, 45 to 59, 60 or more) and sex.

(f) Heterogeneity test for a difference between Caucasian and non-Caucasian among IC patients from London, adjusted for age (<45, 45 to 59, 60 or more) and sex.

(g) Exact Fisher's test.

Table 6.9: Multiple HPV seropositivity by ethnicity (Caucasian and non-Caucasian), type of studies (transplant, dialysis and immunocompetent individuals) and centre

			Alpha-mucosal		Alpha-cutaneous		Beta		Gamma		Rest	
		number	%neg / %pos to 2 or more	CR (95%CI)	%neg / %pos to 2 or more	CR (95%CI)	%neg / %pos to 2 or more	CR (95%CI)	%neg / %pos to 2 or more	CR (95%CI)	%neg / %pos to 2 or more	CR (95%CI)
STUDIES & IMMUNOLOGICAL STATUS												
A) Caucasian	London Tx	243	54/14	ref	65/14	ref	42/39	ref	49/26	ref	49/25	ref
	Oxford Tx	182	65/8	0.7 (0.5 to 1.0)	70/7	0.8 (0.6 to 1.0)	47/36	0.8 (0.6 to 1.1)	58/24	0.9 (0.7 to 1.2)	62/14	0.6 (0.5 to 0.8)
	P-het.			*0.02*		*0.07*		*0.1*		*0.4*		*<0.001*
B) Both centres-Caucasian	Tx	425	59/11	ref	67/11	ref	44/38	ref	53/25	ref	55/20	ref
	IC	182	63/10	1.0 (0.8 to 1.2)	74/7	0.8 (0.6 to 1.1)	42/40	1.0 (0.8 to 1.4)	43/30	1.2 (0.9 to 1.6)	55/21	1.0 (0.8 to 1.3)
	P-het.			*0.8*		*0.2*		*0.8*		*0.1*		*0.8*
C) Oxford-Caucasian	Tx	182	65/8	ref	70/7	ref	47/36	ref	58/24	ref	62/14	ref
	EPIC	80	84/3	1.6 (0.8 to 2.9)	73/8	0.8 (0.4 to 1.3)	43/38	0.9 (0.5 to 1.4)	49/25	0.7 (0.5 to 1.2)	63/13	0.9 (0.6 to 1.5)
	P-het.			*0.2*		*0.4*		*0.6*		*0.2*		*0.7*
D) London-Caucasian	Tx	243	54/14	ref	65/14	ref	42/39	ref	49/26	ref	49/25	ref
	Dialysis	222	53/14	1.1 (0.9 to 1.4)	74/10	0.8 (0.6 to 1.1)	41/36	1.1 (0.8 to 1.5)	46/28	1.1 (0.9 to 1.5)	50/16	0.9 (0.7 to 1.1)
	IC	102	46/16	1.2 (0.9 to 1.5)	75/7	0.6 (0.4 to 1.0)	42/41	0.9 (0.6 to 1.3)	38/33	1.2 (0.9 to 1.7)	50/28	1.0 (0.7 to 1.3)
	P-het.			*0.6*		*0.09*		*0.6*		*0.4*		*0.6*
E) London- non-Caucasian	Tx	166	61/10	ref	73/8	ref	41/36	ref	55/19	ref	63/19	ref
	Dialysis	145	43/15	1.5 (1.2 to 2.0)	61/14	1.5 (1.0 to 2.2)	30/46	1.3 (0.9 to 1.8)	46/28	1.4 (1.0 to 2.0)	50/16	1.0 (0.7 to 1.5)
	IC	50	48/14	1.3 (0.8 to 1.9)	54/12	1.5 (0.9 to 2.6)	28/44	1.4 (0.9 to 2.2)	48/24	1.2 (0.8 to 1.9)	56/24	1.2 (0.7 to 2.0)
	P-het.			*0.01*		*0.07*		*0.2*		*0.08*		*0.7*
ETHNICITY												
F) Both centres - transplant	Caucasian	425	59/11	ref	67/11	ref	44/38	ref	53/25	ref	55/20	ref
	Non-Caucasian	201	62/10	0.9 (0.7 to 1.1)	72/10	0.8 (0.6 to 1.1)	41/35	1.1 (0.8 to 1.4)	55/20	0.9 (0.7 to 1.2)	62/18	0.9 (0.7 to 1.2)
	P-het.			*0.3*		*0.2*		*0.7*		*0.6*		*0.5*
G) OXFORD - transplant	Caucasian	182	65/8	ref	70/7	ref	47/36	ref	58/24	ref	62/14	ref
	Non-Caucasian	35	66/14	1.1 (0.7 to 1.9)	69/17	1.3 (0.7 to 2.2)	43/31	1.6 (0.9 to 3)	57/26	1.2 (0.7 to 2.1)	57/17	1.6 (0.9 to 2.6)
	P-het.			*0.7*		*0.4*		*0.1*		*0.6*		*0.09*
H) LONDON - transplant	Caucasian	243	54/14	ref	65/14	ref	42/39	ref	49/26	ref	49/25	ref
	Non-Caucasian	166	61/10	0.8 (0.6 to 1.1)	73/8	0.7 (0.5 to 1.0)	41/36	0.9 (0.7 to 1.3)	55/19	0.9 (0.6 to 1.2)	63/19	0.8 (0.6 to 1.0)
	P-het.			*0.2*		*0.08*		*0.7*		*0.3*		*0.08*
I) LONDON - dialysis	Caucasian	222	53/14	ref	74/10	ref	41/36	ref	40/30	ref	57/17	ref
	Non-Caucasian	145	43/15	1.2 (0.9 to 1.5)	61/14	1.5 (1.0 to 2.2)	30/46	1.1 (0.8 to 1.6)	46/28	1.1 (0.9 to 1.5)	50/16	0.9 (0.7 to 1.3)
	P-het.			*0.2*		*0.03*		*0.4*		*0.4*		*0.7*
J) LONDON - IC	Caucasian	102	46/16	ref	75/7	ref	42/41	ref	38/33	ref	50/28	ref
	Non-Caucasian	50	48/14	0.8 (0.5 to 1.3)	54/12	1.5 (0.9 to 2.7)	28/44	1.4 (0.8 to 2.3)	48/24	0.9 (0.5 to 1.3)	56/24	0.9 (0.6 to 1.5)
	P-het.			*0.4*		*0.1*		*0.2*		*0.5*		*0.7*

[1] Using negative binomial regression adjusted for age, sex, time since transplantation (where appropriate) and centre (where appropriate). P-value for heterogeneity
CR: Count ratio; CI: confidence interval; HPV: Human papillomavirus; CI: confidence interval; Pos: number seropositive samples; Neg: number seronegative samples
Tx: Transplant patients; IC: Immunocompetent patients.

CHAPTER 7

Human papillomavirus in relation to cutaneous squamous and basal cell carcinoma

7.1 Introduction

This chapter summarises results from the pilot prospective study nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford) in Section 7.2 and from the Oxford and London case-control studies among transplant patients in Section 7.3. It includes reports on the association between SCC and BCC in relation to various risk factors from questionnaire data and also on the relationship between antibodies against HPV-L1 antigens for 38 HPV types (34 types in the London and Oxford studies) with sera collected prior to and after diagnosis of the first SCC or BCC. The chapter ends with a discussion.

7.2 A prospective pilot study nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford)

7.2.1 Statistical methods

Fisher exact tests and unpaired Student t-tests were used to compare response proportions and means. To examine the association between incident SCC and seropositivity to each HPV type, logistic regression was used to derive odds ratios (OR) and their 95% confidence intervals (CI) adjusted for age at recruitment (<55, 55-64, ≥65+), sex and region of cancer registry categorised as Oxfordshire and other since most individuals were registered in Oxfordshire. When fewer than 5 patients were seropositive to an HPV type, P-values were derived using Fisher's exact.

To examine the association between multiple HPV seropositivity and SCC, negative binomial regression adjusted for the same factors was used since over-dispersion was observed when Poisson models were fitted and ORs were also derived using logistic regression based on categorical data (seronegative to all, seropositive to 1 type, seropositive to 2 or more types).

A small number of patients already had prevalent SCC, descriptive statistics (number and percentage) relating to associations between seropositivity to a single HPV type and disease among these patients were also examined.

Missing value categories were added to adjustment variables with incomplete information in order to retain all the observations in the analysis. Likelihood ratio tests were used to assess heterogeneity tests. All P-values are derived from two-sided tests of statistical significance. Statistical analyses were carried out using STATA 9 (StataCorp, 2005).

7.2.2 Results

Table 7.1 shows the distribution by age, sex, and other descriptive variables for incident SCC and controls. There were no statistically significant differences in the distribution of any of the variables between the incident SCC and the control group. The mean follow-up time was similar (9 years) for incident SCC (SD: 1.0 years) and the control group (SD: 2.0 years) (P=0.2). Among controls, there were no statistically significant associations between any HPV type or genus, with age, sex or location of residence at recruitment (Table 7.2).

In Table 7.3, the association between each HPV type and incident SCC was examined. Seroprevalence data were also reported in patients with prevalent SCC. There were no statistically significant differences in the seroprevalence of antibodies against any of the 38 HPV types examined between incident SCC and controls. Among prevalent SCC, the prevalence of antibodies against HPV was generally higher for the majority of the 38 types examined compared to the incident SCC or to the control group. For betaHPV types specifically, the prevalence of antibodies was higher among prevalent SCC than among incident SCC for 10 of 16 types examined (63%). However, the numbers of SCC examined were small and differences were not statistically significant. For HPV-8 (which has been linked to SCC in previous studies), the seroprevalence of antibodies was 20% (16/80) in controls, 23% (9/39) among incident SCC (OR 1.1, 95% CI 0.4-3.0; P=1.0) and 40% (6/15) among prevalent SCC (P-value for controls versus prevalent SCC=0.2). Among the incident SCC only, the seroprevalence of some beta types was higher for individuals who were diagnosed close to the time of blood collection. For example, the seroprevalence of antibodies against HPV-8 was 16% (5/32) among those who were diagnosed 18+ months after blood collection, but 57% (4/7) for those who were diagnosed

within 18 months of blood collection. In addition, although only 7 SCC had their blood taken less than 18 months before diagnosis, three were seropositive to HPV-38 (43%) and four were seropositive for HPV types 9 and 17 (57%).

In Table 7.4 the association between the presence of antibodies against multiple HPV types (seronegative, positive to one or positive to two or more types) and the risk of SCC is shown for incident SCC and controls. Results using negative binomial distribution are reported in Table 7.5. Antibodies against any HPV types were frequently detected: 67% of the incident SCC were found seropositive to at least two HPV types compared to 66% of the controls; the differences were not statistically significant. For beta types, the equivalent seroprevalence was 44% in incident SCC and 38% in controls; again, the differences were not statistically significant.

	Controls N=80	Incident SCC N=39	*Prevalent SCC N=15*
Sex			
male	42 (53%)	24 (62%)	*5 (33%)*
female	38 (47%)	15 (38%)	*10 (67%)*
Age at recruitment (years)			
<55	17 (21%)	7 (18%)	*3 (20%)*
55-	33 (41%)	16 (41%)	*5 (33%)*
65+	30 (38%)	16 (41%)	*7 (47%)*
mean (SD)	61.5 (7.2)	61.7 (7.0)	*62.0 (8.3)*
range	42 to 76	48 to 76	*43 to 75*
Region of residence			
Oxfordshire	46 (58%)	21 (54%)	*11 (73%)*
other	34 (43%)	18 (46%)	*4 (27%)*
Follow-up time (years)			
mean (SD)	9.0 (2.0)	9.4 (1.0)	*8.7 (2.0)*
range	0 to 11	6 to 12	*2 to 10*
Age at diagnosis (years)			
mean (SD)		66.6 (8.2)	*58.2 (9.1)*
range	NA	48 to 85	*39 to 71*
Time from blood collection to diagnosis of cancer (years)			
mean (SD)	NA	4.6 (2.6)	*-4.2 (4.5)*
range		1mth to 10	*-15 to -0.2*

SD: Standard deviation; N: number; NA: Not available; mth: month.

SCC: squamous cell carcinoma

Table 7.1: Descriptive statistics of the controls and of incident and prevalent cutaneous squamous by age at recruitment, sex, region of residence, age at diagnosis, follow-up time and time from blood collection to diagnosis of first SCC

Table 7.2: Human papillomavirus seropositivity among control subjects by age, sex and region of residence (N=80)

Variables	Total	Any HPV seropositive (38 HPV types[3]) No POS	Any Alpha seropositive (11 HPV types) No POS	Any Beta seropositive (16 HPV types) No POS	Any Gamma seropositive (6 HPV types) No POS	Nu seropositive (1 HPV type) No POS	Any Mu seropositive (2 HPV types) No POS	Any ND seropositive (2 HPV types) No POS
Overall	80	69 (86%)	46 (58%)	46 (58%)	41 (51%)	11 (14%)	22 (28%)	6 (8%)
Sex[1]								
Female	38	33 (87%)	23 (61%)	19 (50%)	19 (50%)	3 (8%)	10 (26%)	3 (8%)
Male	42	36 (86%)	23 (55%)	27 (64%)	22 (52%)	8 (19%)	12 (29%)	3 (7%)
		P=1.0	P=0.7	P=0.3	P=1.0	P=0.2	P=1.0	P=1.0
Age (years)[2]								
<55	17	12 (71%)	12 (71%)	9 (53%)	9 (53%)	1 (6%)	2 (12%)	0 (0%)
55-	33	30 (91%)	19 (58%)	21 (64%)	17 (52%)	3 (9%)	10 (30%)	2 (6%)
65+	30	27 (90%)	15 (50%)	16 (53%)	15 (50%)	7 (23%)	10 (33%)	4 (13%)
		P=0.2	P=0.2	P=1.0	P=1.0	P=0.2	P=0.2	P=0.1
Region of residence[1]								
Oxfordshire	46	39 (85%)	28 (61%)	27 (59%)	27 (59%)	4 (9%)	10 (22%)	2 (4%)
other	34	30 (88%)	18 (53%)	19 (56%)	14 (41%)	7 (21%)	12 (35%)	4 (12%)
		P=0.8	P=0.5	P=0.8	P=0.2	P=0.2	P=0.2	P=0.4

[1] Fisher's exact test

[2] P-value for linear trend

[3] Alpha genus types: 2,3,7,10,11,13,16,18,27,57,77; Beta genus types: 5,8,9,15,17,20,23,24,36,38,49,75,76,92,93,96; Gamma genus types: 4, 48, 50, 60, 65, 95; Nu genus type: 41; Mu genus types: 1, 63 ; new types: 101, 103

HPV: Human papillomvairus; No POS: number of HPV seropositive plasma samples; ND: Not defined

HPV			Controls N=80	Incident SCC N=39			Prevalent SCC N=15
Genus	species	type	No POS (%)	No POS (%)	OR[1] (95% CI)	P-value*	No POS (%)
alpha	2	3	7 (9)	8 (21)	2.8 (0.9-8.6)	**0.1**	1 (7)
		10	5 (6)	2 (5)	-	**1.0**	1 (7)
		77	13 (16)	5 (13)	0.8 (0.2-2.3)	**0.6**	0 (0)
	4	2	8 (10)	3 (8)	-	**1.0**	1 (7)
		27	10 (13)	3 (8)	-	**0.5**	3 (20)
		57	6 (8)	0 (0)	-	**0.2**	0 (0)
	7	18	5 (6)	3 (8)	-	**0.7**	4 (27)
	8	7	5 (6)	1 (3)	-	**0.7**	1 (7)
	9	16	8 (10)	3 (8)	-	**1.0**	3 (20)
	10	11	13 (16)	7 (18)	1.2 (0.4-3.4)	**0.7**	1 (7)
		13	7 (9)	4 (10)	-	**0.7**	1 (7)
	1	5	16 (20)	5 (13)	0.5 (0.2-1.6)	**0.3**	1 (7)
		8	16 (20)	9 (23)	1.1 (0.4-2.9)	**0.8**	6 (40)
		20	17 (21)	9 (23)	1.1 (0.4-2.7)	**0.9**	3 (20)
		24	16 (20)	5 (13)	0.5 (0.2-1.6)	**0.3**	2 (13)
		36	13 (16)	6 (15)	0.8 (0.3-2.5)	**0.8**	1 (7)
		93	3 (4)	2 (5)	-	**0.7**	0 (0)
beta	2	9	16 (20)	8 (21)	1.0 (0.4-2.7)	**1.0**	4 (27)
		15	15 (19)	4 (10)	-	**0.3**	3 (20)
		17	12 (15)	8 (21)	1.5 (0.5-4.0)	**0.5**	2 (13)
		23	9 (11)	1 (3)	-	**0.7**	3 (20)
		38	14 (18)	10 (26)	1.5 (0.6-3.9)	**0.4**	5 (33)
		49	16 (20)	8 (21)	0.8 (0.2-2.4)	**0.6**	4 (27)
	3	75	10 (13)	3 (8)	-	**0.5**	3 (20)
		76	14 (18)	4 (10)	-	**0.4**	3 (20)
	4	92	9 (11)	4 (10)	-	**1.0**	4 (27)
	5	96	14 (18)	6 (15)	0.8 (0.3-2.3)	**0.7**	4 (27)
gamma	1	4	25 (31)	16 (41)	1.6 (0.7-3.5)	**0.3**	4 (27)
		65	18 (23)	8 (21)	0.8 (0.3-2.2)	**0.7**	5 (33)
		95	16 (20)	8 (21)	1.0 (0.4-2.6)	**1.0**	3 (20)
	2	48	15 (19)	4 (10)	-	**0.3**	2 (13)
	3	50	7 (9)	3 (8)	-	**0.6**	3 (20)
	4	60	3 (4)	0 (0)	-	**0.5**	0 (0)
nu	1	41	11 (14)	2 (5)	-	**0.2**	1 (7)
mu	1	1	18 (23)	8 (21)	0.9 (0.3-2.3)	**0.8**	4 (27)
	2	63	12 (15)	5 (13)	0.8 (0.2-2.4)	**0.7**	3 (20)
ND		101	2 (3)	0 (0)	-	**1.0**	1 (7)
		103	5 (6)	2 (5)	-	**0.6**	1 (7)

[1] Adjusted for age at recruitment (<55, 55-, 65+), sex and region of residence (Oxfordshire, other).

No POS: number of HPV seropositive plasma samples; ND: Not defined

N: number; OR: odds ratio; CI: confidence interval

*: When the number of HPV seropositive patient was less than 5, P-value was derived using Fisher's exact test

Table 7.3: Antibodies against capsid L1 protein of 38 human papillomavirus (HPV) types among controls, incident and prevalent SCC

Seropositive against [1]	Controls N= 80 No POS (%)	Incident SCC N=39 No POS (%)	OR[2] (95% CI)	P[3]
Alpha-cutaneous				
0	46 (58)	23 (59)	ref	
1	20 (25)	11 (28)	1.1 (0.5-2.8)	
2 or more	14 (18)	5 (13)	0.7 (0.2-2.3)	*0.7*
Alpha-mucosal				
0	58 (73)	26 (67)	ref	
1	15 (19)	10 (26)	1.6 (0.6-4.2)	
2 or more	7 (9)	3 (8)	1.0 (0.2-4.1)	*0.7*
Beta				
0	34 (43)	18 (46)	ref	
1	16 (20)	4 (10)	0.5 (0.1 to 1.7)	
2 or more	30 (38)	17 (44)	1.0 (0.4 to 2.5)	*1.0*
Beta1				
0	48 (60)	23 (59)	ref	
1	14 (18)	8 (21)	1.2 (0.4-3.2)	
2 or more	18 (23)	8 (21)	0.8 (0.3-2.3)	*0.8*
Beta2				
0	50 (63)	23 (59)	ref	
1	16 (20)	9 (23)	1.2 (0.5-3.2)	
2 or more	14 (18)	7 (18)	1.1 (0.4-3.0)	*0.8*
Beta3				
0	60 (75)	29 (74)	ref	
1	6 (8)	6 (15)	1.9 (0.6-6.6)	
2 or more	14 (18)	4 (10)	0.6 (0.2-1.9)	*0.6*
Gamma				
0	39 (49)	20 (51)	ref	
1	21 (26)	11 (28)	1.0 (0.4 to 2.7)	
2 or more	20 (25)	8 (21)	0.8 (0.3 to 2.2)	*0.7*
Gamma1				
0	47 (59)	21 (54)	ref	
1	16 (20)	10 (26)	1.3 (0.5-3.5)	
2 or more	17 (21)	8 (21)	1.0 (0.4-2.8)	*0.8*
Nu				
0	69 (86)	37 (95)	ref	
1	11 (14)	2 (5)	0.3 (0.03 to 1.6)	*0.2*
Mu				
0	58 (73)	30 (77)	ref	
1	14 (18)	5 (13)	0.7 (0.2 to 2.3)	
2	8 (10)	4 (10)	0.9 (0.2 to 3.7)	*0.8*
ND				
0	74 (93)	37 (95)	ref	
1	5 (6)	2 (5)	0.7 (0.1 to 4.7)	
2 or more	1 (1)	0 (0)	NA	*0.7*
Any HPV				
0	11 (14)	8 (21)	ref	
1	16 (20)	5 (13)	0.4 (0.1 to 1.9)	
2 or more	53 (66)	26 (67)	0.7 (0.2 to 2.1)	*0.7*

[1] Alpha genus types: 2,3,7,10,11,13,16,18,27,57,77; Beta genus types: 5,8,9,15,17,20,23,24,36,38,49,75,76,92,93,96; Gamma genus types: 4, 48, 50, 60, 65, 95; Nu genus type: 41; Mu genus types: 1, 63 ; new types: 101, 103

[2] Adjusted for age at recruitment (<55, 55-, 65+), sex and region of residence (Oxfordshire, other).

[3] P-value for linear trend.

No POS: number of HPV seropositive plasma samples; SCC: squamous cell carcinoma

N: number; OR: odds ratio; CI: confidence interval; ND: Not defined; HPV: human papillomavirus

Beta1, 2 or 3: Positive to HPV types from species 1, 2 or 3 respectively of genus beta. Gamma1: Positive to HPV types from species 1 of genus gamma.

Table 7.4: Antibodies against capsid L1 protein of multiple human papillomavirus (HPV) types among controls and incident SCC (using logistic regression)

Table 7.5: Antibodies against capsid L1 protein of multiple human papillomavirus (HPV) types among controls and incident SCC (using negative binomial regression)

	Control	Squamous cell carcinoma					
		incident SCC N=39			prevalent SCC N=15		
HPV	%neg / %pos to 2 or more	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value
alpha - mucosal	73/9	67/8	1.0 (0.5-2.1)	**0.9**	53/7	1.4 (0.5-3.6)	**0.5**
alpha - cutaneous	58/18	59/13	0.8 (0.5-1.4)	**0.5**	73/13	0.7 (0.3-1.7)	**0.4**
beta	43/38	46/44	0.9 (0.5-1.6)	**0.7**	33/40	1.4 (0.5-3.6)	**0.5**
gamma	49/25	51/21	0.9 (0.5-1.6)	**0.8**	40/27	1.0 (0.5-2.2)	**0.9**
nu, mu and ND	63/12	72/10	0.7 (0.4-1.3)	**0.3**	53/7	1.2 (0.5-3.0)	**0.6**

HPV: Human papillomavirus; SCC: squamous cell carcinoma; N: number; CR: Count ratio; CI: confidence interval.

Neg: seronegative: Pos: seropositive

[1] Using negative binomial regression adjusted for sex, age at recruitment, time since transplantation, skin type and centre (where appropriate).

7.3 The London and Oxford case-control studies

7.3.1 Statistical methods

Since non-melanoma skin cancers occur mainly in Caucasian populations, analyses looking at the association between HPV seropositivity or risk factor from the questionnaire and SCC / BCC were restricted to Caucasian patients. Analyses examining the association between seropositivity to HPV16 and a self-reported history of abnormal smear test and between seropositivity to HPV6 and self-reported history of genital warts were not restricted to Caucasian patients. Odds ratios (OR) were estimated to measure the association between SCC and risk factors from the questionnaire using conditional on centre logistic regression adjusted for sex, age at recruitment ($\leq$44, 45-59, $\geq$60), time since transplantation ($\leq$5 years, 5 to 9 years, $\geq$10 years) and skin type (I and II, III and IV). Conditional logistic regression on centre was used to deal with the potential for bias due to confounding by center. Age, sex and time since transplantation were included in the model due to their association with both the outcome and the exposure variable, HPV. Skin type was included in the model as it is strongly associated with the outcome variable.

As the number of patients who answered the questionnaire differed from the ones who gave specimens (Figure 5.2), the figures in tables do not always compare directly between analyses involving HPV results and those based on questionnaire only. Where results are presented in the form of plots, black circles indicate the odds ratios and horizontal lines represent 95% confidence intervals (CI).

To examine the relationship between seropositivity to a single HPV type or multiple HPV seropositivity (seronegative to all, seropositive to 1 type, seropositive to 2 or more) in cases (SCC and BCC only), odds ratios were estimated using conditional logistic regres-

sion on centre and adjusted for sex, age at recruitment (≤44, 45-59, ≥60), time since transplantation (≤5 years, 5 to 9 years,≥10 years) and skin type (I and II, III and IV). Multiple HPV seropositivity was also examined by species within genus. Negative binomial distribution (using multiple seropositivity as continuous variables) was also used with adjustment for the same factors as over-dispersion was observed in Poisson models. All analyses were also performed for each centre separately using unconditional logistic regression adjusted for the same factors. Five sensitivity analyses were performed to explore further the association between HPV and prevalent SCC i) with CIS included as SCC; ii) restricted to SCC only; iii) restricted to patients transplanted for 5 years or more; iv) restricted to SCC diagnosed four years before recruitment.

Descriptive statistics (number and percentage) were reported when figures were too low to derive adjusted estimates (<5 patients). To deal with multiple significance tests, agreement between results of the 2 centres was used to detect genuine associations and the level of statistical significance was set to 1%. Missing value categories were added to adjustment variables with incomplete information in order to retain all the observations in the analysis. Likelihood ratio tests were used to assess heterogeneity and trend tests (obtained by treating the categorical variable as a continuous variable in the model). All P-values were two-sided. Statistical analyses were carried out using STATA 9 (StataCorp, 2005).

7.3.2 Results for risk factors based on questionnaire data

- *Baseline characteristics of participants*

  The characteristics of study populations are shown in Table 7.6 and Figure 7.1. There was no statistically significant difference between London and Oxford in terms of sex, age at recruitment, time since transplantation and skin type (more

details in Chapter 8). The mean time between transplantation and the development of the first skin cancer was 11.9 years [SD: 7.3 years] for SCC and 10.0 years [SD: 6.7 years] for BCC. The median time between diagnosis of prevalent SCC and recruitment was -4 years [IQ25= -2years and IQ75= -7years]. Patients with SCC had an average of 5.2 lesions [SD: 8.5] and those with BCC had 3.5 lesions [SD: 4.2]. As expected, BCC were more often detected on non sun-exposed skin only (25%) than SCC (12%) (P= 0.01). For SCC, the main body sites involved were the head (39%), hand/wrist (28%), arms (12%) whereas for BCC, lesions tended to occur on the head (58%), the back (19%) and on the chest/shoulder (8%). In contrast to SCC, BCC were uncommon on hands (2%).

- *Risk factors from the questionnaire in relation to SCC*

Figures 7.2 and 7.3 show the odds ratios for OTR with SCC (140 patients) compared to controls (454 patients) for various risk factors examined using data from the questionnaire. Men were more likely to develop SCC than women (P=0.02) and odds ratios for SCC also increased with increasing age at recruitment (P-trend<0.001) and with time since transplantation (P-trend<0.001). Patients with SCC were 13 times (95% CI: 6.3 to 28.1) more likely than controls to have been transplanted more than 10 years prior to recruitment. Patients with skin type I and II were also more likely to develop SCC than those with skin type III and IV (P<0.001). A history of actinic keratoses (AK) or cutaneous warts was positively associated with the development of SCC. Transplant data variables and self-reported history of non-cutaneous cancer, psoriasis, genital warts or herpes zoster was not associated with the development of SCC; nor were any of the data for women relating to sexual and reproductive factors. Being born first compared to being born second or third

or higher order was associated with increased odds of developing SCC (P-value for trend= 0.02). A self report of being married or currently living with a partner was associated with an increased risk of SCC (OR: 2.1, 95% CI: 1.2 to 3.6: P= 0.01). Current smokers appeared less likely to develop SCC than controls but this finding was driven by data from only one centre (London). There was no significant association between SCC and types of academic qualification, eye or hair colour, birth country, body mass index, physical activity or markers of crowding and proximity after controlling for age, sex, time since transplantation, skin type and centre. Regarding UV radiation exposure, the number of sunburns before the age of 18 years was positively associated with SCC (P-value for trend<0.002; 3 or more burns versus never: OR: 3.3, 95% CI: 1.8 to 6.2). Cases with SCC were more likely to have had holidays in sunny countries after transplantation but no clear dose-relationship was detected (P-trend=0.08). Patients with SCC and controls did not differ in terms of other markers of UV exposure. Conditional on centre logistic regression with all potential confounding variables in the model (sex, age at recruitment [less than 44, 45-59,60 or more], time since transplantation [less than 5 years, 5 to 9 years, 10 or more years], skin type [I and II, III and IV], number of sunburns before the age of 18 [none, 1 to 2, 3 or more], birth order [1,2, 3 or more] and currently living with partner or being married [yes, no]) did not produce any material difference to any of the results (Table 7.7).

- *Risk factors from the questionnaire in relation to BCC*

Figures 7.4 and 7.5 show the odds ratios for OTR with BCC only (33 patients) compared to controls (454 patients) for various risk factors examined using data from the questionnaire. Men were more likely to develop BCC only than women

and the age at recruitment was positively associated with the occurrence of BCC. Patients transplanted more than 10 years ago prior to recruitment were 6 times more likely to develop BCC compared to those transplanted for 5 years or less (95% CI: 1.9 to 17.8). Patients with skin type I and II were also more likely to develop BCC only than those with skin type III and IV.

No association was found between a history of actinic keratoses and warts and the presence of BCC only; nor were any of the data for women relating to sexual or reproductive factors, transplant data (i.e organ type, donor relationship), cancer history, psoriasis, genital warts or herpes zoster. There was also no statistically significant association between cases with BCC only and the type of academic qualification, eye or hair colour, birth country, body mass index, physical activity or markers of proximity. However, it should be borne in mind that only 33 patients with BCC only were involved in these analyses. Regarding markers of UV exposure, patients who reported going on holidays in sunny countries after transplantation were more likely to develop BCC only (OR: 5.7; 95% CI: 1.9 to 17.4; P=0.0004). Patients with BCC only and controls did not differ in terms of other markers of UV exposure.

- *Risk factors from the questionnaire in relation to SCC and BCC, by centre*

  Tables 7.8, 7.9, 7.10, 7.11 show results restricted to each centre. All the findings after pooling the data were also observed in Oxford and London separately. Several additional factors were associated with cases in London. Patients with SCC from London were more likely to report a history of genital warts than controls. In Oxford, markers of proximity and crowding were associated with NMSC, in particular SCC development. Cases were 2.6 times more likely to have shared a bed or a room as a child than the control group (P=0.03 and P= 0.02 respectively), and were twice

as likely to have shared a room as a child than the control group. The association between smoking status and SCC was stronger in London than Oxford. Numbers were too small to test for interaction and in particular to look into details of patients with BCC.

7.3.3 Results on serological data

- *Laboratory validation: HPV16 and HPV6 in relation to history of cytological abnormalities and genital warts respectively*

  Table 7.12 shows the odds ratio for HPV16 seropositivity associated with a self-reported history of abnormal smear tests and for HPV6 seropositivity associated with self-reported history of genital warts using data from all study participants (irrespective of ethnic group) and for each centre separately. As expected, highly statistically significant associations ($P \leq 0.003$) were observed after adjustment for confounding variables both when data were pooled and when each centre was examined separately.

- *Seropositivity to a single HPV type in relation to SCC and BCC*

  In Table 7.13, the seroprevalence of HPV types is examined among cases and controls. There was no statistically significant difference at the 1% level between the prevalence of antibodies against any of the HPV types between cases with prevalent SCC and controls. The seroprevalence of antibodies against mucosal HPV types in all patients with prevalent SCC were similar than in the control group but age was a strong confounder of the association (as discussed in Chapter 6) producing a two-fold increased risk of prevalent SCC in patients seropositive to any of the three mucosal HPV types examined.

Tables 7.14 and 7.15 show results by centre. The prevalence of antibody to HPV5 was statistically significantly higher in patients with prevalent SCC from Oxford (P=0.02) but not in those from London (P=0.5). In London, higher seroprevalence were observed for several HPV types from alpha (types: 6, 13 and 16), beta (17 and 49) and gamma (48, 50 and 60) genera in SCC compared to controls but associations were only statistically significant at the 5% level. Across centre, the seroprevalence of antibodies against betaHPV types 17 and 49 was consistently higher among patients with prevalent or incident SCC compared to controls.

Patients with incident SCC were all from London. A statistically significant positive association was observed between seropositivity to HPV 60 of the gamma genus and the 20 patients with incident SCC (OR: 10.8; 95% CI 2.6 to 45.4; P=0.001). Higher seroprevalence were also observed among cases with incident SCC for alphaHPV 3 (15%), betaHPV 8 (35%), betaHPV 24 (25%), gammaHPV 4 and 65 (45%) compared to controls from London.

Among the 31 patients with BCC only, 26 were prevalent cases and 5 were incident ones. None of the alphaHPV types was associated with the occurrence of BCC in both centres. Several seroprevalence were found higher in patients with prevalent BCC only compared to controls: gammaHPV 4(12/26, 46% versus 25%), muHPV 41 (6/26, 23% versus 11%), nuHPV 63 (10/26, 38% versus 23%). Three out the 5 cases with incident BCC only were also seropositive to HPV 63 and 101. The small numbers did not allow examination by centre.

- *Seropositivity to multiple HPV types in relation to SCC and BCC*

In Tables 7.16, 7.17 and 7.18 the relationship between the presence of antibodies against multiple HPV types (seronegative, positive to one or positive to 2 or more)

and the risk of prevalent or incident SCC or BCC only is shown for cases and for controls among all patients and by centre.

Multiple HPV seropositivity was common in the control group with about 70% of all patients without skin cancers being seropositive to 2 or more HPV types (63% in Oxford and 75% in London).

Overall, there was no statistically significant difference in multiple HPV seroprevalence for any of the examined genera between prevalent SCC and controls at the 1% level except for mucosal HPV types due to the strong confounding effect of age. The 20 London patients with incident SCC were more likely to be seropositive to 2 or more HPV types of the gamma genus (11/20; 55%) than the control group (64/243; 25%). Multiple seropositivity to HPV types from other genus were not associated with the occurrence of incident SCC compared to those without the tumour. Higher multiple seroprevalence was observed among patients with prevalent or incident SCC but result among cases with prevalent SCC was not corroborated by the Oxford data. Patients with BCC only were also more likely to have multiple seropositivity than controls for nuHPV, muHPV and the 2 not-defined types.

- *Fully adjusted results and sensitivity analyses for prevalent SCC*

Table 7.19 shows results of the sensitivity analyses on the association between prevalent SCC and seropositivity to each HPV type after excluding or including CIS or other skin cancer in the case group. Stronger positive associations were found between prevalent SCC only (with or without CIS) and HPV16 (P=0.02), HPV6 ($P<0.001$), HPV5 (P= 0.03) and HPV 4 (P=0.05). Overall, analyses looking at the association between HPV and SCC with different case status did not produce any material differences in results.

Results on further sensitivity analyses and fully adjusted model for factors from the questionnaire that were found associated with SCC are shown in Table 7.20. Fully adjusted (for sex, age at recruitment [less than 44, 45-59,60 or more], time since transplantation [less than 5 years, 5 to 9 years, 10 or more years], skin type [I and II, III and IV], number of sunburns before the age of 18 [none, 1 to 2, 3 or more], birth order [1,2, 3 or more] and currently living with partner or being married [yes, no]) conditional on centre logistic regression did not produce any material difference nor did the sensitivity analyses restricted to patients who were transplanted for 5 years or more or the restricted analyses to patients with diagnosis within 4 years of blood collection. Self-history of viral warts was not included in the fully adjusted model as it might be on the pathway of HPV and SCC.

- *HPV seropositivity in non-Caucasian and Caucasian patients with prevalent SCC who gave blood only (but did not provide a questionnaire)*

  – Caucasian patients:

    Of the 3 Caucasian patients who only gave blood, 1 patient had prevalent SCC and 2 others had prevalent SCC and BCC. Seropositivity was as follows:

    - patient 1: HPV types 3, 2, 27, 16, 6, 5, 9, 17, 38, 4

    - patient 2: HPV types 95

    - patient 3: HPV types: 6, 63

  – Non-Caucasian patients:

    Two non-Caucasian patients who only gave blood were diagnosed with SCC in these studies. Seropositivity was as follows:

    - patient 1: HPV types 15

- patient 2: HPV types 13, 5, 8, 23, 49, 92, 48, 60, 41 and 101

7.4 Discussion

The prospective pilot study was the first to report on the seroprevalence of antibodies against HPV-L1 in relation to cutaneous squamous cell carcinoma in apparently immuno-competent individuals in which blood was taken prior to the diagnosis of the tumour and the two case-control studies are the first studies conducted in a population of OTR that has examined the relationship between antibodies against HPV-L1 in relation to the risk of cutaneous SCC and BCC.

However, these 3 studies had a number of limitations. Firstly, the pilot study includes only small numbers of cases, although it may be possible in the future to include other centres within the EPIC study. Secondly, cases in the pilot study were identified via linkage to Cancer Registry records and it is well recognised that there is substantial under-reporting of non-melanoma skin cancers in the United Kingdom, in part, because these lesions are rarely life threatening [332]. We were, therefore, unlikely to have identified all available cases and may also have missed skin cancer diagnoses among the controls. We had no information on the severity of SCC, nor did we have details of the occurrence of multiple tumours in the same individual. Thirdly, information on important confounders, such as sun exposure and skin type, was not available for the pilot study, although we do know that all participants were of Caucasian origin. Despite the fact that OTR are at a greatly increased risk of SCC compared to the general population, the 2 case-control studies have limited power to examine associations with all the different HPV types, in part because the prevalence of some is low. Consequently, some associations might not have

reached statistical significance. However, the study was conducted in two separate centres that were nonetheless in close geographical proximity, both to increase numbers and to compare and validate findings across centres.

In accordance with all of the published literature, patients with SCC were more likely to be older, to have fairer skin, to have been transplanted for a longer period of time and to have had a history of AKs than controls. We also found that men were more likely to have SCC than women, a finding that has also been identified in some other studies [98], but not in all [141]. While the risk of SCC is thought to increase with lifetime cumulative sun exposure [333], a higher number of sunburns before the age of 18 has also been associated with SCC in Australia [334] and a moderate association was also observed in a recent multi-centre study [148]. The increased odds of having SCC in patients being married or living with a partner is likely to be a screening effect a partner may identify lesions not seen by the patient themselves [335, 336]. To our knowledge, the association between birth order and the presence of cutaneous SCC has not been reported elsewhere. The possibility of a chance finding is reduced by the fact that the trend was observed in both centres. Birth order has also been inversely associated with allergic disease or eczema [337, 338] in some studies. In this context, it is hypothesised that individuals born first lack early exposure to infectious agents and are therefore more susceptible to develop certain conditions than their siblings [339]. However, its significance in this context remains highly speculative.

Very few patients developed BCC only and figures were too small to examine thoroughly potential risk factors from the questionnaire. However, our findings were in line with published literature with higher occurrence of BCC only in men, older patients, individuals

with fairer skin, with increasing time after transplantation and with increasing age. The only marker of UV exposure that was found to be associated with the presence of BCC only was a history of sunny holidays abroad after transplantation. This result indicates that intermittent sun exposure might increase the risk of BCC as it has been suggested in the published literature [66, 67]. No association was found with any other risk factors from the questionnaire.

In line with previous DNA prevalence studies [281, 340], the seropositivity to any HPV types was high with only 14% of OTR and IC patients without skin cancer being seronegative to all of the 34 and 38 HPV types tested respectively (more details in Chapter 6). As expected, we found a statistically significant (and well-established) association between self-reported history of cervical cytological abnormalities and/or genital warts and HPV16 or HPV6 respectively. This was present in both centres and validated the methodology used for HPV serological detection. HPV is an established cause of cervical cancer and cases have been found to have both a higher prevalence and titre of antibodies against certain HPV types (such as HPV-16), prior to diagnosis, as compared to controls [243, 245, 244]. In one prospective study of antibody levels against HPV-16, women who subsequently developed cervical cancers up to 10 years after blood collection were more than twice as likely to be HPV-16 seropositive than controls [243]. Comparable findings have been reported in prospective studies of other established oncogenic viruses such as human herpesvirus-8 (in relation to Kaposi's sarcoma) and hepatitis B (in relation to hepatocellular carcinoma), in which a higher than expected prevalence and titre of antibodies has been identified in blood taken years before diagnosis of cancer [245, 244].

Thus, results reported here for the incident and prevalent SCC differ from those found for

other known oncogenic infections, as we found no consistent relationship between the presence of antibodies against single and multiple HPV types and prevalent or incident SCC, even, after adjustment for multiple confounding factors. For prevalent SCC among OTR patients, only associations between seropositivity to mucosal HPV types (single or multiple) and the presence of SCC were observed. Younger patients tend to have higher seropositivity to mucosal HPV types, as a group, than those older (Chapter 6). Moreover, independently of HPV, younger people have a lower risk of SCC than older people. Hence, age is a strong positive confounder of the association between HPV seroprevalence of mucosal types and SCC suggesting that the association observed can probably be accounted for by a strong unmeasured (or inadequately measured) confounder such as UV exposure. Higher seroprevalence in patients with incident SCC from the pilot study observed for some beta types where blood samples were obtained closer to diagnosis might indicate that either these patients already have skin cancer prior to blood collection and should have been grouped with the fifteen prevalent cases or that the antibody levels against some beta types rises closer to diagnosis. Consequently, the pilot study suggests that, if HPV was involved in the carcinogenic process, this is reflected in an antibody response very close to the time of diagnosis of SCC, or that the antibody response does not fully reflect oncogenic viral activity. We cannot distinguish whether the antibody response observed in prevalent SCC was a consequence of tumour development or reflects a causal association. Higher seroprevalence for several HPV types (i.e HPV4 or 63) were found in patients with BCC compared to controls but there was no clear association between antibodies to a single or multiple HPV types as only 31 patients develop BCC only. Larger studies are needed to detect genuine associations.

Until recently only antibodies against HPV-L1 types 5, 8, 9, 15, 20, 23, 24, 36 and 38

of the beta genus, HPV 16 of the alpha genus and HPV1 of the mu genus have been examined in the context of skin cancer (reviewed in Chapter 4) [316, 318, 71, 271, 146, 81, 319, 297, 153, 330]. With the introduction of Luminex technology, antibodies against up to 100 HPV types can now be tested simultaneously [319, 153, 330]. Andersson et al (2008) did not report any differences in seropositivity between 72 immunocompetent individuals with SCC and 121 controls for any of the 14 HPV types they examined (HPV types: 1, 5, 6, 8, 9, 10, 15, 16, 20, 24, 32, 36, 38, and 57) [153] whereas Karagas et al (2006) examined 16 HPV types (HPV types: 1, 2, 3, 5, 6, 8, 9, 10, 15, 16, 20, 24, 32, 36, 38, 57) in 252 immunocompetent patients with SCC and 461 controls and found a two-fold increase risk of SCC in patients who were seropositive to HPV5 compared to those who were seronegative [319]. In a small study also using Luminex technology, Waterboer et al (2008) re-tested sera from 43 immunocompetent patients with SCC from Italy and from 77 controls for antibodies against 31 HPV types and found an association between prevalence of antibodies against HPV-L1 15, 17 and 38 (beta genus species 2) and also with HPV-L1 50 (gamma genus) and the presence of SCC [330]. These findings were consistent with a study in which HPV-DNA from the beta genus of species 2 predominated in SCC compared to healthy skin samples [293]. In summary, there is no consistent associations in the published literature.

The natural history of cutaneous HPV types is not well understood [320] and the concordance between HPV-DNA in skin biopsies [153] or in plucked hairs [297] and antibody detection has been low. In comparison with the clear results relating HPV 16 and 6 to self-reported histories of cervical cytological abnormalities or genital warts, the role of any of the 34 (and 38) HPV types examined in relation to SCC remains unclear. HPV might only be latently present in the skin [311]. The prevalence of antibodies against different HPV-

L1 antigens varies from study to study and any association might be a consequence of increased viral replication following cell proliferation in patients with cutaneous SCC (this may also explain associations identified for other proliferative skin lesions, such as psoriasis, burns and wound healing) [271]. Low HPV-DNA copy numbers in tumour cells [341] and the lack of HPV integration to the host-DNA [234] would also support this theory. It is possible that the increased risk of SCC observed in OTR is simply a result of immunosuppression impairing the normal capacity to repair UV-damaged DNA. On the other hand, inhibition of UV-induced apoptosis leading to increased capacity for cells to accumulate UV-induced mutations has been shown in one study to be a general effect of multiple HPV types [230] such that diverse HPV types may play a contributing role, rather than there existing specific high-risk HPV types as shown for cervical cancer. Equally, few sero-epidemiological studies have used the recently developed multiplexed and high throughput technologies such as Luminex and new HPV types or combinations of types [330] might still be found to be associated with SCC development in the future.

In conclusion, our serological data do not support a role for any of the 38 HPV types examined in the aetiology of cutaneous SCC. Further research is needed to clarify the association between SCC, BCC and HPV and to allow direct comparison between sero-epidemiological, HPV DNA detection [342] and functional studies. In particular, large prospective studies, with recording of possible confounding variables, are necessary to elucidate any genuine associations.

Table 7.6: Characterisics of case and control participants among Caucasian transplant patients from Oxford and London

Characteristics - Both centres	Controls	Squamous cell carcinoma			Basal cell carcinoma (only)		
		All	prevalent	incident	All	prevalent	incident
Number	**425**	**139**	**119**	**20**	**31**	**26**	**5**
Sex (ratio M/F)	1.6 (260/165)	2.2 (95/44)	2.1 (81/38)	2.3 (14/6)	3.4 (24/7)	2.7 (19/7)	
Age at recruitments (mean[SD}, years)	48 [13]	59 [10]	60 [10]	56 [13]	55 [11]	55 [11]	
Time since transplantation (mean[SD], years)	9 [7]	16 [7]	16 [7]	13 [7]	12 [6]	12 [7]	
Skin type (% types I & II)	133 (33%)	79 (58%)	67 (57%)	12 (60%)	15 (48%)	13 (50%)	
Body mass index (mean [SD], kg/m²)	26 [5]	26 [5]	25 [4]	27 [6]	25 [4]	25 [3]	
Current smokers (%)	139 (35%)	47 (35%)	40 (34%)	11 (35%)	16 (52%)	13 (50%)	
Alcohol consumption (% never drinker)	69 (18%)	18 (14%)	17 (15%)	1 (5%)	1 (3%)	1 (4%)	
Characteristics - London							
Number	**243**	**89**	**69**	**20**	**22**	**18**	**4**
Sex (ratio M/F)	1.6 (150/93)	2.2 (61/18)	2.1 (47/22)	*2.3 (14/6)*	3.4 (17/5)	2.6 (13/5)	
Age at recruitments (mean[SD}, years)	47 [13]	58 [10]	59 [9]	*56 [13]*	53 [11]	52 [11]	
Time since transplantation (mean[SD], years)	10 [7]	15 [7]	16 [6]	*13 [7]*	12 [6]	15 [7]	
Skin type (% types I & II)	87 (38%)	58 (66%)	46 (68%)	*12 (60%)*	11 (50%)	9 (50%)	
Body mass index (mean [SD], kg/m²)	26 [5]	25 [4]	25 [4]	*27 [6]*	25 [4]	25 [3]	
Current smokers (%)	81 (36%)	29 (33%)	22 (33%)	*11 (35%)*	11 (50%)	8 (44%)	
Alcohol consumption (% never drinker)	39 (18%)	6 (7%)	5 (8%)	*1 (5%)*	1 (5%)	1 (6%)	
Characteristics - Oxford							
Number	**182**	**50**	**50**	**0**	**9**	**8**	**1**
Sex (ratio M/F)	1.5 (110/72)	2.1 (34/16)	*2.1 (34/16)*		3.5 (7/2)	3.0 (6/2)	
Age at recruitments (mean[SD], years)	48 [13]	61 [10]	*61 [10]*		60 [9]	61 [9]	
Time since transplantation (mean[SD], years)	9 [7]	17 [9]	*17 [9]*		12 [8]	12 [9]	
Skin type (% types I & II)	46 (26%)	21 (43%)	*21 (43%)*		4 (44%)	4 (50%)	
Body mass index (mean [SD], kg/m²)	26 [5]	26 [5]	*26 [5]*		25 [3]	26 [4]	
Current smokers (%)	58 (33%)	18 (37%)	*18 (37%)*		5 (56%)	5 (63%)	
Alcohol consumption (% never drinker)	30 (17%)	12 (25%)	*12 (25%)*		0 (0%)	0 (0%)	

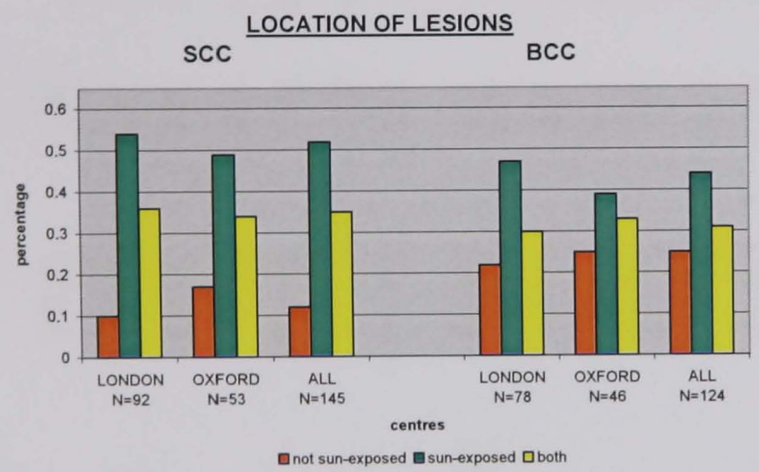
F: female; M: Male; SD: standard deviation

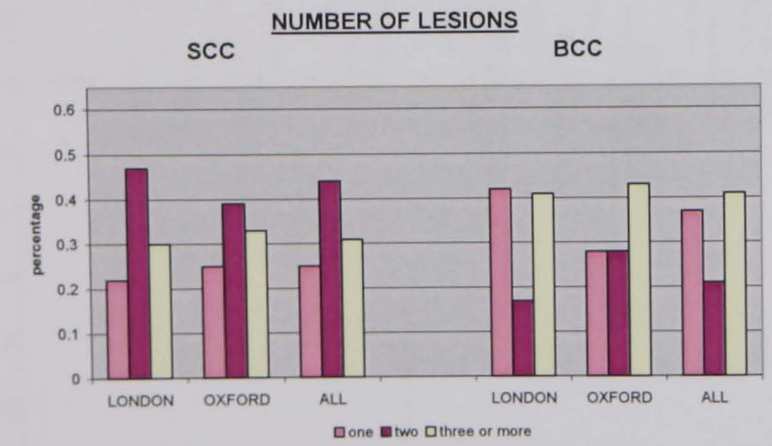
Missing values are excluded from the calculation of percentages.

	SCC			BCC		
	LONDON N=92	OXFORD N=53	TOTAL N=145**	LONDON N=78	OXFORD N=46	TOTAL N=124**.
NUMBER,no(%)						
one	33 (36)	15 (28)	48 (33)	33 (42)	13 (28)	46 (37)
two	17 (18)	12 (23)	29 (20)	13 (17)	13 (28)	26 (21)
three or more	42 (47)	26 (49)	68 (47)	32 (41)	19 (43)	51 (41)
range	*1 to 57*	*1 to 35*	*1 to 57*	1 to 22	1 to 21	1 to 22
mean [SD]	*5.9 [9.7]*	*3.8 [5.4]*	*5.2 [8.5]*	*3.6 [4.5]*	*3.3 [3.7]*	*3.5 [4.2]*
TIME BETWEEN TRANSPLANTATION AND FIRST LESION						
mean [SD], years	11.8 [6.4]	12.2 [8.6]	11.9 [73]	9.7 [6.3]	10.4 [7.5]	10.0 [67]

*: 1 patient from Oxford with also 1 anal SCC; no: number; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma
**. Based on all patients (including patients with other skin cancers)

	SCC			BCC		
	LONDON N=92	OXFORD N=53	TOTAL N=145	LONDON N=78	OXFORD N=46	TOTAL N=124
LOCATION, no (%)						
not sun-exposed	9 (10)	9 (17)	18 (12)	17 (22)	13 (25)	30 (25)
sun-exposed*	50 (54)	26 (49)	76 (52)	36 (47)	18 (39)	54 (44)
both	33 (36)	18 (34)	51 (35)	23 (30)	15 (33)	38 (31)
missing				2	0	2





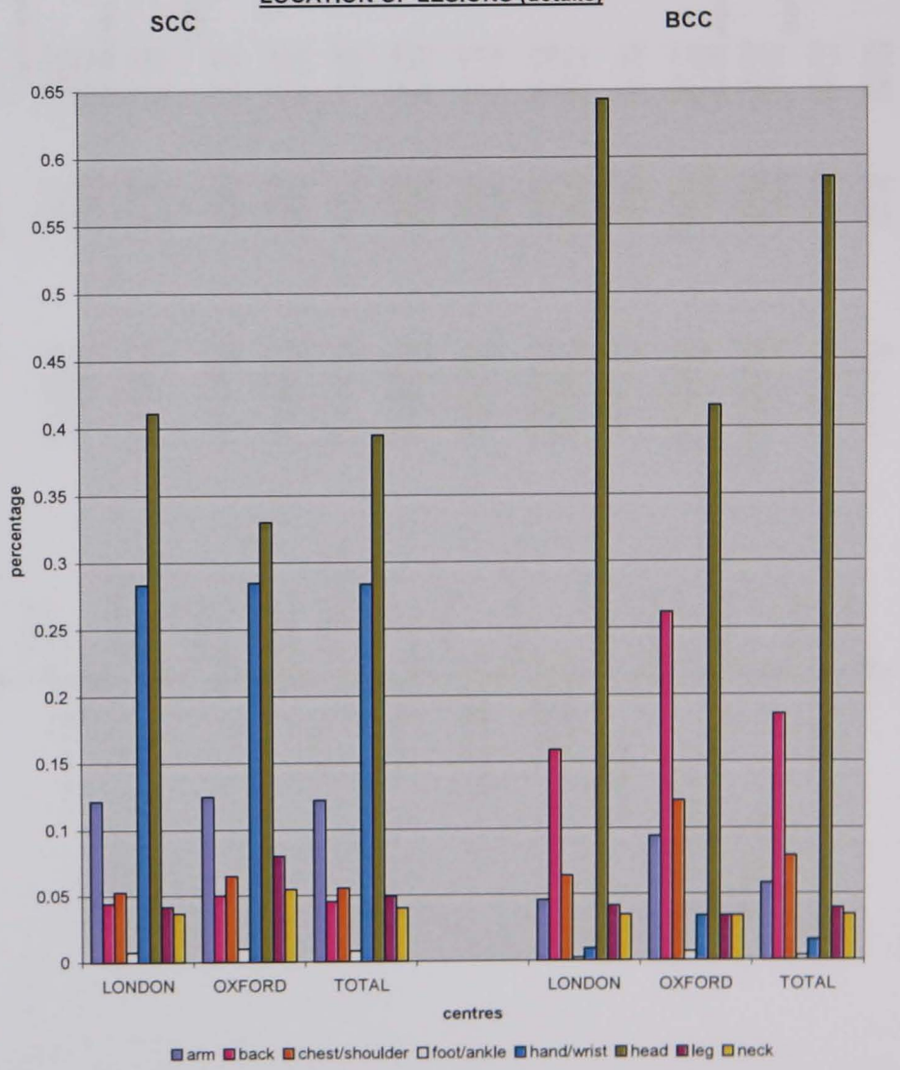
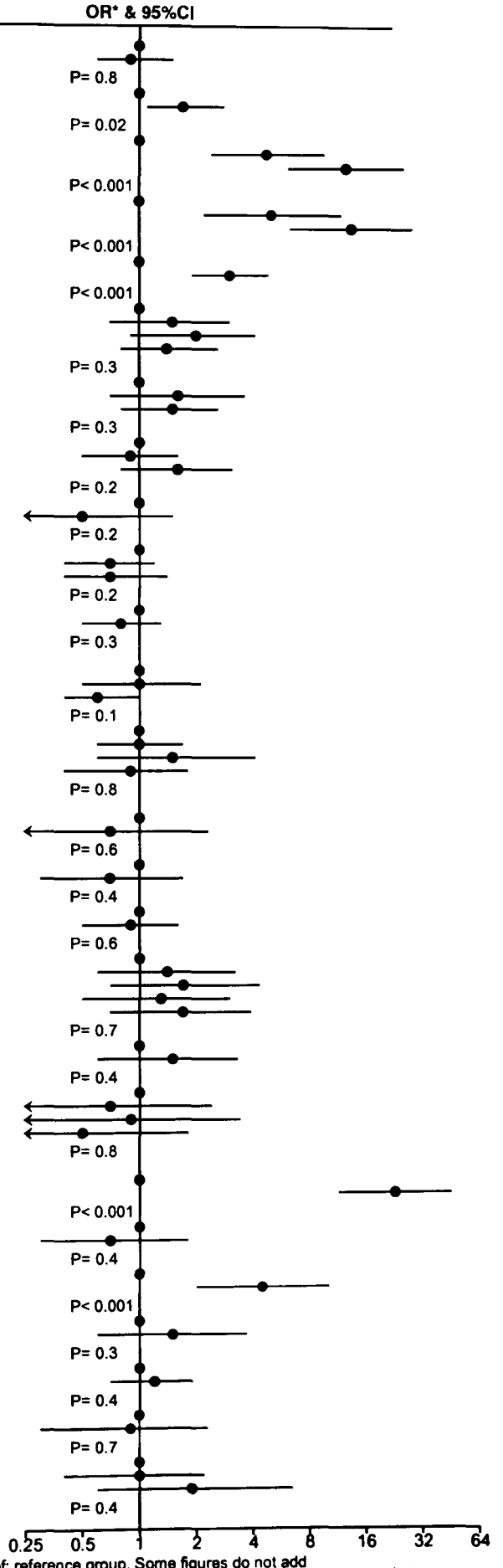


Figure 7.1: Number and body site of SCC and BCC among Caucasian OTR

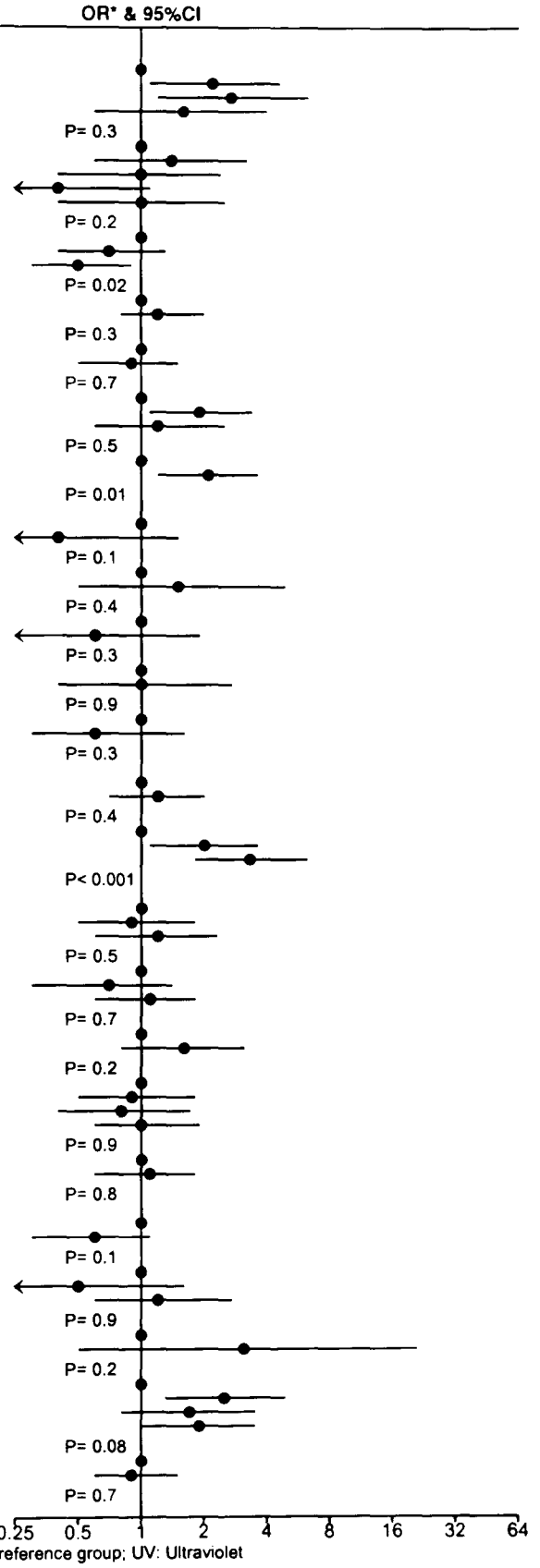
	Number control(%) N=454	Number case(%) N=140	OR* & 95%CI
Centre			
London	271 (60)	89 (64)	ref
Oxford	183 (40)	51 (36)	0.9 (0.6-1.5)
Sex			P= 0.8
Female	171 (38)	44 (31)	ref
Male	283 (62)	96 (69)	1.7 (1.1-2.8)
Age at recruitment (years)			P= 0.02
<45	192 (42)	12 (9)	ref
45-59	175 (39)	56 (40)	4.7 (2.4-9.5)
60 or more	87 (19)	72 (51)	12.4 (6.1-25.1)
Time since transplantation (years)			P< 0.001
<5	186 (41)	9 (6)	ref
5-9	107 (24)	24 (17)	5.0 (2.2-11.7)
10 or more	161 (35)	107 (76)	13.3 (6.3-28.1)
Skin type			P< 0.001
III-IV	312 (69)	60 (43)	ref
I-II	137 (31)	79 (57)	3.0 (1.9-4.8)
Qualification**			P< 0.001
O level/A level	114 (26)	36 (26)	ref
College/University	134 (30)	27 (20)	1.5 (0.7-3.0)
Clerical/commercial	68 (15)	26 (19)	2.0 (0.9-4.1)
Other	129 (29)	48 (35)	1.4 (0.8-2.6)
Eye colour**			P= 0.3
Brown/Hazel	161 (38)	32 (26)	ref
Green	60 (14)	15 (12)	1.6 (0.7-3.6)
Blue	204 (48)	77 (62)	1.5 (0.8-2.6)
Hair colour**			P= 0.3
Black/Brown	237 (52)	59 (42)	ref
Light brown	149 (33)	46 (33)	0.9 (0.5-1.6)
Red or blonde	66 (15)	34 (24)	1.6 (0.8-3.1)
Birth country			P= 0.2
Other	13 (3)	6 (4)	ref
Europe	441 (97)	134 (96)	0.5 (0.1-1.5)
Body mass index			P= 0.2
<25	197 (44)	72 (54)	ref
25-	164 (37)	42 (32)	0.7 (0.4-1.2)
30 or more	87 (19)	19 (14)	0.7 (0.4-1.4)
Exercise (/week)			P= 0.2
Never/Rarely/once	160 (36)	60 (43)	ref
twice or more	280 (64)	78 (57)	0.8 (0.5-1.3)
SMOKING AND ALCOHOL			P= 0.3
Tobacco use**			
Never	226 (50)	78 (56)	ref
Past users	69 (15)	14 (10)	1.0 (0.5-2.1)
Current	159 (35)	48 (34)	0.6 (0.4-1.0)
Alcohol consumption (day/week)			P= 0.1
<1	166 (38)	44 (32)	ref
once or more	168 (38)	64 (47)	1.0 (0.6-1.7)
Former drinker	25 (6)	10 (7)	1.5 (0.6-4.1)
Never	81 (18)	19 (14)	0.9 (0.4-1.8)
TRANSPLANT INFORMATION			P= 0.8
Organ type			
Kidney	435 (96)	135 (96)	ref
Other	19 (4)	5 (4)	0.7 (0.2-2.3)
Donor			P= 0.6
Cadaveric	369 (82)	130 (93)	ref
Living	80 (18)	10 (7)	0.7 (0.3-1.7)
Number of transplant			P= 0.4
1	382 (84)	114 (81)	ref
2 or more	72 (16)	26 (19)	0.9 (0.5-1.6)
Primary diagnosis**			P= 0.6
Unknown	70 (15)	15 (11)	ref
Glomerulonephritis	117 (26)	43 (31)	1.4 (0.6-3.2)
Pyelonephritis	64 (14)	21 (15)	1.7 (0.7-4.3)
Cystic	81 (18)	25 (18)	1.3 (0.5-3.0)
Other	122 (27)	36 (26)	1.7 (0.7-3.9)
Dialysis (Oxford only)			P= 0.7
Yes	162 (89)	41 (80)	ref
No	21 (11)	10 (20)	1.5 (0.6-3.3)
Type of dialysis **(Oxford only)			P= 0.4
Never	21 (12)	10 (20)	ref
Haemodialysis	61 (34)	23 (45)	0.7 (0.2-2.4)
Peri	52 (29)	10 (20)	0.9 (0.2-3.4)
Both	46 (26)	8 (16)	0.5 (0.1-1.8)
OTHER INFORMATION			P= 0.8
Actinic keratoses			
No	427 (94)	49 (35)	ref
Yes	27 (6)	91 (65)	23.1 (11.5-46.1)
Previous cancer			P< 0.001
No	416 (93)	129 (93)	ref
Yes	30 (7)	9 (7)	0.7 (0.3-1.8)
Warts			P= 0.4
No	150 (33)	8 (6)	ref
Yes	302 (67)	129 (94)	4.5 (2.0-10.2)
Genital warts			P< 0.001
No	412 (93)	125 (93)	ref
Yes	32 (7)	10 (7)	1.5 (0.6-3.7)
Shingles			P= 0.3
No	314 (71)	82 (59)	ref
Yes	128 (29)	56 (41)	1.2 (0.7-1.9)
Psoriasis			P= 0.4
No	424 (94)	132 (95)	ref
Yes	26 (6)	7 (5)	0.9 (0.3-2.3)
Family history of skin cancer**			P= 0.7
No	374 (83)	117 (85)	ref
Yes	51(11)	12 (9)	1.0 (0.4-2.2)
Not sure	26 (6)	9 (7)	1.9 (0.6-6.5)
			P= 0.4



N: number; SCC: squamous cell carcinoma; OR: odds ratio; CI: confidence interval; Ref: reference group. Some figures do not add up to total due to missing data. * Adjusted for age at recruitment, sex, time since transplantation, skin type and stratified by centres. **P-values for heterogeneity

Figure 7.2: Potential risk factors from the questionnaire and their association with SCC

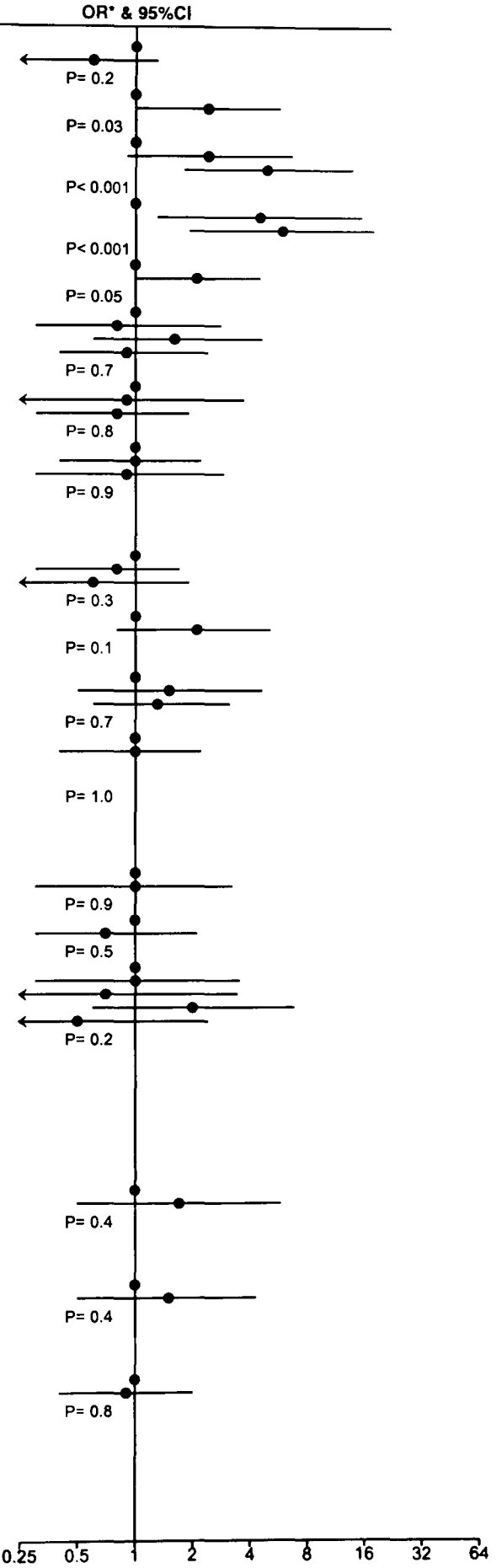
	Number control(%) N=454	Number case(%) N=140	OR* & 95%CI
PROXIMITY			
Number in household			
1	70 (16)	14 (10)	ref
2	176 (40)	81 (59)	2.2 (1.1-4.6)
3	87 (20)	29 (21)	2.7 (1.2-6.3)
4 or more	107 (24)	14 (10)	1.6 (0.6-4.0)
Number of siblings			P= 0.3
0	41 (9)	16 (11)	ref
1	138 (30)	46 (33)	1.4 (0.6-3.2)
2	113 (25)	32 (23)	1.0 (0.4-2.4)
3	64 (14)	11 (8)	0.4 (0.1-1.1)
4 or more	98 (22)	35 (25)	1.0 (0.4-2.5)
Birth order			P= 0.2
1st	179 (40)	66 (47)	ref
2nd	142 (31)	40 (29)	0.7 (0.4-1.3)
3rd or more	132 (29)	33 (24)	0.5 (0.3-0.9)
Share a room as a child			P= 0.02
Yes	286 (64)	83 (59)	ref
No	163 (36)	57 (41)	1.2 (0.8-2.0)
Share a bed as a child			P= 0.3
Yes	99 (22)	50 (36)	ref
No	353 (78)	89 (64)	0.9 (0.5-1.5)
Number of children			P= 0.7
0	173 (38)	26 (19)	ref
1-2	198 (44)	86 (61)	1.9 (1.1-3.4)
3 or more	83 (18)	28 (20)	1.2 (0.6-2.5)
Partner			P= 0.5
No	144 (32)	24 (17)	ref
Yes	310 (68)	116 (83)	2.1 (1.2-3.6)
WOMEN INFORMATION**			P= 0.01
Abnormal smear test			
No	124 (77)	39 (91)	ref
Yes	38 (23)	4 (9)	0.4 (0.1-1.5)
Still menstruating			P= 0.1
No	90 (20)	34 (24)	ref
Yes	68 (15)	9 (6)	1.5 (0.5-4.9)
Hysterectomy			P= 0.4
No	129 (30)	36 (26)	ref
Yes	23 (5)	6 (4)	0.6 (0.2-1.9)
Ever used oral contraceptive			P= 0.3
No	57 (13)	25 (18)	ref
Yes	100 (23)	18 (13)	1.0 (0.4-2.7)
Ever used hormone replacement			P= 0.9
No	118 (27)	29 (21)	ref
Yes	38 (9)	13 (9)	0.6 (0.3-1.6)
UV EXPOSURE			P= 0.3
Ever had outdoor occupation			
Yes	300 (66)	82 (59)	ref
No	153 (34)	57 (41)	1.2 (0.7-2.0)
Sunburns before 18			P= 0.4
Never	216 (48)	54 (39)	ref
1-2	132 (29)	39 (28)	2.0 (1.1-3.6)
3 or more	103 (23)	45 (33)	3.3 (1.8-6.2)
UV EXPOSURE- BEFORE TRANSPLANTATION			P< 0.001
Sunburn			
Never	93 (21)	21 (15)	ref
1-2	177 (39)	52 (38)	0.9 (0.5-1.8)
3 or more	183 (40)	65 (47)	1.2 (0.6-2.3)
Sunbathing			P= 0.5
Never	176 (40)	55 (40)	ref
1-2	78 (18)	19 (14)	0.7 (0.3-1.4)
3 or more	189 (43)	62 (46)	1.1 (0.6-1.8)
Sunbed			P= 0.7
Never	368 (82)	119 (86)	ref
Ever	83 (18)	20 (14)	1.6 (0.8-3.1)
Sunny Holidays			P= 0.2
Never	96 (21)	37 (27)	ref
1-2	87 (19)	31 (22)	0.9 (0.5-1.8)
3-4	80 (18)	21 (15)	0.8 (0.4-1.7)
6 or more	185 (41)	49 (36)	1.0 (0.6-1.9)
Outdoor hobbies			P= 0.9
Yes	297 (66)	97 (70)	ref
No	155 (34)	42 (30)	1.1 (0.6-1.8)
UV EXPOSURE- AFTER TRANSPLANTATION			P= 0.8
Sunburn			
Never	331 (83)	107 (86)	ref
Ever	70 (17)	18 (14)	0.6 (0.3-1.1)
Sunbathing			P= 0.1
Never	351 (82)	121 (88)	ref
1-2	33 (8)	4 (3)	0.5 (0.1-1.6)
3 or more	46 (11)	12 (9)	1.2 (0.6-2.7)
Sunbed			P= 0.9
Never	420 (99)	134 (99)	ref
Ever	3 (1)	2 (1)	3.1 (0.5-21)
Sunny Holidays			P= 0.2
Never	203 (48)	35 (26)	ref
1-2	75 (18)	29 (21)	2.5 (1.3-4.9)
3-4	62 (15)	22 (16)	1.7 (0.8-3.5)
6 or more	85 (20)	49 (36)	1.9 (1.0-3.5)
Outdoor hobbies			P= 0.08
Yes	235 (55)	82 (60)	ref
No	194 (45)	54 (40)	0.9 (0.6-1.5)
			P= 0.7



N: number; SCC: squamous cell carcinoma;OR: odds ratio; CI: confidence interval; Ref: reference group; UV: Ultraviolet
Some figures do not add up to total due to missing data.
* Adjusted for age at recruitment, sex, time since transplantation, skin type and stratified by centres. All P-values are for trend
** Restricted to women (number of controls and cases: 171 and 44 respectively)

Figure 7.3: Potential risk factors from the questionnaire and their association with SCC (continued)

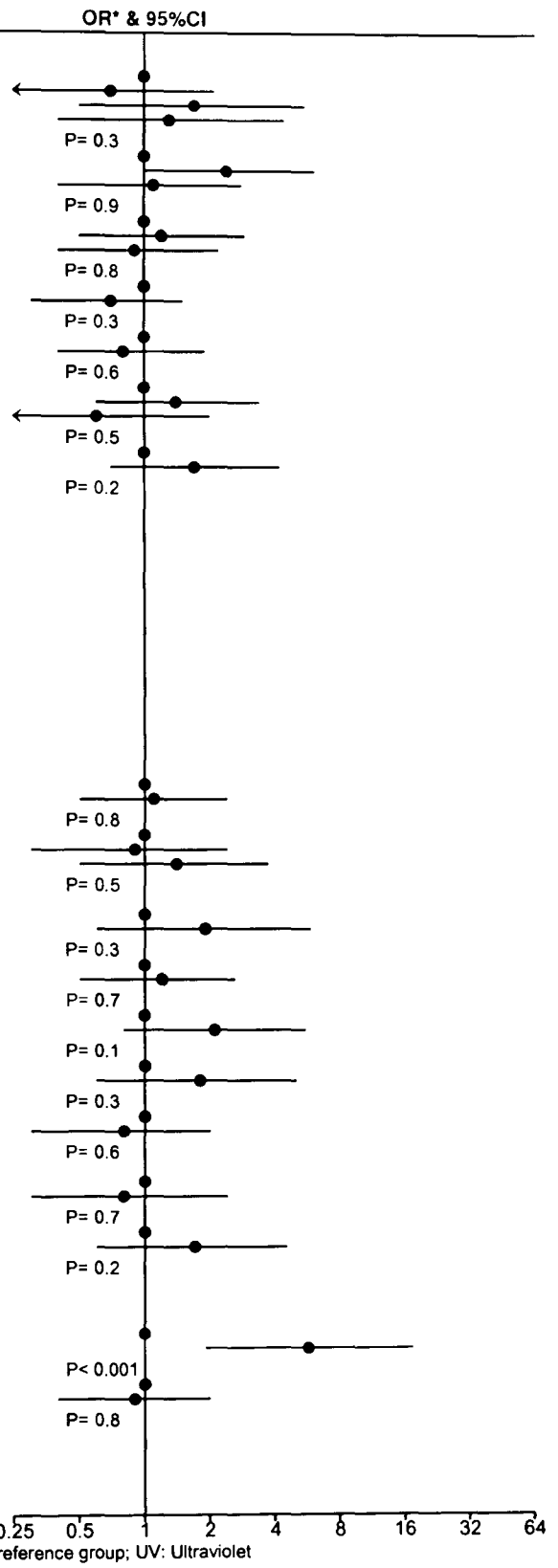
	Number control(%) N=454	Number case(%) N=33	OR* & 95%CI
Centre			
London	271 (60)	24 (73)	ref
Oxford	183 (40)	9 (27)	0.6 (0.2 to 1.3)
Sex			P= 0.2
Female	171 (38)	8 (24)	ref
Male	283 (62)	25 (76)	2.4 (1.0-5.7)
Age at recruitment (years)			P= 0.03
<45	192 (42)	6 (18)	ref
45-59	175 (39)	14 (42)	2.4 (0.9-6.6)
60 or more	87 (19)	13 (39)	4.9 (1.8-13.8)
Time since transplantation (years)			P< 0.001
<5	186 (41)	4 (12)	ref
5-9	107 (24)	9 (27)	4.5 (1.3-15.4)
10 or more	161 (35)	20 (61)	5.9 (1.9-17.8)
Skin type			P< 0.001
III-IV	312 (69)	17 (52)	ref
I-II	137 (31)	16 (48)	2.1 (1.0-4.5)
Qualification**			P= 0.05
O level/A level	114 (26)	10 (30)	ref
College/University	134 (30)	5 (15)	0.8 (0.3-2.8)
Clerical/commercial	68 (15)	8 (24)	1.6 (0.6-4.6)
Other	129 (29)	10 (30)	0.9 (0.4-2.4)
Eye colour**			P= 0.7
Brown/Hazel	161 (38)	12 (40)	ref
Green	60 (14)	3 (10)	0.9 (0.2-3.7)
Blue	204 (48)	15 (50)	0.8 (0.3-1.9)
Hair colour**			P= 0.8
Black/Brown	237 (52)	17 (52)	ref
Light brown	149 (33)	12 (36)	1.0 (0.4-2.2)
Red or blonde	66 (15)	4 (12)	0.9 (0.3-2.9)
Birth country			P= 0.9
Other	13 (3)	1 (3)	-
Europe	441 (97)	32 (97)	-
Body mass index			
<25	197 (44)	17 (52)	ref
25-	164 (37)	11 (33)	0.8 (0.3-1.7)
30 or more	87 (19)	5 (15)	0.6 (0.2-1.9)
Exercise (/week)			P= 0.3
Never/Rarely/once	160 (36)	7 (22)	ref
twice or more	280 (64)	25 (78)	2.1 (0.8-5.1)
SMOKING AND ALCOHOL			P= 0.1
Tobacco use**			
Never	226 (50)	12 (36)	ref
Past users	69 (15)	5 (15)	1.5 (0.5-4.6)
Current	159 (35)	16 (48)	1.3 (0.6-3.1)
Alcohol consumption (day/week)			P= 0.7
<1	166 (38)	13 (39)	ref
once or more	168 (38)	18 (55)	1.0 (0.4-2.2)
Former drinker	25 (6)	1 (3)	-
Never	81 (18)	1 (3)	-
TRANSPLANT INFORMATION			P= 1.0
Organ type			
Kidney	434 (96)	32 (97)	-
Other	20 (4)	1 (3)	-
Donor			
Cadaveric	369 (82)	29 (88)	ref
Living	80 (18)	4 (12)	1.0 (0.3-3.2)
Number of transplant			P= 0.9
1	382 (84)	28 (85)	ref
2 or more	72 (16)	5 (15)	0.7 (0.3-2.1)
Primary diagnosis**			P= 0.5
Unknown	70 (15)	4 (12)	ref
Glomeronephritis	117 (26)	10 (30)	1.0 (0.3-3.5)
Pyeonephritis	64 (14)	3 (9)	0.7 (0.1-3.4)
Cystic	81 (18)	12 (36)	2.0 (0.6-6.8)
Other	122 (27)	4 (12)	0.5 (0.1-2.4)
Dialysis (Oxford only)			P= 0.2
Yes	162 (89)	7 (78)	-
No	21 (11)	2 (22)	-
Type of dialysis **(Oxford only)			
Never	21 (12)	2 (25)	-
Haemodialysis	61 (34)	4 (50)	-
Peri	52 (29)	2 (25)	-
Both	46 (26)	0 (0)	-
OTHER INFORMATION			
Actinic keratoses			
No	427 (94)	28 (85)	ref
Yes	27 (6)	5 (15)	1.7 (0.5 to 5.8)
Previous cancer			P= 0.4
No	416 (93)	31 (94)	-
Yes	30 (7)	2 (6)	-
Warts			
No	150 (33)	5 (15)	ref
Yes	302 (67)	28 (85)	1.5 (0.5-4.3)
Genital warts			P= 0.4
No	412 (93)	30 (97)	-
Yes	32 (7)	1 (3)	-
Shingles			
No	314 (71)	23 (70)	ref
Yes	128 (29)	10 (30)	0.9 (0.4-2.0)
Psoriasis			P= 0.8
No	424 (94)	32 (100)	-
Yes	26 (6)	0 (0)	-
Family history of skin cancer**			
No	374 (83)	31 (94)	-
Yes	51 (11)	1 (3)	-
Not sure	26 (6)	1 (3)	-



N: number; BCC: basal cell carcinoma; OR: odds ratio; CI: confidence interval; Ref: reference group. Some figures do not add up to total due to missing data. * Adjusted for age at recruitment, sex, time since transplantation, skin type and stratified by centres. **P-values for heterogeneity

Figure 7.4: Potential risk factors from the questionnaire and their association with BCC only

	Number control(%) N=454	Number case(%) N=33	OR* & 95%CI
PROXIMITY			
Number in household			
1	70 (16)	6 (18)	ref
2	176 (40)	11 (33)	0.7 (0.2-2.1)
3	87 (20)	9 (27)	1.7 (0.5-5.5)
4 or more	107 (24)	7 (21)	1.3 (0.4-4.4)
			P= 0.3
Number of siblings			
0 or 1	179 (39)	9 (27)	ref
2	113 (25)	13 (39)	2.4 (1.0-6.1)
3 or more	162 (36)	11 (33)	1.1 (0.4-2.8)
			P= 0.9
Birth order			
1st	179 (40)	12 (36)	ref
2nd	142 (31)	11 (33)	1.2 (0.5-2.9)
3rd or more	132 (29)	10 (30)	0.9 (0.4-2.2)
			P= 0.8
Share a room as a child			
Yes	286 (64)	23 (70)	ref
No	163 (36)	10 (30)	0.7 (0.3-1.5)
			P= 0.3
Share a bed as a child			
Yes	99 (22)	11 (33)	ref
No	353 (78)	22 (67)	0.8 (0.4-1.9)
			P= 0.6
Number of children			
0	173 (38)	9 (27)	ref
1-2	198 (44)	20 (61)	1.4 (0.6-3.4)
3 or more	83 (18)	4 (12)	0.6 (0.2-2.0)
			P= 0.5
Partner			
No	144 (32)	7 (21)	ref
Yes	310 (68)	26 (79)	1.7 (0.7-4.2)
			P= 0.2
WOMEN INFORMATION**			
Abnormal smear test			
No	124 (77)	7 (88)	-
Yes	38 (23)	1 (13)	-
Still menstruating			
No	90 (20)	4 (12)	-
Yes	68 (15)	4 (12)	-
Hysterectomy			
No	129 (30)	8 (24)	-
Yes	23 (5)	0 (0)	-
Ever used oral contraceptive			
No	57 (13)	2 (6)	-
Yes	100 (23)	6 (18)	-
Ever used hormone replacement			
No	118 (27)	6 (18)	-
Yes	38 (9)	2 (6)	-
UV EXPOSURE			
Ever had outdoor occupation			
Yes	300 (66)	19 (58)	ref
No	153 (34)	14 (42)	1.1 (0.5-2.4)
			P= 0.8
Sunburns before 18			
Never	216 (48)	18 (55)	ref
1-2	132 (29)	7 (21)	0.9 (0.3-2.4)
3 or more	103 (23)	8 (24)	1.4 (0.5-3.7)
			P= 0.5
UV EXPOSURE- BEFORE TRANSPLANTATION			
Sunburn			
Never	93 (21)	4 (12)	ref
Ever	360 (79)	29 (88)	1.9 (0.6-5.8)
			P= 0.3
Sunbathing			
Never	176 (40)	13 (39)	ref
Ever	267 (60)	20 (61)	1.2 (0.5-2.6)
			P= 0.7
Sunbed			
Never	368 (82)	26 (79)	ref
Ever	83 (18)	7 (21)	2.1 (0.8-5.5)
			P= 0.1
Sunny Holidays			
Never	96 (21)	5 (15)	ref
Ever	352 (79)	28 (85)	1.8 (0.6-5.0)
			P= 0.3
Outdoor hobbies			
Yes	297 (66)	25 (76)	ref
No	155 (34)	8 (24)	0.8 (0.3-2.0)
			P= 0.6
UV EXPOSURE- AFTER TRANSPLANTATION			
Sunburn			
Never	331 (83)	22 (81)	ref
Ever	70 (17)	5 (19)	0.8 (0.3-2.4)
			P= 0.7
Sunbathing			
Never	351 (82)	27 (82)	ref
Ever	79 (18)	6 (18)	1.7 (0.6-4.5)
			P= 0.2
Sunbed			
Never	420 (99)	32 (100)	-
Ever	3 (1)	0 (0)	-
Sunny Holidays			
Never	203 (48)	4 (13)	ref
Ever	222 (52)	28 (88)	5.7 (1.9-17.4)
			P< 0.001
Outdoor hobbies			
Yes	235 (55)	21 (64)	ref
No	194 (45)	12 (36)	0.9 (0.4-2.0)
			P= 0.8



N: number; SCC: squamous cell carcinoma;OR; odds ratio; CI: confidence interval; Ref: reference group; UV: Ultraviolet
Some figures do not add up to total due to missing data.
* Adjusted for age at recruitment, sex, time since transplantation, skin type and stratified by centres. All P-values are for trend
** Restricted to women (number of controls and cases: 171 and 8 respectively)

Figure 7.5: Potential risk factors from the questionnaire and their association with BCC only (continued)

risk factors	Controls n (%) N=454	SCC n (%) N=140	OR* (95%CI)
Sex			
female	171 (38)	44 (31)	ref
male	283 (62)	96 (69)	1.6 (1.0-2.7)
P-value het.			***0.06***
Age at recruitment			
<45	192 (42)	12 (9)	ref
45-59	175 (39)	56 (40)	5.0 (2.4-10.3)
60 or more	87 (19)	72 (51)	15.9 (7.3-34.4)
P-value trend			***P<0.001***
Time since transplantation			
<5	186 (41)	9 (6)	ref
5 to 9	107 (24)	24 (17)	5.5 (2.3-13.2)
10 or more	161 (35)	107 (76)	17.6 (8.0-38.8)
P-value trend			***P<0.001***
Skin type			
III or IV	137 (31)	79 (57)	ref
I or II	312 (69)	60 (43)	2.7 (1.7-4.5)
P-value het.			***P<0.001***
Sunburn before 18			
never	93 (21)	54(39)	ref
1 to 2	177 (39)	39 (28)	1.8 (1.0-3.4)
3 or more	183 (40)	45 (33)	3.2 (1.7-6.1)
P-value trend			***P<0.001***
Birth order			
1st	179 (40)	66 (47)	ref
2nd	142 (31)	40 (29)	0.7 (0.4-1.3)
3rd or more	132 (29)	33 (24)	0.5 (0.3-0.9)
P-value trend			***0.03***
Partner			
no	144 (32)	24 (17)	ref
yes	310 (68)	116 (83)	1.9 (1.1-3.4)
P-value het.			***0.03***

* P-values were calculated using logistic regression and adjusted for all factors in the table

N: total number; n: number; Het.: Heterogeneity test; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: confidence interval; OR: Odds ratio

Table 7.7: Fully adjusted analyses for risk factors associated with SCC among Caucasian transplant patients

Table 7.8: Potential risk factors from the questionnaire and their association with SCC and BCC only among Caucasian transplant patients, by centre

risk factors	Controls OXFORD n (%) N=183	SCC n (%) N=51	SCC OR* (95%CI)	BCC only n (%) N=9	Controls LONDON n (%) N=271	SCC n (%) N=89	SCC OR* (95%CI)	BCC only n (%) N=24
Sex								
female	72 (39)	16 (31)	ref	15 (63)	99 (37)	28 (31)	ref	6 (24)
male	111 (61)	35 (69)	1.4 (0.6 to 3.2)	9 (38)	172 (63)	61 (69)	1.8 (1.0 to 3.3)	18 (75)
P-value het.			***0.4***				***0.06***	
Age at recruitment								
<45	78 (43)	3 (6)	ref	2 (8)	114 (42)	9 (10)	ref	5 (21)
45-59	68 (37)	19 (37)	5.8 (1.6-21.7)	10 (42)	107 (37)	37 (42)	4.4 (1.98-10.1)	11 (46)
60 or more	37 (20)	29 (57)	22.2 (5.9-83.7)	12 (50)	50 (18)	43 (48)	9.6 (4.1-22.8)	8 (33)
P-value trend			***<0.001***				***<0.001***	
Time since transplantation								
<5	70 (38)	4 (8)	ref	3 (13)	116 (43)	5 (6)	ref	3 (13)
5 to 9	49 (27)	5 (10)	2.3 (0.5-10.1)	3 (13)	58 (21)	19 (21)	7.4 (2.5-21.8)	6 (25)
10 or more	64 (35)	42 (82)	15.0 (4.6-49.1)	18 (75)	97 (36)	65 (73)	13.1 (4.9-35.1)	15 (63)
P-value trend			***<0.001***				***<0.001***	
Skin type								
I to II	136 (75)	30 (59)	ref	14 (58)	92 (34)	58 (66)	ref	12 (50)
III to IV	45 (25)	21 (41)	2.3 (1.0 to 5.2)	10 (42)	176 (66)	30 (34)	3.4 (1.9 to 6.1)	12 (50)
P-value het.			***0.05***				***<0.001***	

* P-values were calculated using logistic regression and adjusted for sex, time since transplantation, age at recruitment and skin type.

N: total number; n: number; Het.: Heterogeneity test; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: confidence interval; OR: Odds ratio

Fitzpatrick classification scale as follows (I) never tans, always burns, (II) rarely tans, usually burns, (III) usually tans, can burn and (IV) always tans, rarely burns

risk factors	Controls OXFORD number (%) N=183	SCC number (%) N=51	SCC OR* (95%CI)	BCC only number (%) N=9	Controls LONDON number (%) N=271	SCC number (%) N=89	SCC OR* (95%CI)	BCC only number (%) N=24
TRANSPLANT INFORMATION								
Organ type								
kidney	171 (93)	51 (100)		9 (100)	263 (97)	83 (93)	ref	23 (96)
other	12 (7)	0 (0)		0 (0)	8 (3)	6 (7)	1.0 (0.3-3.6)	1 (4)
P-value het.							***1.0***	
Cadaveric or living donor								
cadaveric	149 (83)	47 (92)	ref	9 (100)	220 (82)	83 (93)	ref	20 (83)
living	31 (17)	4 (8)	1.0 (0.3-3.9)	0 (0)	49 (18)	6 (7)	0.7 (0.2-1.9)	4 (17)
P-value het.			***1.0***				***0.4***	
Number of transplantations								
1	146 (80)	36 (71)	ref	7 (78)	236 (87)	78 (88)	ref	21 (88)
2 or more	37 (20)	15 (29)	0.9 (0.4-2.3)	2 (22)	35 (13)	11 (12)	0.8 (0.3-1.8)	3 (13)
P-value het.			***0.9***				***0.6***	
Primary diagnosis								
unknown	36 (20)	6 (12)	ref	1 (11)	34 (13)	9 (10)	ref	3 (13)
Glomerulonephritis	35 (19)	15 (29)	2.9 (0.8-10.9)	2 (22)	82 (30)	28 (31)	0.7 (0.2-2.1)	8 (33)
Pyelonephritis	23 (13)	9 (18)	3.5 (0.8-15.3)	2 (22)	41 (15)	12 (13)	0.9 (0.3-3.1)	1 (4)
Cystic	33 (18)	9 (18)	1.3 (0.3-5.0)	4 (44)	48 (18)	16 (18)	1.0 (0.3-3.1)	8 (33)
Other	56 (31)	12 (24)	2.4 (0.6-9.1)	0 (0)	66 (24)	24 (27)	1.1 (0.4-3.3)	4 (17)
P-value het.			***0.3***				***0.9***	
WARTS AND CANCER HISTORY								
Previous cancer								
no	166 (92)	47 (94)		7 (78)	250 (94)	82 (93)	ref	24 (100)
yes	15 (8)	3 (6)		2 (22)	15 (6)	6 (7)	1.1 (0.3-3.6)	0 (0)
P-value het.							***0.9***	
Warts								
no	74 (40)	7 (14)	ref	3 (33)	76 (28)	1 (1)		2 (8)
yes	109 (60)	44 (86)	2.8 (1.0-7.8)	6 (67)	193 (72)	85 (99)		22 (92)
P-value het.			***0.03***					
Genital warts								
no	163 (89)	48 (94)		9 (100)	249 (95)	77 (92)	ref	21 (95)
yes	20 (11)	3 (6)		0 (0)	12 (5)	7 (8)	3.1 (0.9-10.0)	1 (5)
P-value het.							***0.07***	
Shingles								
no	119 (65)	29 (57)	ref	5 (56)	195 (75)	53 (61)	ref	18 (75)
yes	63 (35)	22 (43)	1.1 (0.5-2.5)	4 (44)	65 (25)	34 (39)	1.3 (0.7-2.5)	6 (25)
P-value het.			***0.8***				***0.4***	
Psoriasis								
no	171 (93)	49 (96)		9 (100)	253 (95)	83 (94)	ref	23 (100)
yes	12 (7)	2 (4)		0 (0)	14 (5)	5 (6)	1.2 (0.3-4.1)	0 (0)
P-value het.							***0.8***	
Family history of other cancers								
no	79 (46)	23 (49)	ref	6 (67)	123 (48)	50 (58)	ref	12 (50)
yes	91 (54)	24 (51)	0.9 (0.4-2.1)	3 (33)	135 (52)	36 (42)	0.6 (0.3-1.2)	12 (50)
P-value het.			***0.8***				***0.2***	
WOMEN INFORMATION								
Abnormal smear								
no	50 (70)	15 (94)		2 (100)	74 (81)	24 (89)		5 (83)
yes	21 (30)	1 (6)		0 (0)	17 (19)	3 (11)		1 (17)
P-value het.								
Still menstruating								
no	38 (21)	13 (25)		2 (22)	52 (20)	21 (24)	ref	2 (8)
yes	34 (19)	3 (6)		0 (0)	34 (13)	6 (7)	1.0 (0.2-4.3)	4 (17)
P-value het.							***1.0***	
Hysterectomy								
no	60 (33)	15 (29)		2 (22)	69 (27)	21 (24)	ref	6 (25)
yes	11 (6)	1 (2)		0 (0)	12 (5)	5 (6)	1.0 (0.3-3.8)	0 (0)
P-value het.							***1.0***	
Ever used oral contraceptive								
no	24 (13)	10 (20)	ref	0 (0)	33 (13)	15 (17)	ref	2 (8)
yes	48 (26)	6 (12)	1.3 (0.3-6.1)	2 (22)	52 (20)	12 (14)	0.6 (0.1-2.3)	4 (17)
P-value het.			***0.8***				***0.5***	
Ever used hormone replacement therapy								
no	53 (29)	12 (24)	ref	1 (11)	65 (25)	17 (20)	ref	5 (21)
yes	18 (10)	4 (8)	0.4 (0.1-2.5)	1 (11)	20 (8)	9 (10)	0.6 (0.2-1.9)	1 (4)
P-value het.			***0.3***				***0.4***	

* P-values were calculated using logistic regression and adjusted for sex, time since transplantation, age at recruitment and skin type.

N: total number; Het.: Heterogeneity test; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: confidence interval; OR: Odds ratio

When seropostivity if less than 5, no estimate was derived.

Table 7.9: Potential risk factors from the questionnaire and their association with SCC and BCC only among Caucasian transplant patients, by centre (continued 1)

risk factors	Controls OXFORD number (%) N=183	SCC number (%) N=51	OR* (95%CI)	BCC only number (%) N=9	Controls LONDON number (%) N=271	SCC number (%) N=89	OR* (95%CI)	BCC only number (%) N=24
Eye color								
brown/hazel	59 (33)	19 (38)	ref	6 (67)	102 (42)	13 (18)	ref	6 (29)
green	28 (16)	5 (10)	0.6 (0.2-2.3)	1 (11)	32 (13)	10 (14)	3.7 (1.2-11.3)	2 (10)
blue	93 (52)	26 (52)	0.6 (0.2-1.4)	2 (22)	111 (45)	51 (69)	3.1 (1.4-6.9)	13 (62)
P-value het.			***0.5***				***0.01***	
Hair color								
black/dark brown	90 (49)	22 (43)	ref	5 (56)	147 (54)	37 (42)	ref	12 (50)
light brown	62 (34)	19 (37)	0.9 (0.4-2.1)	1 (11)	87 (32)	27 (31)	0.9 (0.5-1.8)	11 (46)
red or blonde	30 (16)	10 (20)	1.4 (0.4-4.6)	3 (33)	36 (13)	24 (27)	1.8 (0.8-4.2)	1 (4)
P-value het.			***0.8***				***0.2***	
Birth country								
Other	4 (2)	3 (6)		0 (0)	9 (3)	3 (3)		1 (4)
UK/Europe/Ireland	179 (98)	48 (94)		9 (100)	262 (97)	86 (97)		23 (96)
P-value het.								
Body mass index								
<22.5	76 (43)	27 (55)	ref	4 (44)	121 (45)	45 (54)	ref	13 (54)
22.5-	65 (37)	18 (37)	1.0 (0.4-2.4)	5 (56)	99 (37)	24 (29)	0.6 (0.3-1.2)	6 (25)
25.0-	36 (20)	4 (8)	0.5 (0.1-1.7)	0 (0)	51 (19)	15 (18)	0.9 (0.4-1.9)	5 (21)
P-value trend			***0.3***				***0.5***	
Job								
any	146 (80)	25 (49)	ref	5 (56)	180 (66)	51 (57)	ref	18 (75)
unemployed	11 (6)	2 (4)	1.0 (0.2-5.5)	1 (11)	54 (20)	6 (7)	0.5 (0.2-1.5)	1 (4)
retired	26 (14)	24 (47)	2.1 (0.7-6.1)	3 (33)	37 (14)	32 (36)	1.3 (0.6-2.8)	5 (21)
P-value het.			***0.4***				***0.3***	
Qualification								
O level	28 (16)	3 (6)	ref	3 (33)	86 (32)	33 (37)	ref	7 (29)
A level/Coll./uni	74 (42)	11 (23)	1.2 (0.3-5.6)	2 (22)	60 (22)	16 (18)	1.8 (0.8-4.4)	3 (13)
Clerical/commercial	23 (13)	16 (33)	2.8 (0.6-13.3)	2 (22)	45 (17)	10 (11)	1.5 (0.6-3.9)	6 (25)
Other	51 (29)	18 (38)	2.0 (0.5-8.9)	2 (22)	78 (29)	30 (34)	1.2 (0.6-2.3)	8 (33)
P-value het.			***0.4***				***0.6***	
Exercise								
never/rarely/<1	72 (40)	28 (56)	ref	2 (22)	88 (34)	32 (36)	ref	5 (22)
once or more	109 (60)	22 (44)	0.8 (0.3-1.7)	7 (78)	171 (66)	56 (64)	0.7 (0.4-1.4)	18 (78)
P-value het.			***0.5***				***0.4***	
ALCOHOL AND TOBACCO CONSUMPTION								
Tobacco use								
never	98 (54)	31 (61)	ref	3 (33)	128 (47)	47 (53)	ref	9 (38)
past	25 (14)	2 (4)	0.8 (0.1-4.5)	1 (11)	44 (16)	12 (13)	0.9 (0.4-2.3)	4 (17)
current	60 (33)	18 (35)	0.9 (0.4-2.1)	5 (56)	99 (37)	30 (34)	0.5 (0.2-0.9)	11 (46)
P-value het.			***1.0***				***0.08***	
Alcohol consumption								
<1day	72 (40)	14 (28)	ref	4 (44)	94 (36)	30 (34)	ref	9 (38)
1+	73 (40)	23 (46)	0.9 (0.4-2.3)	5 (56)	95 (37)	41 (47)	1.0 (0.5-2.1)	13 (54)
former	4 (2)	0 (0)	-	0 (0)	21 (8)	10 (11)	1.6 (0.5-4.7)	1 (4)
Never	33 (18)	13 (26)	2.1 (0.7-6.2)	0 (0)	48 (19)	6 (7)	0.4 (0.1-1.1)	1 (4)
P-value het.			***0.2***				***0.2***	
PROXIMITY								
Number in household								
1	34 (19)	6 (12)	ref	2 (22)	36 (14)	8 (9)	ref	4 (17)
2	71 (39)	29 (57)	2.6 (0.8-8.4)	4 (44)	105 (41)	52 (60)	2.3 (0.9-6.0)	7 (29)
3	34 (19)	12 (24)	3.9 (1.0-15.2)	2 (22)	53 (21)	17 (20)	2.4 (0.8-7.5)	7 (29)
4 or more	44 (24)	4 (8)	1.3 (0.3-6.3)	1 (11)	63 (25)	10 (11)	1.8 (0.5-5.9)	6 (25)
P-value trend			***0.5***				***0.5***	
Number of siblings								
0	15 (8)	7 (14)	ref	1 (11)	26 (10)	9 (10)	ref	1 (4)
1	60 (33)	21 (41)	1.5 (0.4-5.7)	3 (33)	78 (29)	25 (28)	1.4 (0.5-4.1)	4 (17)
2	49 (27)	12 (24)	1.0 (0.2-4.0)	4 (44)	64 (24)	20 (22)	1.2 (0.4-3.6)	9 (38)
3	24 (13)	6 (12)	0.8 (0.2-4.1)	1 (11)	40 (15)	5 (6)	0.3 (0.1-1.2)	6 (25)
4 or more	35 (19)	5 (10)	0.4 (0.1-1.9)	0 (0)	63 (23)	30 (34)	1.6 (0.5-4.7)	4 (17)
P-value trend			***0.06***				***0.9***	
Birth order								
1st	77 (42)	25 (50)	ref	6 (67)	102 (38)	41 (46)	ref	6 (25)
2nd	61 (33)	19 (38)	0.6 (0.3-1.5)	1 (11)	81 (30)	21 (24)	0.7 (0.3-1.4)	10 (42)
3rd or more	45 (25)	6 (12)	0.4 (0.1-1.2)	2 (22)	87 (32)	27 (30)	0.5 (0.3-1.1)	8 (33)
P-value trend			***0.08***				***0.07***	
Share a room as a child								
yes	119 (66)	22 (43)	ref	4 (44)	167 (62)	61 (69)	ref	19 (79)
no	62 (34)	29 (57)	2.4 (1.1-5.3)	5 (56)	101 (38)	28 (31)	0.7 (0.4-1.3)	5 (21)
P-value het.			***0.03***				***0.3***	
Share a bed as a child								
yes	35 (19)	10 (20)	ref	2 (22)	64 (24)	40 (45)	ref	9 (38)
no	148 (81)	40 (80)	2.6 (0.9-7.5)	7 (78)	205 (76)	49 (55)	0.6 (0.3-1.1)	15 (63)
P-value het.			***0.07***				***0.08***	
Number of children								
none	74 (40)	11 (22)	ref	2 (22)	99 (37)	15 (17)	ref	7 (29)
1 or 2	74 (40)	29 (57)	1.8 (0.7-4.5)	6 (67)	124 (46)	57 (64)	1.9 (0.9-4.2)	14 (58)
3+	35 (19)	11 (22)	0.9 (0.3-3.2)	1 (11)	48 (18)	17 (19)	1.3 (0.5-3.3)	3 (13)
P-value trend			***1.0***				***0.6***	
Partner								
no	60 (33)	8 (16)	ref	2 (22)	84 (31)	16 (18)	ref	5 (21)
yes	123 (67)	43 (84)	2.8 (1.1-7.6)	7 (78)	187 (69)	73 (82)	1.9 (0.9-3.8)	19 (79)
P-value het.			***0.03***				***0.07***	

* P-values were calculated using logistic regression and adjusted for sex, time since transplantation, age at recruitment and skin type.

N: total number; Het.: Heterogeneity test; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: confidence interval; OR: Odds ratio

When seropostivity if less than 5, no estimate was derived.

Table 7.10: Potential risk factors from the questionnaire and their association with SCC and BCC only among Caucasian transplant patients, by centre (continued 2)

risk factors	Controls OXFORD number (%) N=183	SCC number (%) N=51	OR* (95%CI)	BCC only number (%) N=9	Controls LONDON number (%) N=271	SCC number (%) N=89	OR* (95%CI)	BCC only number (%) N=24
ULTRAVIOLET RADIATION EXPOSURE								
Sunburn before 18								
never	41 (23)	11 (22)	ref	2 (22)	175 (65)	43 (48)	ref	16 (67)
1 to 2	77 (42)	19 (39)	1.5 (0.5-4.4)	3 (33)	55 (20)	20 (22)	2.0 (0.9-4.5)	4 (17)
3 or more	64 (35)	19 (39)	3.1 (1.0-9.8)	4 (44)	39 (14)	26 (29)	3.5 (1.6-7.6)	4 (17)
P-value trend			***0.04***				***<0.001***	
Ever used sunbed								
never	149 (81)	45 (88)	ref	8 (89)	219 (81)	75 (84)	ref	18 (75)
ever	34 (19)	6 (12)	1.2 (0.4-4.0)	1 (11)	51 (19)	14 (16)	1.8 (0.8-4.1)	6 (25)
P-value het.			***0.7***				***0.2***	
Sunbed use and sun bath								
none	46 (25)	17 (33)	ref	0 (0)	106 (40)	35 (40)	ref	9 (38)
either	105 (57)	28 (55)	0.9 (0.3-2.2)	8 (89)	128 (48)	43 (49)	0.8 (0.4-1.5)	12 (50)
both	32 (17)	6 (12)	1.2 (0.3-4.5)	1 (11)	32 (12)	10 (11)	2.0 (0.7-5.8)	3 (13)
P-value trend			***0.9***				***0.6***	
Ever had outdoor hobbies								
no	45 (25)	7 (14)	ref	1 (11)	72 (28)	20 (23)	ref	5 (21)
yes	137 (75)	43 (86)	2.1 (0.7-5.9)	8 (89)	188 (72)	67 (77)	1.0 (0.5-2.0)	19 (79)
P-value het.			***0.2***				***0.9***	
Ever had outdoor occupation								
no	126 (69)	28 (55)	ref	5 (56)	174 (64)	54 (61)	ref	14 (58)
yes	57 (31)	23 (45)	1.4 (0.6-3.3)	4 (44)	96 (36)	34 (39)	1.0 (0.5-1.9)	10 (42)
P-value het.			***0.4***				***0.9***	
Ever lived abroad								
yes	45 (25)	10 (20)	ref	2 (22)	41 (15)	14 (16)	ref	9 (38)
no	138 (75)	41 (80)	1.5 (0.6-3.7)	7 (78)	230 (85)	75 (84)	1.0 (0.4-2.3)	15 (63)
P-value het.			***0.4***				***1.0***	
BEFORE TRANSPLANTATION								
Sunburn								
never	25 (14)	5 (10)	ref	1 (11)	68 (25)	16 (18)	ref	3 (13)
1 to 2	63 (34)	17 (35)	1.6 (0.4-6.1)	3 (33)	114 (42)	35 (39)	0.8 (0.3-1.7)	9 (38)
3 or more	95 (52)	27 (55)	2.0 (0.5-7.3)	5 (56)	88 (33)	38 (43)	1.0 (0.5-2.3)	12 (50)
P-value trend			***0.3***				***0.8***	
Sunbathing								
never	52 (29)	17 (35)	ref	0 (0)	124 (47)	38 (44)	ref	13 (54)
1 to 2	28 (15)	7 (14)	0.6 (0.2-2.3)	2 (22)	50 (19)	12 (14)	0.7 (0.3-1.7)	0 (0)
3 or more	101 (56)	25 (51)	1.3 (0.5-3.5)	7 (78)	88 (34)	37 (43)	1.1 (0.6-2.1)	11 (46)
P-value trend			***0.6***				0.5 (0.2-0.9)	
Sunbed								
never	150 (82)	44 (88)	ref	8 (89)	218 (81)	75 (84)	ref	18 (75)
ever	33 (18)	6 (12)	1.4 (0.4-4.8)	1 (11)	50 (19)	14 (16)	1.8 (0.8-4.1)	6 (25)
P-value het.			***0.6***				***0.2***	
Sunny holidays								
never	44 (24)	17 (35)	ref	3 (33)	52 (20)	20 (22)	ref	2 (8)
1 to 2	29 (16)	9 (18)	1.1 (0.4-3.7)	1 (11)	58 (22)	22 (25)	1.0 (0.4-2.3)	2 (8)
3 to 5	39 (21)	7 (14)	0.7 (0.2-2.4)	0 (0)	41 (15)	14 (16)	1.0 (0.4-2.6)	5 (21)
6 or more	71 (39)	16 (33)	1.4 (0.5-4.1)	5 (56)	114 (43)	33 (37)	1.0 (0.5-2.2)	15 (63)
P-value trend			***0.7***				***1.0***	
Outdoor hobbies								
yes	121 (66)	35 (70)	ref	8 (89)	176 (65)	62 (70)	ref	17 (71)
no	61 (34)	15 (30)	1.1 (0.4-2.5)	1 (11)	94 (35)	27 (30)	1.1 (0.6-2.2)	7 (29)
P-value het.			***0.9***				***0.7***	
AFTER TRANSPLANTATION								
Sunburn								
never	145 (79)	44 (88)	ref	7 (78)	186 (85)	63 (84)	ref	15 (83)
ever	38 (21)	6 (12)	0.9 (0.3-2.7)	2 (22)	32 (15)	12 (16)	0.5 (0.2-1.2)	3 (17)
P-value het.			***0.8***				***0.1***	
Sunbathing								
never	138 (75)	43 (84)	ref	7 (78)	213 (86)	78 (91)	ref	20 (83)
1 to 2	16 (9)	3 (6)	1.4 (0.3-7.2)	0 (0)	17 (7)	1 (1)	0.2 (0.0-1.4)	2 (8)
3 or more	29 (16)	5 (10)	0.7 (0.2-2.5)	2 (22)	17 (7)	7 (8)	1.8 (0.6-5.5)	2 (8)
P-value trend			***0.7***				***0.8***	
Sunbed								
never	180 (99)	50 (98)		9 (100)	240 (100)	84 (99)		23 (100)
ever	2 (1)	1 (2)		0 (0)	1 (0)	1 (1)		0 (0)
P-value het.								
Sunny holidays								
never	82 (45)	17 (33)	ref	3 (33)	121 (50)	18 (21)	ref	1 (4)
1 to 2	38 (21)	12 (24)	1.9 (0.7-5.5)	2 (22)	37 (15)	17 (20)	3.1 (1.2-7.8)	4 (17)
3 to 5	29 (16)	9 (18)	1.5 (0.5-4.7)	1 (11)	33 (14)	13 (15)	1.9 (0.7-5.2)	5 (22)
6 or more	34 (19)	13 (25)	1.4 (0.5-4.1)	3 (33)	51 (21)	36 (43)	2.4 (1.1-5.3)	13 (57)
P-value trend			***0.5***				***0.06***	
Outdoor hobbies								
ever	104 (57)	35 (70)	ref	6 (67)	131 (53)	47 (55)	ref	15 (63)
never	78 (43)	15 (30)	0.6 (0.3-1.5)	3 (33)	116 (47)	39 (45)	1.2 (0.6-2.1)	9 (38)
P-value het.			***0.3***				***0.6***	

* P-values were calculated using logistic regression and adjusted for sex, time since transplantation, age at recruitment and skin type.

N: total number; Het.: Heterogeneity test; SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: confidence interval; OR: Odds ratio

When seropostivity if less than 5, no estimate was derived.

Table 7.11: Potential risk factors from the questionnaire and their association with SCC and BCC only among Caucasian transplant patients, by centre (continued 3)

Table 7.12: History of abnormal cervical smear and genital warts in relation to HPV16 and HPV6 (respectively)

		Self-reported abnormal smear test [2,3]			
Risk factor	**centre (No. never/ever)**	**Never No. POS (%)**	**Ever No. POS (%)**	**adjusted OR [1] (95%CI)**	**P-value**
HPV16	Both (215/56)	32 (15)	28 (50)	5.1 (2.6-10.2)	<0.001
	Oxford (83/27)	9 (11)	13 (48)	8.6 (2.5-29.4)	<0.001
	London (132/29)	23 (17)	15 (52)	4.3 (1.8-10.5)	0.001

		Self-reported history of genital warts [3]			
Risk factor	**centre (No. never/ever)**	**Never No. POS (%)**	**Ever No. POS (%)**	**adjusted OR [1] (95%CI)**	**P-value**
HPV6	Both (688/53)	172 (25)	31 (58)	4.0 (2.2-7.2)	<0.001
	Oxford (270/26)	51 (19)	15 (58)	4.6 (1.9-11.2)	<0.001
	London (418/27)	121 (29)	16 (59)	3.4 (1.5-7.6)	0.003

HPV: human papillomavirus

OR: Odds ratio; CI: Confidence interval; No. POS: Number of seropositive samples; No: number

[1] Analyses are stratified by centres (where appropriate) and adjusted for age at recruitment, time since transplantation, and sex .

[2] Restricted to women only. [3] No restriction for ethnicity.

Table 7.13: Odds ratio of squamous cell carcinoma and basal cell carcinoma in patients who are HPV seropositive to one type compared to those who are seronegative to the same type, among Caucasian transplant patients in both centres

genus	species	type	controls N=425	SQUAMOUS CELL CARCINOMA all N=139			prevalent N=119			incident N=20	BASAL CELL CARCINOMA (only) all N=31			prevalent N=26	incident N=5
			No. (%)	No. (%)	OR[1] (95% CI)	P-value*	No. (%)	OR[1] (95% CI)	P-value*	No. (%)	No. (%)	OR[1] (95% CI)	P-value*	No. (%)	No.
alpha	2	3	36 (8)	13 (9)	1.5 (0.7 to 3.3)	*0.3*	10 (8)	1.2 (0.5 to 2.9)	*0.7*	3 (15)	2 (6)	-	*0.5*	2 (8)	0
	4	2	61 (14)	22 (16)	1.4 (0.7 to 2.6)	*0.3*	18 (15)	1.3 (0.7 to 2.7)	*0.4*	4 (20)	6 (19)	1.5 (0.5 to 4.0)	*0.5*	4 (15)	2
		27	73 (17)	23 (17)	1.2 (0.6 to 2.2)	*0.6*	19 (16)	1.2 (0.6 to 2.4)	*0.6*	4 (20)	5 (16)	1.0 (0.4 to 2.9)	*1.0*	4 (15)	1
	8	7	36 (8)	7 (5)	0.6 (0.2 to 1.5)	*0.3*	4 (3)	-	*0.07*	3 (15)	4 (13)	1.6 (0.5 to 5.3)	*0.4*	4 (15)	0
	9	16	67 (16)	24 (17)	1.6 (0.9 to 3.1)	*0.1*	22 (18)	1.8 (0.9 to 3.6)	*0.09*	2 (10)	2 (6)	-	*0.2*	1 (4)	1
	10	6	128 (30)	45 (32)	1.7 (1.0 to 2.8)	*0.05*	38 (32)	2.0 (1.1 to 3.5)	*0.02*	7 (35)	9 (29)	1.1 (0.5 to 2.7)	*0.8*	8 (31)	1
		13	42 (10)	16 (12)	1.7 (0.8 to 3.7)	*0.2*	14 (12)	2.2 (1.0 to 5.1)	*0.06*	2 (10)	2 (6)	-	*0.8*	2 (8)	0
	1	5	39 (9)	22 (16)	2.0 (1.0 to 4.0)	*0.05*	18 (15)	1.9 (0.9 to 4.0)	*0.09*	4 (20)	5 (16)	1.7 (0.6 to 4.9)	*0.4*	5 (19)	0
		8	91 (21)	34 (24)	1.4 (0.8 to 2.4)	*0.3*	27 (23)	1.2 (0.6 to 2.1)	*0.6*	7 (35)	10 (32)	1.7 (0.7 to 3.9)	*0.2*	7 (27)	3
		20	61 (14)	19 (14)	1.0 (0.5 to 1.8)	*0.9*	15 (13)	0.7 (0.4 to 1.5)	*0.4*	4 (20)	5 (16)	0.9 (0.3 to 2.4)	*0.8*	3 (12)	2
		24	47 (11)	17 (12)	1.6 (0.8 to 3.1)	*0.2*	12 (10)	1.1 (0.5 to 2.5)	*0.8*	5 (25)	4 (13)	-	*0.8*	2 (8)	2
		36	50 (12)	10 (7)	0.5 (0.2 to 1.2)	*0.1*	8 (7)	0.4 (0.2 to 1.1)	*0.06*	2 (10)	6 (19)	1.5 (0.5 to 4.1)	*0.5*	4 (15)	2
		93	12 (3)	8 (6)	1.9 (0.6 to 5.5)	*0.3*	6 (5)	2.0 (0.6 to 6.6)	*0.3*	2 (10)	1 (3)	-	*0.6*	1 (4)	0
beta		9	65 (15)	23 (17)	1.1 (0.6 to 2.1)	*0.7*	19 (16)	1.0 (0.5 to 2.0)	*1.0*	4 (20)	5 (16)	1.0 (0.3 to 2.8)	*1.0*	4 (15)	1
		15	116 (27)	38 (27)	1.0 (0.6 to 1.7)	*1.0*	34 (29)	1.0 (0.6 to 1.7)	*0.9*	4 (20)	10 (32)	1.3 (0.6 to 2.9)	*0.6*	8 (31)	2
	2	17	100 (24)	43 (31)	1.6 (1.0 to 2.7)	*0.07*	37 (31)	1.7 (1.0 to 2.9)	*0.07*	6 (30)	6 (19)	0.8 (0.3 to 2.1)	*0.6*	4 (15)	2
		23	41 (10)	13 (9)	1.1 (0.5 to 2.3)	*0.8*	9 (8)	0.9 (0.4 to 2.1)	*0.7*	4 (20)	3 (10)	-	*1.0*	3 (12)	0
		38	95 (22)	32 (23)	0.9 (0.6 to 1.6)	*0.9*	28 (24)	0.9 (0.5 to 1.6)	*0.7*	4 (20)	10 (32)	1.5 (0.6 to 3.4)	*0.4*	8 (31)	2
		49	84 (20)	37 (27)	1.5 (0.9 to 2.6)	*0.1*	32 (27)	1.4 (0.8 to 2.5)	*0.3*	5 (25)	6 (19)	0.9 (0.3 to 2.4)	*0.9*	4 (15)	2
	3	75	50 (12)	22 (16)	1.4 (0.7 to 2.7)	*0.3*	17 (14)	1.1 (0.5 to 2.2)	*0.8*	5 (25)	6 (19)	1.4 (0.5 to 3.9)	*0.5*	4 (15)	2
		76	42 (10)	18 (13)	1.2 (0.6 to 2.4)	*0.6*	16 (13)	1.1 (0.5 to 2.4)	*0.7*	2 (10)	4 (13)	-	*0.5*	2 (8)	2
	4	92	52 (12)	20 (14)	0.9 (0.5 to 1.8)	*0.8*	14 (12)	0.7 (0.3 to 1.4)	*0.3*	6 (30)	6 (19)	1.1 (0.4 to 3.1)	*0.8*	4 (15)	2
	5	96	62 (15)	21 (15)	1.1 (0.6 to 2.1)	*0.7*	16 (13)	0.9 (0.4 to 1.7)	*0.7*	5 (25)	4 (13)	-	*1.0*	2 (8)	2
gamma		4	108 (25)	47 (34)	1.5 (0.9 to 2.5)	*0.1*	38 (32)	1.3 (0.8 to 2.2)	*0.4*	9 (45)	14 (45)	2.4 (1.1 to 5.3)	*0.04*	12 (46)	2
	1	65	119 (28)	38 (27)	1.2 (0.7 to 2.0)	*0.6*	29 (24)	1.0 (0.6 to 1.7)	*1.0*	9 (45)	12 (39)	1.7 (0.8 to 3.8)	*0.2*	10 (38)	2
		95	91 (21)	28 (20)	0.9 (0.5 to 1.5)	*0.6*	23 (19)	0.7 (0.4 to 1.4)	*0.3*	5 (25)	7 (23)	0.9 (0.4 to 2.3)	*0.9*	5 (19)	2
	2	48	66 (16)	25 (18)	1.3 (0.7 to 2.5)	*0.4*	20 (17)	1.3 (0.7 to 2.6)	*0.4*	5 (25)	5 (16)	1.0 (0.4 to 3.0)	*0.9*	3 (12)	2
	3	50	32 (8)	16 (12)	2.0 (0.9 to 4.4)	*0.09*	14 (12)	2.1 (0.9 to 4.8)	*0.1*	2 (10)	1 (3)	-	*0.7*	1 (4)	0
	4	60	19 (4)	12 (9)	1.9 (0.8 to 4.8)	*0.2*	7 (6)	1.2 (0.4 to 3.6)	*0.7*	5 (25)	2 (6)	-	*0.6*	2 (8)	0
mu	1	41	46 (11)	19 (14)	1.5 (0.7 to 2.9)	*0.3*	16 (13)	1.5 (0.7 to 3.2)	*0.3*	3 (15)	7 (23)	2.3 (0.9 to 5.8)	*0.1*	6 (23)	1
nu	1	1	124 (29)	39 (28)	0.9 (0.5 to 1.5)	*0.6*	34 (29)	0.9 (0.5 to 1.6)	*0.8*	5 (25)	12 (39)	1.4 (0.6 to 3.1)	*0.4*	10 (38)	2
	2	63	98 (23)	31 (22)	1.0 (0.6 to 1.7)	*1.0*	26 (22)	1.0 (0.5 to 1.8)	*0.9*	5 (25)	13 (42)	2.3 (1.0 to 5.1)	*0.05*	10 (38)	3
ND		101	30 (7)	19 (14)	1.7 (0.8 to 3.7)	*0.2*	16 (13)	1.8 (0.8 to 3.9)	*0.2*	3 (15)	6 (19)	2.2 (0.8 to 6.3)	*0.2*	3 (12)	3
		103	26 (6)	8 (6)	0.8 (0.3 to 2.1)	*0.7*	5 (4)	0.6 (0.2 to 1.7)	*0.3*	3 (15)	2 (6)	-	*0.6*	2 (8)	0

OR: odds ratio N: total number; No.: number; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; ND: not defined; Incident SCC are from London only.

[1] P-values and odds ratios were calculated using conditional (on centre) logistic regression and adjusted for sex, age at recruitment, time since transplantation and skin type.

*. When the number of seropositive patient was less than 5, P-value was derived using Fisher's exact test

Table 7.14: Odds ratio of squamous cell carcinoma and basal cell carcinoma in patients who are HPV seropositive to one type compared to those who are seronegative to the same type, among Caucasian transplant patients in London

genus	species	type	controls N=243	SQUAMOUS CELL CARCINOMA all N=89			prevalent N=69			incident N=20	BASAL CELL CARCINOMA (only) all N=22	prevalent N=18	incident N=4
			No. (%)	No. (%)	OR (95% CI)	P-value	No. (%)	OR (95% CI)	P-value*	No. (%)	No. (%)	No. (%)	No.
alpha	2	3	21 (9)	12 (13)	2.8 (1.1 to 7.5)	***0.04***	9 (13)	2.4 (0.8 to 7.1)	***0.1***	3 (15)	1 (5)	1 (6)	0
	4	2	41 (17)	18 (20)	1.7 (0.8 to 3.6)	***0.2***	14 (20)	1.7 (0.7 to 4.0)	***0.2***	4 (20)	5 (23)	3 (17)	2
		27	51 (21)	18 (20)	1.3 (0.6 to 2.7)	***0.5***	14 (20)	1.5 (0.6 to 3.4)	***0.4***	4 (20)	4 (18)	3 (17)	1
	8	7	23 (9)	5 (6)	0.5 (0.2 to 1.6)	***0.2***	2 (3)	-	***0.08***	3 (15)	3 (14)	3 (17)	0
	9	16	40 (16)	18 (20)	2.3 (1.0 to 5.1)	***0.04***	16 (23)	2.7 (1.1 to 6.7)	***0.02***	2 (10)	2 (9)	1 (6)	1
	10	6	81 (33)	35 (39)	1.8 (1.0 to 3.3)	***0.06***	28 (41)	2.4 (1.2 to 4.9)	***0.02***	7 (35)	8 (36)	7 (39)	1
		13	32 (13)	15 (17)	1.9 (0.9 to 4.5)	***0.1***	13 (19)	2.8 (1.1 to 7.1)	***0.03***	2 (10)	1 (5)	1 (6)	0
	1	5	27 (11)	15 (17)	1.6 (0.7 to 3.7)	***0.3***	11 (16)	1.4 (0.5 to 3.5)	***0.5***	4 (20)	2 (9)	2 (11)	0
		8	58 (24)	26 (29)	1.7 (0.8 to 3.2)	***0.14***	19 (28)	1.4 (0.6 to 2.9)	***0.4***	7 (35)	8 (36)	5 (28)	3
		20	39 (16)	11 (12)	0.9 (0.4 to 2.2)	***0.9***	7 (10)	0.6 (0.2 to 1.7)	***0.4***	4 (20)	4 (18)	2 (11)	2
		24	26 (11)	12 (13)	2.4 (1.0 to 5.9)	***0.05***	7 (10)	1.7 (0.6 to 4.9)	***0.4***	5 (25)	3 (14)	1 (6)	2
		36	36 (15)	6 (7)	0.4 (0.1 to 1.0)	***0.04***	4 (6)	-	***0.06***	2 (10)	4 (18)	2 (11)	2
		93	11 (5)	7 (8)	1.6 (0.5 to 5.1)	***0.4***	5 (7)	1.5 (0.4 to 5.6)	***0.5***	2 (10)	0 (0)	0 (0)	0
beta	2	9	45 (19)	13 (15)	0.9 (0.4 to 1.9)	***0.7***	9 (13)	0.6 (0.3 to 1.6)	***0.4***	4 (20)	3 (14)	2 (11)	1
		15	64 (26)	20 (22)	1.0 (0.5 to 2.1)	***0.9***	16 (23)	1.0 (0.5 to 2.2)	***0.9***	4 (20)	8 (36)	6 (33)	2
		17	61 (25)	29 (33)	1.8 (0.9 to 3.4)	***0.09***	23 (33)	1.9 (0.9 to 3.9)	***0.09***	6 (30)	5 (23)	3 (17)	2
		23	29 (12)	9 (10)	1.0 (0.4 to 2.6)	***0.9***	5 (7)	0.7 (0.2 to 2.1)	***0.5***	4 (20)	3 (14)	3 (17)	0
		38	56 (23)	20 (22)	1.1 (0.5 to 2.2)	***0.8***	16 (23)	1.1 (0.5 to 2.4)	***0.8***	4 (20)	8 (36)	6 (33)	2
		49	46 (19)	22 (25)	2.1 (1.0 to 4.2)	***0.05***	17 (25)	2.0 (0.9 to 4.5)	***0.09***	5 (25)	4 (18)	2 (11)	2
	3	75	33 (14)	15 (17)	1.7 (0.8 to 3.8)	***0.2***	10 (14)	1.3 (0.5 to 3.2)	***0.6***	5 (25)	5 (23)	3 (17)	2
		76	29 (12)	11 (12)	1.2 (0.5 to 3.0)	***0.7***	9 (13)	1.2 (0.5 to 3.2)	***0.7***	2 (10)	4 (18)	2 (11)	2
	4	92	37 (15)	16 (18)	1.0 (0.5 to 2.2)	***1.0***	10 (14)	0.6 (0.2 to 1.5)	***0.3***	6 (30)	5 (23)	3 (17)	2
	5	96	36 (15)	15 (17)	1.5 (0.7 to 3.4)	***0.3***	10 (14)	1.1 (0.4 to 2.7)	***0.8***	5 (25)	3 (14)	1 (6)	2
gamma	1	4	66 (27)	32 (36)	1.9 (1.0 to 3.6)	***0.05***	23 (33)	1.7 (0.8 to 3.5)	***0.2***	9 (45)	11 (50)	9 (50)	2
		65	74 (30)	24 (27)	1.1 (0.6 to 2.2)	***0.7***	15 (22)	0.8 (0.4 to 1.8)	***0.7***	9 (45)	10 (45)	8 (44)	2
		95	54 (22)	16 (18)	0.9 (0.4 to 1.9)	***0.8***	11 (16)	0.7 (0.3 to 1.7)	***0.4***	5 (25)	4 (18)	2 (11)	2
	2	48	39 (16)	21 (24)	2.1 (1.0 to 4.4)	***0.05***	16 (23)	2.3 (1.0 to 5.3)	***0.05***	5 (25)	5 (23)	3 (17)	2
	3	50	19 (8)	12 (13)	2.2 (0.8 to 5.8)	***0.1***	10 (14)	2.4 (0.8 to 7.0)	***0.1***	2 (10)	1 (5)	1 (6)	0
	4	60	12 (5)	12 (13)	3.6 (1.2 to 10.3)	***0.02***	7 (10)	2.1 (0.6 to 7.1)	***0.2***	5 (25)	2 (9)	2 (11)	0
nu	1	41	32 (13)	14 (16)	1.5 (0.7 to 3.4)	***0.3***	11 (16)	1.7 (0.7 to 4.4)	***0.3***	3 (15)	5 (23)	4 (22)	1
nu	1	1	81 (33)	25 (28)	0.8 (0.4 to 1.5)	***0.5***	20 (29)	0.9 (0.4 to 1.7)	***0.7***	5 (25)	8 (36)	6 (33)	2
nu	2	63	67 (28)	22 (25)	0.9 (0.5 to 1.7)	***0.7***	17 (25)	0.8 (0.4 to 1.7)	***0.6***	5 (25)	9 (41)	6 (33)	3
ND		101	25 (10)	14 (16)	1.6 (0.7 to 3.7)	***0.3***	11 (16)	1.6 (0.6 to 4.2)	***0.3***	3 (15)	5 (23)	2 (11)	3
ND		103	18 (7)	6 (7)	0.8 (0.2 to 2.4)	***0.6***	3 (4)	-	***0.6***	3 (15)	1 (5)	1 (6)	0

OR: odds ratio; N: total number; n: number; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; ND: not defined; Incident SCC are from London only.

¹ P-values and odds ratios were calculated using conditional (on centre) logistic regression and adjusted for sex, age at recruitment, time since transplantation and skin type.

* When the number of seropositive patient was less than 5, P-value was derived using Fisher's exact test

Table 7.15: Odds ratio of squamous cell carcinoma and basal cell carcinoma in patients who are HPV seropositive to one type compared to those who are seronegative to the same type, among Caucasian transplant patients in Oxford

genus	species	type	controls N=182	SQUAMOUS CELL CARCINOMA all N=50			prevalent N=50			incident N=0	BASAL CELL CARCINOMA (only) all N=9	prevalent N=8	incident N=1
			No. (%)	No. (%)	OR (95% CI)	P-value*	No. (%)	OR (95% CI)	P-value	No. (%)	No. (%)	No. (%)	No.
alpha	2	3	15 (8)	1 (2)	-	***0.2***					1	1	0
	4	2	20 (11)	4 (8)	-	***0.8***					1	1	0
		27	22 (12)	5 (10)	0.8 (0.2-3.1)	***0.8***					1	1	0
	8	7	13 (7)	2 (4)	-	***0.5***					1	1	0
	9	16	27 (15)	6 (12)	1.0 (0.3-3.5)	***1.0***					0	0	0
	10	6	47 (26)	10 (20)	1.5 (0.6-4.0)	***0.4***					1	1	0
		13	10 (5)	1 (2)	-	***0.5***					1	1	0
	1	5	12 (7)	7 (14)	4.8 (1.3-17.3)	***0.02***					3	3	0
		8	33 (18)	8 (16)	1.0 (0.4-2.9)	***1.0***					2	2	0
		20	22 (12)	8 (16)	0.9 (0.3-2.6)	***0.8***					1	1	0
		24	21 (12)	5 (10)	0.9 (0.3-3.0)	***0.8***					1	1	0
		36	14 (8)	4 (8)	-	***1.0***					2	2	0
		93	1 (1)	1 (2)	-	***0.4***					1	1	0
beta	2	9	20 (11)	10 (20)	2.3 (0.8-7.0)	***0.1***					2	2	0
		15	52 (29)	18 (36)	1.1 (0.5-2.5)	***0.8***					2	2	0
		17	39 (21)	14 (28)	1.6 (0.7-4.0)	***0.3***					1	1	0
		23	12 (7)	4 (8)	-	***0.8***					0	0	0
		38	39 (21)	12 (24)	0.8 (0.3-2.0)	***0.7***					2	2	0
		49	38 (21)	15 (30)	1.0 (0.4-2.4)	***1.0***					2	2	0
	3	75	17 (9)	7 (14)	1.3 (0.4-4.1)	***0.7***					1	1	0
		76	13 (7)	7 (14)	1.3 (0.4-4.2)	***0.7***					0	0	0
	4	92	15 (8)	4 (8)	-	***1.0***					1	1	0
	5	96	26 (14)	6 (12)	0.7 (0.2-2.1)	***0.5***					1	1	0
gamma	1	4	42 (23)	15 (30)	1.0 (0.4-2.3)	***0.9***					3	3	0
		65	45 (25)	14 (28)	1.3 (0.5-3.1)	***0.6***					2	2	0
		95	37 (20)	12 (24)	0.7 (0.3-1.9)	***0.5***					3	3	0
	2	48	27 (15)	4 (8)	-	***0.2***					0	0	0
	3	50	13 (7)	4 (8)	-	***0.8***					0	0	0
	4	60	7 (4)	0 (0)	-	***0.4***					0	0	0
mu	1	41	14 (8)	5 (10)	1.4 (0.4-5.2)	***0.6***					2	2	0
nu	1	1	43 (24)	14 (28)	0.9 (0.4-2.3)	***0.9***					4	4	0
	2	63	31 (17)	9 (18)	1.4 (0.5-3.8)	***0.5***					4	4	0
ND		101	5 (3)	5 (10)	2.8 (0.6-13.8)	***0.2***					1	1	0
		103	8 (4)	2 (4)	-	***1.0***					1	1	0

OR: odds ratio; N: total number; n: number; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; ND: not defined; Incident SCC are from London only.

¹ P-values and odds ratios were calculated using conditional (on centre) logistic regression and adjusted for sex, age at recruitment, time since transplantation and skin type.

*. When the number of seropositive patient was less than 5, P-value was derived using Fisher's exact test

	CONTROLS	SQUAMOUS CELL CARCINOMA					BASAL CELL CARCINOMA				
	N= 425	All N=139		Prevalent N=119		Incident N=20	All N=31		Prevalent N=26		Incident N=5
genus	No. (%)	No. (%)	OR[1] (95%CI)	No. (%)	OR[1] (95%CI)	No. (%)	No. (%)	OR[1] (95%CI)	No. (%)	OR[1] (95%CI)	No.
alpha-mucosal											
negative	250 (59)	77 (55)	ref	67 (56)	ref	10 (50)	20 (65)	ref	17 (65)	ref	3
1	127 (30)	46 (33)	1.5 (0.9-2.6)	37 (31)	1.7 (1.0-3.0)	9 (45)	10 (32)	1.0 (0.5-2.4)	8 (31)	1.0 (0.4-2.6)	2
2 or more	48 (11)	16 (12)	2.2 (1.0-4.9)	15 (13)	2.8 (1.2-6.6)	1 (5)	1 (3)	0.4 (0.0-3.1)	1 (4)	0.4 (0.1-3.5)	0
P-trend			*0.02*		*0.007*			*0.4*		*0.4*	
alpha-cutaneous											
negative	285 (67)	102 (73)	ref	90 (76)	ref	12 (60)	21 (68)	ref	18 (69)	ref	3
1	92 (22)	16 (12)	0.5 (0.3-1.1)	12 (10)	0.4 (0.2-0.9)	4 (20)	5 (16)	0.7 (0.3-2.0)	4 (15)	0.7 (0.2-2.1)	1
2 or more	48 (11)	21 (15)	1.6 (0.8-3.3)	17 (14)	1.6 (0.7-3.4)	4 (20)	5 (16)	1.6 (0.5-4.8)	4 (15)	1.5 (0.5-4.9)	1
P-trend			*0.6*		*0.6*			*0.7*		*0.8*	
beta											
negative	188 (44)	59 (42)	ref	51 (43)	ref	8 (40)	11 (35)	ref	10 (38)	ref	1
1	76 (18)	21 (15)	0.9 (0.5-1.7)	18 (15)	0.9 (0.4-1.9)	3 (15)	5 (16)	1.0 (0.3-3.0)	5 (19)	1.1 (0.3-3.4)	0
2 or more	161 (38)	59 (42)	1.3 (0.8-2.2)	50 (42)	1.2 (0.7-2.1)	9 (45)	15 (48)	1.6 (0.7-3.7)	11 (42)	1.3 (0.5-3.3)	4
P-trend			*0.3*		*0.5*			*0.3*		*0.6*	
beta1											
negative	292 (69)	91 (65)	ref	80 (67)	ref	11 (55)	18 (58)	ref	17 (65)	ref	1
1	58 (14)	20 (14)	1.2 (0.6-2.5)	18 (15)	1.2 (0.6-2.5)	2 (10)	6 (19)	1.9 (0.7-5.3)	5 (19)	1.7 (0.6-5.0)	1
2 or more	75 (18)	28 (20)	1.4 (0.8-2.6)	21 (18)	1.1 (0.6-2.1)	7 (35)	7 (23)	1.3 (0.5-3.4)	4 (15)	0.8 (0.3-2.6)	3
P-trend			*0.2*		*0.7*			*0.4*		*1.0*	
beta2											
negative	248 (58)	76 (55)	ref	64 (54)	ref	12 (60)	16 (52)	ref	14 (54)	ref	2
1	73 (17)	24 (17)	1.1 (0.6-2.0)	21 (18)	1.1 (0.6-2.3)	3 (15)	6 (19)	1.1 (0.4-3.2)	5 (19)	1.1 (0.4-3.3)	1
2 or more	104 (24)	39 (28)	1.3 (0.8-2.2)	34 (29)	1.3 (0.7-2.3)	5 (25)	9 (29)	1.4 (0.6-3.4)	7 (27)	1.3 (0.5-3.4)	2
P-trend			*0.4*		*0.4*			*0.5*		*0.7*	
beta3											
negative	328 (77)	99 (71)	ref	85 (71)	ref	14 (70)	24 (77)	ref	21 (81)	ref	3
1	48 (11)	15 (11)	1.0 (0.5-2.1)	14 (12)	1.1 (0.5-2.3)	1 (5)	2 (6)	0.6 (0.1-2.6)	2 (8)	0.7 (0.1-3.0)	0
2 or more	49 (12)	25 (18)	1.6 (0.9-3.1)	20 (17)	1.3 (0.6-2.6)	5 (25)	5 (16)	1.2 (0.4-3.4)	3 (12)	0.8 (0.2-3.0)	2
P-trend			*0.2*		*0.5*			*1.0*		*0.7*	
gamma											
negative	225 (53)	61 (44)	ref	57 (48)	ref	4 (20)	10 (32)	ref	8 (31)	ref	2
1	92 (22)	34 (24)	1.3 (0.7-2.3)	29 (24)	1.2 (0.6-2.2)	5 (25)	11 (35)	2.9 (1.1-7.4)	10 (38)	3.2 (1.2-8.8)	1
2 or more	108 (25)	44 (32)	1.7 (1.0-3.0)	33 (28)	1.3 (0.7-2.4)	11 (55)	10 (32)	2.1 (0.8-5.4)	8 (31)	2.1 (0.7-6.1)	2
P-trend			*0.05*		*0.3*			*0.09*		*0.1*	
gamma1											
negative	247 (58)	72 (52)	ref	65 (55)	ref	7 (35)	10 (32)	ref	8 (31)	ref	2
1	86 (20)	34 (24)	1.2 (0.7-2.1)	30 (25)	1.2 (0.7-2.2)	4 (20)	12 (39)	3.5 (1.4-8.8)	11 (42)	4.0 (1.5-10.9)	1
2 or more	92 (22)	33 (24)	1.4 (0.8-2.4)	24 (20)	1.0 (0.5-1.9)	9 (45)	9 (29)	2.3 (0.9-6.1)	7 (27)	2.3 (0.8-6.7)	2
P-trend			*0.3*		*0.9*			*0.06*		*0.08*	
nu											
negative	379 (89)	120 (86)	ref	103 (87)	ref	17 (85)	24 (77)	ref	20 (77)	ref	4
positive	46 (11)	19 (14)	1.5 (0.7-2.9)	16 (13)	1.5 (0.7-3.2)	3 (15)	7 (23)	2.3 (0.9-5.8)	6 (23)	2.3 (0.9-6.4)	1
P-het.			*0.3*		*0.3*			*0.1*		*0.1*	
mu											
negative	264 (62)	88 (63)	ref	74 (62)	ref	14 (70)	16 (52)	ref	14 (54)	ref	2
1	100 (24)	32 (23)	1 (0.6-1.7)	30 (25)	1.1 (0.6-2.0)	2 (10)	5 (16)	1.0 (0.3-2.8)	4 (15)	0.9 (0.3-2.8)	1
2 or more	61 (14)	19 (14)	0.9 (0.5-1.8)	15 (13)	0.8 (0.4-1.8)	4 (20)	10 (32)	2.3 (0.9-5.7)	8 (31)	2.1 (0.8-5.7)	2
P-trend			*0.8*		*0.8*			*0.1*		*0.2*	
ND											
negative	378 (89)	118 (85)	ref	102 (86)	ref	16 (80)	25 (81)	ref	23 (88)	ref	1
1	38 (9)	15 (11)	1.2 (0.6-2.6)	13 (11)	1.2 (0.5-2.7)	2 (10)	4 (13)	1.3 (0.4-4.1)	1 (4)	0.3 (0.0-2.7)	2
2 or more	9 (2)	6 (4)	1.4 (0.4-5.1)	4 (3)	1.1 (0.3-4.8)	2 (10)	2 (6)	1.9 (0.3-10.9)	2 (8)	2.4 (0.4-13)	2
P-trend			*0.5*		*0.7*			*0.4*		*0.9*	
Any types											
negative	59 (14)	12 (9)	ref	12 (10)	ref	1 (5)	1 (3)	ref	0 (0)	ref	1
1 or 2	136 (32)	47 (33)	2.0 (0.9-4.5)	39 (33)	2.0 (0.8-4.7)	7 (35)	9 (29)		9 (35)		0
3 to 6	123 (29)	37 (27)	1.5 (0.6-3.5)	33 (28)	1.7 (0.7-4.0)	4 (20)	10 (32)	1.4 (0.5-3.6)	9 (35)	1.4 (0.5-3.9)	1
7 or more	107 (25)	43 (31)	2.7 (1.2-6.3)	35 (29)	2.4 (1.0-5.8)	8 (40)	11 (35)	2.1 (0.8-5.5)	8 (31)	1.8 (0.6-4.9)	3
P-trend			*0.06*		*0.1*			*0.1*		*0.3*	

OR: Odds ratio, CI: Confidence interval, SCC: Squamous cell carcinoma, BCC: Basal cell carcinoma, HPV: Human papillomavirus, no:number;
N: total number; Het. :heterogeneity; ND: Not defined
*Adjusted for age at recruitment, sex, time since transplantation, skin type and stratified by centres.

**: Beta1, 2 or 3: Positive to HPV types from species 1, 2 or 3 respectively of genus beta. Gamma1: Positive to HPV types from species 1 of genus gamma.

Table 7.16: Association between multiple HPV seropositivity, squamous cell carcinoma and basal cell carcinoma only, among Caucasian transplant patients (using conditional logistic regression)

	OXFORD			LONDON			
	controls	Prevalent SCC		controls	Prevalent SCC		Incident SCC
	N=182	N=50		N=243	N=69		N=20
genus	No. (%)	No. (%)	OR[1] (95%CI)	No. (%)	No. (%)	OR[1] (95%CI)	No. (%)
alpha-mucosal							
negative	119 (65)	36 (72)	ref	131 (54)	31 (45)	ref	10
1	48 (26)	11 (22)	1.1 (0.4-2.7)	79 (33)	26 (38)	2.3 (1.1-4.9)	9
2 or more	15 (8)	3 (6)	2.9 (0.5-16.5)	33 (14)	12 (17)	3.9 (1.4-11.0)	1
P-trend			***0.4***			***P<0.001***	
alpha-cutaneous							
negative	127 (70)	43 (86)	ref	158 (65)	47 (68)	ref	12
1	42 (23)	2 (4)	0.1 (0.0-0.6)	50 (21)	10 (14)	0.7 (0.3-1.7)	4
2 or more	13 (7)	5 (10)	1.6 (0.4-7.0)	35 (14)	12 (17)	1.7 (0.7-4.4)	4
P-trend			***0.4***			***0.5***	
beta							
negative	85 (47)	22 (44)	ref	103 (42)	29 (42)	ref	8
1	31 (17)	10 (20)	1.5 (0.5-4.4)	45 (19)	8 (12)	0.6 (0.2-1.8)	3
2 or more	66 (36)	18 (36)	0.9 (0.4-2.2)	95 (39)	32 (46)	1.6 (0.8-3.4)	9
P-trend			***0.9***			***0.2***	
beta1							
negative	131 (72)	32 (64)	ref	161 (66)	48 (70)	ref	11
1	24 (13)	11 (22)	1.5 (0.5-4.3)	34 (14)	7 (10)	1.0 (0.3-2.9)	2
2 or more	27 (15)	7 (14)	1.3 (0.4-4.0)	48 (20)	14 (20)	1.1 (0.5-2.4)	7
P-trend			***0.5***			***0.9***	
beta2							
negative	108 (59)	29 (58)	ref	140 (58)	35 (51)	ref	12
1	29 (16)	6 (12)	1.1 (0.3-3.5)	44 (18)	15 (22)	1.3 (0.6-3.1)	3
2 or more	45 (25)	15 (30)	1.1 (0.4-2.6)	59 (24)	19 (28)	1.8 (0.8-4.0)	5
P-trend			***0.9***			***0.2***	
beta3							
negative	141 (77)	35 (70)	ref	187 (77)	50 (72)	ref	14
1	23 (13)	6 (12)	0.6 (0.2-2.0)	25 (10)	8 (12)	1.5 (0.5-4.4)	1
2 or more	18 (10)	9 (18)	1.2 (0.4-3.7)	31 (13)	11 (16)	1.7 (0.6-4.3)	5
P-trend			***1.0***			***0.2***	
gamma							
negative	105 (58)	27 (54)	ref	120 (49)	31 (45)	ref	4
1	33 (18)	9 (18)	0.6 (0.2-1.8)	59 (24)	19 (28)	1.9 (0.8-4.3)	5
2 or more	44 (24)	14 (28)	1.0 (0.4-2.4)	64 (26)	19 (28)	1.7 (0.8-3.9)	11
P-trend			***0.8***			***0.1***	
gamma1							
negative	114 (63)	28 (56)	ref	133 (55)	37 (54)	ref	7
1	31 (17)	9 (18)	0.8 (0.3-2.3)	55 (23)	21 (30)	1.7 (0.8-3.7)	4
2 or more	37 (20)	13 (26)	1.0 (0.4-2.7)	55 (23)	11 (16)	0.9 (0.4-2.3)	9
P-trend			***1.0***			***0.8***	
nu							
negative	168 (92)	45 (90)	ref	211 (87)	58 (84)	ref	17
positive	14 (8)	5 (10)	1.4 (0.4-5.2)	32 (13)	11 (16)	1.7 (0.7-4.4)	3
P-het.			***0.6***			***0.3***	
mu							
negative	125 (69)	32 (64)	ref	139 (57)	42 (61)	ref	14
1	40 (22)	13 (26)	1.2 (0.5-2.9)	60 (25)	17 (25)	1.1 (0.5-2.5)	2
2 or more	17 (9)	5 (10)	1.1 (0.3-4.1)	44 (18)	10 (14)	0.7 (0.3-1.8)	4
P-trend			***0.8***			***0.6***	
ND							
negative	169 (93)	44 (88)	ref	209 (86)	58 (84)	ref	16
1	13 (7)	5 (10)	1.2 (0.3-4.7)	25 (10)	8 (12)	1.2 (0.4-3.5)	2
2 or more	0 (0)	1 (2)	-	9 (4)	3 (4)	0.8 (0.2-4.1)	2
P-trend			***0.3***			***1.0***	
Any types							
negative	35 (19)	6 (12)	ref	24 (10)	6 (9)	ref	1
1 or 2	63 (35)	21 (42)	1.9 (0.6-6.4)	73 (30)	18 (26)	1.7 (0.5-6.1)	7
3 to 6	41 (23)	9 (18)	1.2 (0.3-4.5)	82 (34)	24 (35)	2.0 (0.6-7.0)	4
7 or more	43 (24)	14 (28)	1.7 (0.5-6.0)	64 (26)	21 (30)	3.3 (0.9-12.1)	8
P-trend			***0.7***			***0.1***	

OR: Odds ratio, CI: Confidence interval, SCC: Squamous cell carcinoma, BCC: Basal cell carcinoma, HPV: Human papillomavirus, No.:number

N: total number; Het. :heterogeneity; ND: Not defined

**: Beta1, 2 or 3: Positive-HPV types from species 1, 2 or 3 respectively of genus beta. Gamma1: Positive-HPV types from species 1 of genus gamma.

Table 7.17: Association between multiple HPV seropositivity, squamous cell carcinoma among Caucasian transplant patients, by centre (using logistic regression)

Table 7.18: Association between multiple HPV seropositivity, squamous cell carcinoma and basal cell carcinoma only, among Caucasian transplant patients from Oxford and London (using negative binomial regression)

BOTH CENTRES

	CONTROL	SQUAMOUS CELL CARCINOMA									BASAL CELL CARCINOMA					
		all N=139			prevalent N=119			Incident N=20			All N=31			Prevalent N=26		
HPV	N=425 %neg / %pos to 2 or more	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value
alpha - mucosal	59/11	55/12	1.4 (1.0-1.9)	**0.04**	56/13	1.4 (1.0-2.0)	**0.04**	50/5	1.2 (0.7-2.3)	**0.5**	65/3	0.9 (0.5-1.6)	**0.7**	65/4	0.9 (0.5-1.7)	**0.7**
alpha - cutaneous	67/11	73/15	1.1 (0.8-1.6)	**0.5**	76/14	1.0 (0.7-1.5)	**0.9**	60/20	1.6 (0.9-2.9)	**0.2**	68/16	1.3 (0.7-2.2)	**0.4**	69/15	1.2 (0.6-2.2)	**0.6**
beta	44/38	42/42	1.2 (0.8-1.7)	**0.4**	43/42	1.0 (0.7-1.5)	**0.9**	40/45	1.7 (0.8-3.5)	**0.2**	35/48	1.2 (0.7-2.2)	**0.5**	38/42	1.0 (0.5-1.9)	**0.9**
gamma	53/25	44/32	1.3 (0.9-1.8)	**0.1**	48/28	1.2 (0.8-1.6)	**0.4**	20/55	2.0 (1.1-3.6)	**0.03**	32/32	1.4 (0.8-2.4)	**0.2**	31/31	1.3 (0.7-2.3)	**0.3**
nu, mu and ND	55/20	50/22	1.1 (0.8-1.5)	**0.5**	50/21	1.1 (0.8-1.5)	**0.7**	50/30	1.3 (0.7-2.3)	**0.5**	39/39	1.7 (1.1-2.7)	**0.03**	42/38	1.5 (0.9-2.5)	**0.09**

LONDON

		all N=89			prevalent N=69			Incident N=20			All N=31			Prevalent N=26		
HPV	N= 243 %neg / %pos to 2 or more	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value
alpha - mucosal	54/14	46/15	1.4 (1.0-2.1)	**0.04**	45/17	1.6 (1.1-2.4)	**0.02**	50/5	1.0 (0.5-1.9)	**0.9**	59/5	0.9 (0.5-1.7)	**0.7**	61/6	0.8 (0.4-1.7)	**0.6**
alpha - cutaneous	65/14	66/18	1.3 (0.8-2.0)	**0.2**	68/17	1.2 (0.7-1.9)	**0.5**	60/20	1.4 (0.7-2.7)	**0.3**	68/18	1.2 (0.6-2.3)	**0.7**	72/17	1.0 (0.5-2.1)	**1.0**
beta	42/39	42/46	1.2 (0.8-1.9)	**0.3**	42/46	1.0 (0.6-1.7)	**0.9**	40/45	1.6 (0.8-3.5)	**0.2**	27/55	1.2 (0.6-2.5)	**0.6**	33/44	0.8 (0.4-1.8)	**0.6**
gamma	49/26	38/34	1.5 (1.0-2.2)	**0.03**	43/28	1.4 (0.9-2.1)	**0.2**	20/55	2.0 (1.1-3.6)	**0.02**	27/36	1.6 (0.9-2.8)	**0.1**	28/33	1.4 (0.7-2.7)	**0.3**
nu, mu and ND	49/25	47/24	1.0 (0.7-1.5)	**0.9**	46/22	1.0 (0.7-1.5)	**0.9**	50/30	1.0 (0.6-1.9)	**0.9**	36/32	1.4 (0.8-2.3)	**0.3**	44/28	1.1 (0.6-2.0)	**0.7**

OXFORD

		all N=50			prevalent N=50			Incident N=0			All N=9			Prevalent N=8		
HPV	N=182 %neg / %pos to 2 or more	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value	%neg / %pos to 2 or more	CR[1] (95% CI)	P-value
alpha - mucosal	65/8	72/6	1.2 (0.7-2.3)	**0.5**							78/0	0.8 (0.2-3.4)	**0.8**	75/0	0.9 (0.2-4.0)	**0.9**
alpha - cutaneous	70/7	86/10	0.8 (0.4-1.7)	**0.6**							67/11	1.5 (0.5-4.4)	**0.5**	63/13	1.7 (0.6-4.9)	**0.4**
beta	47/36	44/36	1.2 (0.7-2.2)	**0.5**							56/33	0.8 (0.3-2.4)	**0.7**	50/38	0.9 (0.3-2.8)	**0.9**
gamma	58/24	54/28	1.0 (0.6-1.8)	**1.0**							44/22	0.9 (0.3-2.7)	**0.9**	38/25	1.0 (0.3-3.1)	**1.0**
nu, mu and ND	62/14	56/20	1.3 (0.8-2.2)	**0.3**							44/56	2.5 (1.1-5.5)	**0.03**	38/63	2.8 (1.3-6.3)	**0.02**

HPV: Human papillomavirus; SCC: squamous cell carcinoma, BCC: Basal cell carcinoma; N: number; CR: Count ratio; CI: confidence interval.
Neg: seronegative: Pos: seropositive
[1] Using negative binomial regression adjusted for sex, age at recruitment, time since transplantation, skin type and centre (where appropriate).

Table 7.19: Sensitivity analyses 1- Odds ratio of prevalent squamous cell carcinoma (with different case groups) in patients who are HPV seropositive to one type compared to those who are seronegative to the same type, among Caucasian transplant patients from both centres

genus	species	type	Main analyses: CIS excluded, BOTH CENTRES: controls No. pos (%) N=425	prevalent SCC No. pos (%) N=119	OR[1] (95% CI)	P-value*	CIS included in case group, BOTH CENTRES: Prevalent SCC No. pos (%) N=141	OR[1] (95% CI)	P-value*	SCC only (with or without CIS), BOTH CENTRES: Prevalent SCC No. pos (%) N=55	OR[1] (95% CI)	P-value*	SCC only (with/without CIS and with/without BCC), BOTH CENTRES: Prevalent SCC No. pos (%) N=110	OR[1] (95% CI)	P-value*
alpha	2	3	36 (8)	10 (8)	1.2 (0.5-2.9)	*0.7*	10 (7)	1.0 (0.4-2.3)	*1.0*	4 (7)	-	*1.0*	9 (8)	1.1 (0.5-2.8)	*0.8*
	4	2	61 (14)	18 (15)	1.3 (0.7-2.7)	*0.4*	18 (13)	1.1 (0.5-2.1)	*0.9*	7 (13)	1.0 (0.4-2.5)	*1.0*	16 (15)	1.2 (0.6-2.6)	*0.6*
		27	73 (17)	19 (16)	1.2 (0.6-2.4)	*0.6*	21 (15)	1.1 (0.6-2.0)	*0.9*	8 (15)	1.0 (0.4-2.3)	*0.9*	16 (15)	1.1 (0.5-2.2)	*0.8*
	8	7	36 (8)	4 (3)	-	*0.07*	5 (4)	0.4 (0.1-1.2)	*0.09*	3 (5)	-	*0.6*	4 (4)	-	*0.1*
	9	16	67 (16)	22 (18)	1.8 (0.9-3.6)	*0.09*	23 (16)	1.4 (0.7-2.7)	*0.3*	15 (27)	2.4 (1.2-5.2)	*0.02*	20 (18)	1.7 (0.9-3.5)	*0.1*
	10	6	128 (30)	38 (32)	2.0 (1.1-3.5)	*0.02*	42 (30)	1.6 (1.0-2.7)	*0.07*	26 (47)	3.2 (1.6-6.2)	*<0.001*	35 (32)	2.0 (1.1-3.5)	*0.02*
		13	42 (10)	14 (12)	2.2 (1.0-5.1)	*0.06*	16 (11)	1.9 (0.9-4.2)	*0.1*	7 (13)	2.0 (0.7-5.3)	*0.2*	12 (11)	1.9 (0.8-4.6)	*0.1*
		5	39 (9)	18 (15)	1.9 (0.9-4.0)	*0.09*	20 (14)	1.8 (0.9-3.5)	*0.1*	12 (22)	2.3 (1.1-5.8)	*0.03*	16 (15)	1.8 (0.8-3.8)	*0.1*
		8	91 (21)	27 (23)	1.2 (0.6-2.1)	*0.6*	29 (21)	1.0 (0.6-1.8)	*0.9*	14 (25)	1.3 (0.6-2.6)	*0.5*	23 (21)	1.1 (0.6-2.0)	*0.9*
	1	20	61 (14)	15 (13)	0.7 (0.4-1.5)	*0.4*	16 (11)	0.6 (0.3-1.3)	*0.2*	9 (16)	0.9 (0.4-2.1)	*0.8*	14 (13)	0.7 (0.3-1.5)	*0.4*
		24	47 (11)	12 (10)	1.1 (0.5-2.5)	*0.7*	13 (9)	0.9 (0.4-2.0)	*0.8*	6 (11)	1.0 (0.4-2.8)	*0.9*	9 (8)	0.8 (0.4-2.0)	*0.7*
		36	50 (12)	8 (7)	0.4 (0.2-1.1)	*0.06*	8 (6)	0.4 (0.2-0.9)	*0.02*	4 (7)	-	*0.5*	7 (6)	0.4 (0.2-1.1)	*0.05*
		93	12 (3)	6 (5)	2.0 (0.6-6.6)	*0.3*	6 (4)	1.5 (0.5-4.9)	*0.5*	3 (5)	-	*0.4*	5 (5)	1.7 (0.5-6.2)	*0.4*
beta		9	65 (15)	19 (16)	1.0 (0.5-2.0)	*0.7*	21 (15)	1.0 (0.5-1.9)	*0.9*	9 (16)	1.0 (0.5-2.4)	*0.9*	18 (16)	1.0 (0.5-2.0)	*1.0*
		15	116 (27)	34 (29)	1.0 (0.6-1.7)	*0.9*	37 (26)	0.9 (0.5-1.4)	*0.5*	16 (29)	1.0 (0.5-2.0)	*1.0*	30 (27)	0.9 (0.5-1.6)	*0.7*
	2	17	100 (24)	37 (31)	1.7 (1.0-2.9)	*0.07*	37 (26)	1.2 (0.7-2.1)	*0.5*	17 (31)	1.5 (0.8-3.1)	*0.2*	34 (31)	1.6 (0.9-2.8)	*0.1*
		23	41 (10)	9 (8)	0.9 (0.4-2.1)	*0.7*	10 (7)	0.8 (0.3-1.8)	*0.5*	6 (11)	1.1 (0.4-2.9)	*0.9*	9 (18)	1.0 (0.4-2.3)	*0.9*
		38	95 (22)	28 (24)	0.9 (0.5-1.6)	*0.7*	30 (21)	0.8 (0.4-1.3)	*0.3*	15 (27)	1.1 (0.5-2.2)	*0.8*	26 (24)	0.9 (0.5-1.6)	*0.7*
		49	84 (20)	32 (27)	1.4 (0.8-2.5)	*0.3*	33 (23)	1.1 (0.7-2.0)	*0.6*	18 (33)	1.7 (0.8-3.3)	*0.1*	30 (27)	1.4 (0.8-2.5)	*0.3*
	3	75	50 (12)	17 (14)	1.1 (0.5-2.2)	*0.8*	18 (13)	0.9 (0.5-1.8)	*0.9*	7 (13)	0.9 (0.7-2.3)	*0.8*	15 (14)	1.0 (0.5-2.1)	*1.0*
		76	42 (10)	16 (13)	1.1 (0.5-2.4)	*0.7*	16 (11)	0.9 (0.4-1.9)	*0.8*	5 (9)	0.7 (0.3-2.1)	*0.5*	14 (13)	1.0 (0.5-2.2)	*1.0*
	4	92	52 (12)	14 (12)	0.7 (0.3-1.4)	*0.3*	14 (10)	0.5 (0.3-1.1)	*0.09*	6 (11)	0.6 (0.2-1.5)	*0.2*	12 (11)	0.6 (0.3-1.3)	*0.2*
	5	96	62 (15)	16 (13)	0.9 (0.4-1.7)	*0.7*	19 (13)	0.9 (0.5-1.7)	*0.7*	6 (11)	0.6 (0.2-1.7)	*0.3*	14 (13)	0.8 (0.4-1.6)	*0.5*
gamma		4	108 (25)	38 (32)	1.3 (0.8-2.2)	*0.3*	41 (29)	1.1 (0.7-1.9)	*0.6*	23 (42)	1.9 (1.0-3.7)	*0.05*	36 (33)	1.3 (0.8-2.3)	*0.3*
	1	65	119 (28)	29 (24)	1.0 (0.6-1.7)	*1.0*	32 (23)	0.9 (0.5-1.5)	*0.7*	14 (25)	1.0 (0.5-1.9)	*0.9*	27 (25)	1.0 (0.5-1.7)	*0.9*
		95	91 (21)	24 (20)	0.8 (0.4-1.4)	*0.4*	29 (21)	0.8 (0.4-1.4)	*0.4*	15 (27)	1.1 (0.5-2.2)	*0.9*	21 (19)	0.7 (0.4-1.3)	*0.2*
	2	48	66 (16)	20 (17)	1.3 (0.7-2.6)	*0.4*	20 (14)	1.1 (0.6-2.0)	*0.9*	12 (22)	1.7 (0.8-3.7)	*0.2*	17 (15)	1.2 (0.6-2.3)	*0.7*
	3	50	32 (8)	14 (12)	2.1 (0.9-4.8)	*0.1*	14 (10)	1.6 (0.7-3.6)	*0.3*	7 (13)	2.1 (0.8-5.8)	*0.2*	13 (12)	2.1 (0.9-5.1)	*0.09*
	4	60	19 (4)	7 (6)	1.2 (0.4-3.6)	*0.7*	7 (5)	1.0 (0.4-2.8)	*1.0*	5 (9)	1.8 (0.6-5.6)	*0.9*	7 (6)	1.4 (0.5-4.0)	*0.5*
mu	1	41	46 (11)	16 (13)	1.5 (0.7-3.2)	*0.3*	17 (12)	1.3 (0.6-2.6)	*0.5*	10 (18)	1.9 (0.8-4.4)	*0.2*	16 (15)	1.7(0.8-3.6)	*0.2*
nu	1	1	124 (29)	34 (29)	0.9 (0.5-1.6)	*0.8*	39 (28)	1.0 (0.6-1.6)	*0.9*	16 (29)	0.9 (0.5-1.8)	*0.8*	31 (28)	0.9 (0.5-1.5)	*0.6*
	2	63	98 (23)	26 (22)	1.0 (0.5-1.8)	*0.9*	28 (20)	0.9 (0.5-1.5)	*0.6*	13 (24)	1.0 (0.5-2.0)	*0.1*	22 (20)	0.8 (0.5-1.6)	*0.6*
ND		101	30 (7)	16 (13)	1.8 (0.8-3.9)	*0.2*	17 (12)	1.5 (0.7-3.3)	*0.3*	7 (13)	1.6 (0.6-4.3)	*0.4*	13 (12)	1.5 (0.7-3.5)	*0.3*
		103	26 (6)	5 (4)	0.6 (0.2-1.7)	*0.3*	5 (4)	0.5 (0.2-1.5)	*0.2*	4 (7)	-	*0.8*	5 (5)	0.6 (0.2-1.9)	*0.4*

HPV: Human papillomavirus; OR: odds ratio, CI: confidence interval; N: total number; SCC: squamous cell carciNo.ma; No. POS: number of seropositive samples; ND: Not defined.
CIS: Carcinoma in situ, BCC: basal cell carcinoma

[1] Analyses are stratified by centres and adjusted for age at recruitment, time since trasnplantation, sex and skin type.

*: When the number of seropositive patient was less than 5, P-value was derived using Fisher's exact test

Table 7.20: Sensitivity analyses 2- Odds ratio of prevalent squamous cell carcinoma in patients who are HPV seropositive to one type compared to those who are seronegative to the same type, among Caucasian transplant patient from both centres: a) fully adjusted analysis, b) restricted analysis to patients with diagnosis less than 4 years since blood collection/recruitment, c) restricted analysis to patients who have been transplanted for at least 5 years

			MAIN ANALYSES				Fully adjusted analysis		Restricted to patients with time from SCC to blood sample/recruitment less than 4 years			Restricted to patients with time since transplantation greater than 5 years.			
genus	species	type	controls no pos (%) N=425	Prevalent SCC no pos (%) N=119	Adjusted OR[1]	P-value*	Fully adjusted OR[2]	P-value*	Prevalent SCC no pos (%) N=57	Adjusted OR[1]	P-value*	controls no pos (%) N=275	Prevalent SCC no pos (%) N=115	Adjusted OR[1]	P-value*
alpha	2	3	36 (8)	10 (8)	1.2 (0.5 to 2.9)	*0.7*	1.2 (0.5 to 3.2)	*0.7*	4 (7)	0.9 (0.3 to 3.1)	*0.9*	21 (8)	10 (9)	1.3 (0.5 to 3.1)	*0.6*
	4	2	61 (14)	18 (15)	1.3 (0.7 to 2.7)	*0.4*	1.6 (0.7 to 3.5)	*0.2*	8 (14)	1.1 (0.4 to 2.8)	*0.8*	44 (16)	18 (16)	1.4 (0.7 to 2.8)	*0.4*
		27	73 (17)	19 (16)	1.2 (0.6 to 2.4)	*0.6*	1.3 (0.6 to 2.9)	*0.5*	7 (12)	0.9 (0.3 to 2.2)	*0.7*	52 (19)	19 (17)	1.3 (0.6 to 2.5)	*0.5*
	8	7	36 (8)	4 (3)	-	*0.07*	-	*0.07*	3 (5)	-	*0.6*	27 (10)	4 (3)	-	*0.04*
	9	16	67 (16)	22 (18)	1.8 (0.9 to 3.6)	*0.09*	2.0 (1.0 to 4.3)	*0.07*	11 (19)	1.8 (0.8 to 4.2)	*0.2*	43 (16)	22 (19)	1.9 (0.9 to 3.8)	*0.07*
	10	6	128 (30)	38 (32)	2.0 (1.1 to 3.5)	*0.02*	1.9 (1.0 to 3.4)	*0.05*	19 (33)	2.3 (1.1 to 4.6)	*0.02*	81 (29)	35 (30)	1.8 (1.0 to 3.2)	*0.1*
		13	42 (10)	14 (12)	2.2 (1.0 to 5.1)	*0.06*	2.3 (0.9 to 5.5)	*0.07*	7 (12)	2.2 (0.8 to 6.1)	*0.2*	29 (11)	14 (12)	2.3 (1.0 to 5.3)	*0.06*
		5	39 (9)	18 (15)	1.9 (0.9 to 4.0)	*0.09*	1.8 (0.8 to 4.0)	*0.1*	8 (14)	1.6 (0.6 to 4.0)	*0.3*	31 (11)	18 (16)	2.0 (0.9 to 4.2)	*0.1*
		8	91 (21)	27 (23)	1.2 (0.6 to 2.1)	*0.6*	1.2 (0.6 to 2.2)	*0.6*	11 (19)	0.9 (0.4 to 1.9)	*0.7*	67 (24)	27 (23)	1.2 (0.7 to 2.3)	*0.5*
	1	20	61 (14)	15 (13)	0.7 (0.4 to 1.5)	*0.4*	0.7 (0.3 to 1.6)	*0.4*	6 (11)	0.6 (0.2 to 1.6)	*0.3*	45 (16)	15 (13)	0.8 (0.4 to 1.6)	*0.5*
		24	47 (11)	12 (10)	1.1 (0.5 to 2.5)	*0.7*	1.1 (0.5 to 2.5)	*0.9*	2 (4)	-	*0.1*	35 (13)	12 (10)	1.2 (0.6 to 2.7)	*0.6*
		36	50 (12)	8 (7)	0.4 (0.2 to 1.1)	*0.06*	0.6 (0.2 to 1.5)	*0.2*	3 (5)	-	*0.2*	38 (14)	8 (7)	0.4 (0.2 to 1.1)	*0.1*
		93	12 (3)	6 (5)	2.0 (0.6 to 6.6)	*0.3*	2.3 (0.6 to 8.1)	*0.2*	4 (7)	-	*0.1*	9 (3)	5 (4)	1.5 (0.4 to 5.4)	*0.6*
beta		9	65 (15)	19 (16)	1.0 (0.5 to 2.0)	*0.7*	1.0 (0.5 to 2.1)	*1.0*	5 (9)	0.5 (0.2 to 1.4)	*0.2*	46 (17)	19 (17)	1.0 (0.5 to 2.0)	*0.9*
		15	116 (27)	34 (29)	1.0 (0.6 to 1.7)	*0.9*	1.0 (0.6 to 1.9)	*0.9*	12 (21)	0.7 (0.3 to 1.4)	*0.3*	89 (32)	34 (30)	1.0 (0.6 to 1.8)	*0.9*
	2	17	100 (24)	37 (31)	1.7 (1.0 to 2.9)	*0.07*	1.6 (0.9 to 2.9)	*0.1*	11 (19)	0.9 (0.4 to 1.9)	*0.7*	72 (26)	37 (32)	1.8 (1.0 to 3.2)	*0.04*
		23	41 (10)	9 (8)	0.9 (0.4 to 2.1)	*0.7*	0.8 (0.3 to 2.1)	*0.7*	5 (9)	1.0 (0.3 to 2.8)	*0.9*	33 (12)	8 (7)	0.7 (0.3 to 1.8)	*0.5*
		38	95 (22)	28 (24)	0.9 (0.5 to 1.6)	*0.7*	0.8 (0.4 to 1.4)	*0.4*	10 (18)	0.6 (0.3 to 1.4)	*0.3*	74 (27)	28 (24)	0.9 (0.5 to 1.7)	*0.8*
		49	84 (20)	32 (27)	1.4 (0.8 to 2.5)	*0.3*	1.5 (0.8 to 2.8)	*0.2*	14 (25)	1.3 (0.6 to 2.6)	*0.5*	63 (23)	32 (28)	1.5 (0.8 to 2.7)	*0.2*
	3	75	50 (12)	17 (14)	1.1 (0.5 to 2.2)	*0.8*	1.0 (0.4 to 2.1)	*0.9*	7 (12)	0.9 (0.4 to 2.3)	*0.9*	40 (15)	17 (15)	1.1 (0.5 to 2.2)	*0.8*
		76	42 (10)	16 (13)	1.1 (0.5 to 2.4)	*0.7*	1.3 (0.6 to 2.8)	*0.5*	5 (9)	0.7 (0.2 to 2.0)	*0.5*	34 (12)	16 (14)	1.2 (0.6 to 2.4)	*0.7*
	4	92	52 (12)	14 (12)	0.7 (0.3 to 1.4)	*0.3*	0.7 (0.3 to 1.5)	*0.3*	6 (11)	0.5 (0.2 to 1.3)	*0.2*	40 (15)	14 (12)	0.7 (0.3 to 1.4)	*0.3*
	5	96	62 (15)	16 (13)	0.9 (0.4 to 1.7)	*0.7*	0.8 (0.4 to 1.6)	*0.5*	6 (11)	0.6 (0.2 to 1.6)	*0.3*	49 (18)	16 (14)	0.9 (0.5 to 1.8)	*0.8*
gamma		4	108 (25)	38 (32)	1.3 (0.8 to 2.2)	*0.3*	1.4 (0.7 to 2.5)	*0.3*	14 (25)	1.0 (0.5 to 2.0)	*1.0*	82 (30)	38 (33)	1.3 (0.8 to 2.3)	*0.3*
	1	65	119 (28)	29 (24)	1.0 (0.6 to 1.7)	*1.0*	1.1 (0.6 to 2.0)	*0.8*	13 (23)	0.9 (0.4 to 1.9)	*0.8*	86 (31)	29 (25)	1.0 (0.6 to 1.8)	*0.9*
		95	91 (21)	24 (20)	0.8 (0.4 to 1.4)	*0.4*	0.7 (0.4 to 1.4)	*0.4*	7 (12)	0.4 (0.2 to 1.1)	*0.05*	65 (24)	24 (21)	0.8 (0.4 to 1.4)	*0.4*
	2	48	66 (16)	20 (17)	1.3 (0.7 to 2.6)	*0.4*	1.8 (0.9 to 3.8)	*0.1*	7 (12)	0.9 (0.4 to 2.3)	*0.8*	46 (17)	20 (17)	1.4 (0.7 to 2.8)	*0.3*
	3	50	32 (8)	14 (12)	2.1 (0.9 to 4.8)	*0.1*	2.4 (0.9 to 6.5)	*0.09*	5 (9)	1.6 (0.5 to 4.9)	*0.5*	21 (8)	14 (12)	2.2 (0.9 to 5.2)	*0.08*
	4	60	19 (4)	7 (6)	1.2 (0.4 to 3.6)	*0.7*	1.4 (0.4 to 4.4)	*0.6*	4 (7)	-	*0.3*	13 (5)	7 (6)	1.3 (0.4 to 3.6)	*0.7*
mu	1	41	46 (11)	16 (13)	1.5 (0.7 to 3.2)	*0.3*	1.7 (0.8 to 3.7)	*0.2*	9 (16)	1.9 (0.8 to 4.5)	*0.2*	29 (11)	15 (13)	1.4 (0.7 to 3.1)	*0.3*
nu	1	1	124 (29)	34 (29)	0.9 (0.5 to 1.6)	*0.8*	0.9 (0.5 to 1.6)	*0.6*	17 (30)	0.9 (0.5 to 1.9)	*0.9*	86 (31)	34 (30)	1.0 (0.6 to 1.7)	*0.9*
	2	63	98 (23)	26 (22)	1.0 (0.5 to 1.8)	*0.9*	0.9 (0.5 to 1.7)	*0.7*	10 (18)	0.7 (0.3 to 1.5)	*0.3*	74 (27)	26 (23)	1.0 (0.5 to 1.8)	*1.0*
ND		101	30 (7)	16 (13)	1.8 (0.8 to 3.9)	*0.2*	1.6 (0.7 to 3.7)	*0.3*	3 (5)	-	*0.8*	21 (8)	16 (14)	1.8 (0.8 to 4.1)	*0.2*
		103	26 (6)	5 (4)	0.6 (0.2 to 1.7)	*0.3*	0.6 (0.2 to 2.0)	*0.4*	2 (4)	-	*0.6*	20 (7)	5 (4)	0.6 (0.2 to 1.7)	*0.3*

HPV: Human papillomavirus; OR: odds ratio; CI: confidence interval; N: total number; SCC: squamous cell carcinoma; No POS: number of seropositive samples; ND: not defined; NA: Not available

[1] P-values and odds ratios were calculated using conditional (on centre) logistic regression and adjusted for sex, time since transplantation, age at recruitment and skin type.

[2] P-values and odds ratios were calculated using conditional (on centre) logistic regression and adjusted for sex, time since transplantation, age at recruitment, skin type, birth order, number of sunburn before 18 and living with a partner.

*: When the number of seropositive patient was less than 5, P-value was derived using Fisher's exact test

CHAPTER 8

Summary and conclusions

8.1 Introduction

Established risk factors for basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) include exposure to solar ultra-violet radiation and, for SCC in particular, immuno-suppression, such as that experienced by organ transplant recipients (OTR). In addition, there is a suggestion of an association between certain human papillomaviruses (such as betaHPV types 5 and 8) and the development of cutaneous squamous (but not basal) cell carcinoma. To date, more than 118 papillomaviruses have been completely described, of which about a hundred infect humans. The alpha types, particularly HPV-16, 18, 33 and 45 are well established causes of cancer of the uterine cervix; the viral E6 and E7 proteins are associated with the degradation of tumour suppressor proteins p53 and pRb respectively. A role for HPV in the aetiology of skin cancers is uncertain.

Most studies of HPV and cutaneous squamous cell carcinoma have used HPV-DNA de-

tection methods to examine the association. Due to the high sensitivity of PCR methods and the ubiquity of HPV, previously unknown types are often identified. The ability to detect HPV-DNA varies widely between samples from the same patient, in part depending on the type of sample (hair follicle or skin biopsy), the section of the sample examined (the surface or deeper within the specimen) or the location on the body from where the sample originated (sun exposed or not). More recently, studies have used new serological assays that detect antibodies against HPV; all recruited immunocompetent individuals and have used case-control designs in which sera were obtained after the cancer was diagnosed. The main aim of this thesis was to examine the association between squamous and basal cell carcinoma and antibodies against the L1 antigen of 38 HPV types. The main hypothesis was therefore: is human papillomavirus a cause of squamous cell carcinoma? Data came from a small prospective pilot study from the Oxford component the European Prospective Investigation into Cancer and Nutrition and new data from case-control studies nested among high-risk cohorts of OTR from London and from Oxford. Plasma and sera were tested using Luminex technology.

Few data are available on the seroprevalence and risk factors associated with HPV types other than those associated with cancer of uterine cervix. A secondary aim of this thesis was to examine the seroepidemiology of HPV among OTR and to investigate seroprevalence and epidemiology of HPV among different ethnic groups and among people with different immunological status.

8.2 Summary of the findings in this thesis

Risk factors from the questionnaire associated with post-transplant SCC and BCC among Caucasian OTR (Chapter 7)

Using questionnaire data, risk factors for SCC and BCC only were examined among Caucasian OTR. Both SCC and BCC were more common in people with susceptibility to burn easily. For both types of lesions, the risk increased with increasing time since transplantation and age. SCC was more common in men than women and in transplant patients with higher self-reported number of sunburns as a child and with the presence of keratotic lesions (viral warts or AK). In contrast, BCC was associated with a higher number of sunny holidays after transplantation. SCC was more common in patients who were married or living with a partner probably reflecting a screening effect, and the presence of SCC was also inversely related to birth order. This last finding might suggest an early exposure to infectious agents, but its significance in this context remains highly speculative. A larger proportion of SCC was found on sun-exposed areas compared with BCC, which occurred on the back as well as the head. In contrast to SCC, very few BCC were diagnosed on the hand, suggesting a difference in the mechanisms involved in SCC and BCC pathogenesis.

HPV seroprevalence by ethnicity, centre and immune-status in individuals without skin cancer (Chapter 6)

A large body of research has been undertaken on mucosal HPV types but the natural history of cutaneous HPV is not known. This is the first report describing antibody responses in high-risk transplant populations and comparing HPV seroprevalence across groups with different immune-status and with different ethnic origins. Among organ transplant recipients, HPV 5, 93 and 101 were detected more frequently in non-Caucasians than Caucasians and HPV 1 more frequently among Caucasians. For all ethnic groups, HPV4 seroprevalence was lower among OTR than IC or dialysis patients. Overall, between 81% and 94% of individuals were seropositive to at least one HPV type and no

statistically significant difference was observed between the three groups with different immune-status (transplantation, dialysis or immunocompetent patients). The seroprevalence of 8 HPV types differed significantly between the two geographically close centres (London and Oxford). Those individuals seropositive to multiple types of one genus were more likely to be seroreactive to multiple types of another genus independently of immune-status or ethnicity.

Risk factors associated with HPV seropositivity among Caucasian OTR without skin cancer (Chapter 6)

Since NMSC occur mainly in Caucasians, further analyses were restricted to these patients. Among Caucasian control OTR, associations between risk factors from questionnaire and HPV seroprevalence were examined. Around 86% were seropositive to at least one HPV: 57% to alpha types, 56% to beta, 47% to gamma types and 45% to other types. As expected, antibodies against HPV 16 were associated with a self-reported history of an abnormal cervical smear and antibodies against HPV 6 were associated with a self-reported history of genital warts. These findings validated the methodology. As expected, antibodies against mucosal alphaHPV types were more frequent in younger patients and among women. Skin type and self reported markers of exposure to ultraviolet radiation were not consistently associated with any HPV types. No other distinguishing epidemiological features of transplant recipients with antibodies against single or multiple HPV types were identified.

Association between HPV seroprevalence and SCC and BCC among Caucasian OTR (Chapter 7)

In contrast with the results on the association between a self-reported history of abnormal

smears or genital warts and mucosal types 16 and 6 respectively, there were no consistent associations between any of the HPV types examined (including cutaneous betaHPVs) and prevalent or incident SCC in the prospective study or in the case-control study. Nor was seropositivity to multiple types associated with SCC or BCC. Numbers were too small to examine thoroughly the association between BCC and antibodies against HPV.

8.3 Conclusions and suggestions for future work

There was no consistent association between any of the 38 HPV types and SCC. Consequently, our serological data do not support a role for any of the HPV types examined in the aetiology of SCC.

Table 8.1 summarises all HPV-DNA case-control studies that have, to date, examined the association between HPV genotyping and SCC with more than 50 cases. Due to the high sensitivity of PCR methods and the ubiquity of HPV, previously unknown types are often identified. The ability to detect HPV-DNA varies widely between samples from the same patient, in part depending on the type of sample (hair follicle or skin biopsy), the section of the sample examined (the surface or deeper within the specimen), the location on the body from where the sample originated (sun exposed or not) or the choice of the primer. Consequently, studies have not shown consistent associations between the presence of HPV DNA and SCC.

Given the low copy numbers of HPV DNA in skin cancers, the association between HPV and SCC and, the question of high risk might be better adressed using serological methods. Studies detecting antibodies against HPV have all recruited immunocompetent individuals and have used case-control designs in which sera were obtained after the cancer was diagnosed. Table 8.2 summarises the serological data on the association between

HPV and skin cancer available to date together with the results of the thesis. Although possible molecular mechanisms with E6 and E7 betaHPV proteins probably working as a co-factor with ultraviolet radiation early in the development of SCC has been suggested, there is as yet no convincing epidemiological evidence to support such a role. There is also no indication of a hierarchy of high-risk beta-HPV types. It is also possible that the increased risk of SCC observed in OTR is simply a result of immunosuppression impairing the normal capacity to repair UV-damaged DNA.

Difficulties with establishing a role 'epidemiologically', if any, include the ubiquity of HPV, the sensitivity of PCR detection methods and the lack of viral load data. Equally, few sero-epidemiological studies have used the recently developed multiplexed and high throughput technologies such as Luminex and new HPV types or combination of types might still be found to be associated with SCC development in the future. Luminex assay is a powerful tool for sero-epidemiological studies to detect antibodies against up to 100 HPV types simultaneously. It is currently the 'gold standard' method but more research is needed on validation (sensitivity and specificity) of the assay for each HPV type.

In February 2009, following the results of this thesis and work of other groups, the International Agency for Research on Cancer (IARC) published an update on the association between HPV and squamous cell carcinoma. The conclusion was that there is "a need for further research of cutaneous HPV types of the beta and gamma genera. These widespread HPV types were classified in Group 3 on the basis of inconclusive evidence of causing skin cancer in humans and limited mechanistic data. Exceptions were the beta HPV5 and HPV8, which are 'possibly carcinogenic' in patients with epidermodysplasia verruciformis" [343].

Further research is needed to clarify the natural history of cutaneous type using detailed questionnaire, in particular given the growing research interest in the possible role of HPV

in the pathogenesis of cutaneous SCC. Large prospective study with repeated serological measurements and with HPV-DNA and viral load data are needed to elucidate any genuine association between HPV infection and SCC.

Author, year	Number SCC/controls	group	sample location	Results
Struijk, 2003	155/371	IC	Plucked eyebrows	All beta HPV and types 5, 15 and 20
Termorshuizen, 2004	156/320	IC	Plucked eyebrows	All beta HPV and types 5, 15 and 20
Struijk, 2006	64/58	IC	Plucked eyebrows	No association
Forslund, 2007	82/92	IC	Skin biopsies	Beta species 2
Patel, 2008	101/101	IC	Skin biopsies	Beta species 1

IC: immunocompetent

Table 8.1: Case-control studies on cutaneous cell carcinoma in relation to the detection of HPV-DNA ($\geq$ 50 cases; Appendix A)

Author, year	Number SCC/controls	group	assay	incident/ prevalent	Results
Feltkamp, 2003	160/333	IC	ELISA	prevalent	HPV8 (beta-1)
Masini, 2003	46/84	IC	ELISA	prevalent	HPV8 (beta-1)
Struijk, 2003	64/58	IC	ELISA	prevalent	HPV8 (beta-1)
Karagas, 2006	252/461	IC	Luminex	prevalent	HPV5 (beta-1)
Casabonne, 2007	39/80	IC	Luminex	incident	No association
Andersson, 2008	72/121	IC	Luminex	prevalent	No association
Waterboer, 2008	43/77	IC	Luminex	prevalent	beta-2 species combined and gamma species combined
Casabonne, 2009	119/425	OTR	Luminex	prevalent	No association (inconsistent results across 2 centres)

*Incident or prevalent: blood taken prior or after diagnosis of SCC respectively; IC: immunocompetent; OTR: organ transplant recipients.

Table 8.2: Case-control studies on cutaneous squamous cell carcinoma in relation to the detection of antibodies against some beta, gamma, nu and mu HPV types (Figure 4.4).

Appendices

APPENDIX A

Case-control studies that used HPV-DNA methods

Study, Year, Country	Number cases/controls	IC/IS	Sample (primers)	HPV type	OR unadjusted + 95% CI (% positive cases/controls)	OR adjusted + 95% CI (% positive cases/controls)	Adjustment variables/ Matching	Comment
Boxman *et al.*, 1999, The Netherlands	69 cases (BBC:44, SCC:18; both:7) 66 controls	IC	plucked hair from eyebrow, scalp, arm, legs (L1 gene)	5	1.1 (0.4-3.0) 16% / 15%		1:1 matched for age, sunscreen allocation	31 RTRs (Dutch patients) 518 ICs (Australia, the Nambour)
	19 cases 12 controls	IS RTR			0.4 (0.1-2.3) 37% / 58%			
De Jong-Tieben *et al.*, 2000, The Netherlands	5 SCC 4 clinical normal skin in patients without skin cancers	RTR	skin biopsies (L1 gene)	beta	20% / 0%			Higher prevalence in sun-exposed ares but only in patients with skin cancers HPV5,8,9,12,15,24,X1
Boxman *et al.*, 2000, Australia	NMSC: 51 BCC & 25 SCC 89 controls	IC	plucked hair from eyebrow, scalp, arm, legs (L1 gene)	beta	beta-HPV DNA & NMSC: 0.8 (0.3-1.8) 63% / 67% & BCC: 0.6 (0.2-1.5) 61% / 71% & SSC: 2.0 (0.5-8.0) 68% / 56%	-	1:1 matched for age, sunscreen allocation	Multiple seropositivity in patients with SCC (60% vs 45%, P<0.05) Within the cohort of resident of Nambour; Problem of detection
O'connor *et al.*, 2001,Ireland	12 SCC 20 Controls	IC	skin biopsies (L1 gene)	all	**samples**: 28.3 (3.2 to 338.2)			Mainly beta-HPV types HPV38(x3), 36 (x2), X1 (x2), 15 (x2), 20
Struijk *et al.*, 2003, The Netherlands	155 SCC 371 controls	IC	plucked eyebrow hairs (E7 gene)	beta: 5,8, 15,20,24,38 alpha mucosal: 16 Other:2	**All beta-HPV**: 2.1 (1.4-3.2) 75% / 58% *type specific*: 2.0 (1.3-3.1) **HPV5**: 2.8 (1.6 to 5.0) 23% / 13% **HPV8**: 2.6 (1.4 to 4.9) 17% / 10% **HPV15**: 3.2 (1.6 to 6.2) 17% / 8% **HPV20**: 2.1 (1.3 to 3.5) 33% / 24% **HPV24**: 2.6 (1.5 to 4.5) 25% / 15% **HPV38**: 2.0 (1.2 to 3.3) 32% / 25%	1.7 (1.1-2.7) *type specific*: 1.7 (1.1-2.6) **HPV5**: 2.8 (1.6 to 5.0) **HPV8**: 1.8 (0.9to 3.4) **HPV15**: 2.5 (1.3 to 4.8) **HPV20**: 1.7 (1.0 to 2.9) **HPV24**: 1.4 (0.8 to 2.6) **HPV38**: 1.5 (0.9 to 2.6)	Adjusted for age and sex.	HPV 16: all cases were negative HPV2: one individual was positive. Multiple infection more common in patients with SCC (OR: 1.9, 95%CI 1.2 to 3.1) Analysis restricted to HPV-DNA+: none of HPV types stoodd out. No adjustement for sun exposure, skin type, smoking as there are not associated with the presence of HPV DNA in eyebrow hairs.
Iftner, 2003 *et al.*, USA (California)	72 SCC 106 controls	IC	skin biopsies (E1 gene)	alpha mucosal: 16,31,33,35, and 51 beta: 5,8,12,17,19,22, and 36	**All HPV**: 30.0 (10.9 to 83.0) 60% / 5% **In Pos samples**: beta: 9% / 20% cutaneous: 21% / 20%	32.0 (10.0 to 100.0)	Adjusted for age, sex and sun exposure (location)	-Multiple infection more common in patients with SCC -California (most cases), Germany mixed (most controls) -Detection problem (not all types) -Problem missing values. -Sample size New laboratory method: reverse hybridization method
Termorshuizen *et al.*, 2004, the Netherlands	156 SCC 320 controls	IC	plucked eyebrown hairs (E7 gene)	5 8 15 20 24 38	**All beta-HPV**: 2.1 (1.3 to 3.2) 71% / 54% **HPV5**: 2.2 (1.3 to 3.8) 24% / 12% **HPV8**: 1.8 (1.0 to 3.3) 17% / 10% **HPV15**: 2.2 (1.2 to 4.1) 17% / 8% **HPV20**: 1.6 (1.0 to 2.6) 33% / 23% **HPV24**: 1.8 (1.1 to 3.0) 25% / 15% **HPV38**: 1.4 (0.9 to 2.2) 32% / 25%	**HPV5**: 2.0 (P=0.02) **HPV15**: 2.1 (P=0.03) **HPV20**: 1.6 (P=0.06)	Adjusted for age, sex skin type, sun exposure and painful sunburns. Unmatched	
Harwood *et al.*, 2004, the UK	10 NMSC (19 samples) 29 without (36 samples)	IC	skin biopsies (L1 gene)	all	Patients / Samples Any HPV: 7.3 (1.2 to 53.0) / 1.5 (0.4 to 5.3) 70 % / 24% 42% / 33% **In positive samples**: beta: 63% / 25% ; mucosal: 0% / 0%; cutaneous: 63% / 75%	**beta in sample**: 6.4 (1.8 to 22.9) P=0.004 **beta at any site**: 6.8 (1.3 to 36.1) P=0.02 **beta in NSE skin**: 7.3 (1.5 to 35.4) P=0.01 **beta in SE skin**: 4.9 (2.0 to 25.8) P=0.06 Positive NS association for any HPV and NMSC Negative NS association for cutaneous HPV and NMSC	Adjusted for transplant status, sex and either source of sample (sun exposed or not) or number of samples per individual.	Sub-analyses (sun exposed and not exposed) Beta type more prevalent in RTR than IC (OR=21 P<0.001)
	31 NMSC (57 samples) 7 without (10 samples)	IS RTR			Patients / Samples Any HPV: 10.9 (0.9 to 153.2) / 6.9 (1.0 to 42.0) 94% / 57% 91% / 60% **In positive samples**: beta: 94% / 37% ; mucosal: 2% / 0%; cutaneous: 48% / 100%			Sub-analyses (sun exposed and not exposed) Beta types more prevalent in RTR than IC (OR=2.1 P<0.001

SCC: Squamous cell carcinoma; BCC: basal cell carcinoma; IC: Immunocompetent patients; IS: Immunosuppressed patients; OR: Odds ratios; CI: confidence interval; RTR: renal transplant recipient; AK: actinic keratoses; NK:not known; NSE: not sun exposed; SE: sun exposed; NS: Not significant at 5% level;
HPV: human papillomavirus; NMSC: non-melanoma skin cancer

Study, Year, Country	Number cases/controls	IC/IS	Sample	HPV type	OR unadjusted + 95% CI (% positive cases/controls)	OR adjusted + 95% CI (% positive cases/controls)	Adjustment variables/ Matching	Comment
Struijk *et al.*, 2006, Australia	64 SCC 58 tumour-free controls	IC	plucked eyebrow hairs (E7 gene)	5, 8, 15, 16, 20, 24 and 38		**HPV5:** 19% / 13% 0.6 (0.2-2.2) **HPV8:** 3% / 3% 0.4 (0.1-4.1) **HPV15:** 2% / 2% 0.6 (0.04-11.0) **HPV20:** 10% / 17% 0.9 (0.2-3.7) **HPV24:** 12% / 14% 0.8 (0.2-3.1) **HPV38:** 26% / 13% 0.3 (0.1-1..2)	Adjusted for age and sex	Baseline patients who were seronegative to any beta types
Forslund *et al.*, 2007, Sweden	82 SCC 126 BCC 92 benign lesions matched-pair healthy skin	IC	skin biopsies (L1 gene)	all		SCC: **beta species1:** 1.6 (0.7-3.4) **beta species 2**: 4.4 (1.9-10.1) BCC: **beta species1:** 1.2 (0.6-2.5) **beta species 2**: 1.7 (0.7-4.0)	Adjusted for age, sex, skin type, sunburns, eye color, location of lesion (sun exposed or not).	"Stripped" surface Higher HPV-DNA detection in UV-exposed skin. 3 different laboratories: 31% (219/698) samples positive to any HPV types, of whom 17% (120/698) were positive to identical HPV type (detection in at least 2 laboratories). 42 different types were found (37 beta, 3 gamma, 2 alpha). Most prevalent HPV20, HPV21, HPV38.
Luron *et al.*, 2007, France	54 patients with 40 SCC 27 BCC 9 healthy skin	Xeroderma Pigmentosum	skin biopsies (L1 gene)	all	SCC: Any HPV (22% / 50%): 3.5 (0.6- 37.7) Beta HPV (22% / 48%): 3.2 (0.5-34.2) Cutaneous HPV (11% / 8%): 0.6 (0.05-18.3) BCC: Any HPV (22% / 15%): 0.6 (0.07-8.2) Beta HPV (22% / 15%): 0.6 (0.07-8.2) Cutaneous HPV (11% / 4%): 0.1 (0.0-3.1)			Age between 4 and 35 years old. All biopsies from sun-exposed skin. Most prevalent: HPV24 (21%) and HPV 19 (21%) Problem: multiple samples from same patient (i.e HPV24 in 3 SCC from same patient).
Alotaibi *et al.*, 2006, Canada	8 SCC 47 healthy control	IC	swab samples (L1 gene)	beta	SCC: 7 (88%)/41 (87%) 1.0 (0.1-53.8)			All new putative HPV types for SCC.
Asgari *et al.*, 2008, USA	85 SCC (30 were in-situ SCC) Internal control: 72 perilesion 95 age-matched external controls (with two sample on from sun-exposed and one from not-sun exposed skin)	IC	skin biopsies (L1 gene)	all		**Lesion versus perilesion / combined cases-controls** **alpha-types:** 0.7 (0.2-2.4) / 2.8 (0.8-9.8) **beta-types:** 1.3 (0.6-2.7) / 0.7 (0.8-3.5) **gamma-types:** 1.5 (0.4-5.3) / 1.3 (0.5-4.0)	Frequency matched on age and sex Adjusted for propensity of sunburn	
Andersson *et al.*, 2008, Sweden (results on type in Forslund *et al.*, 2007, Sweden)	72 SCC 121 Benign	IC	skin biopsies (L1 gene)	alpha and beta		SCC: Any HPV: 2.1 (1.0-4.2) BCC: Any HPV: 0.96 (0.57-1.62)	Adjusted for sex, age, location tumour, previous sunburn and smoking.	58%, 39% and 39% of patients with SCC, BCC and benign lesions were positive in at least one of the three laboratories. Paired data (lesions and benign sample from same patient)
Patel *et al.*, 2008, USA	101 SCC 101 BCC	IC	skin biopsies (E1 gene)	beta		**SCC versus BCC:** Any beta-types: 1.5 (0.7-3.5) HPV5, 8, 15, 20, 24, 36, and/or 38: 2.6 (1.4-5.1) Beta species 1: 2.0 (1.0-3.6) Beta species 2: 1.4 (0.7-2.5) Beta species 3, 4 or 5: 1.0 (0.5-2.0)	Adjusted for age, sex, education, smoking, skin sensitivity to sunlight and lifetime sunburns.	Case-case design New laboratory method: reverse hybridization method HPV24 (9% versus 15%)

SCC: Squamous cell carcinoma; BCC: basal cell carcinoma; IC: Immunocompetent patients; IS: Immunosuppressed patients; OR: Odds ratios; CI: confidence interval; RTR: renal transplant recipient; AK: actinic keratoses; NK:not known; NSE: not sun exposed; SE: sun exposed; NS: Not significant at 5% level;
HPV: human papillomavirus; NMSC: non-melanoma skin cancer

APPENDIX B

HPV-DNA prevalence in patients with SCC or BCC

Study, Year, Country	Number of patients and/or specimens	Sample	HPV type	Prevalence (samples/patients) SCC		Prevalence (samples/patients) BCC		Primers	Comment
				IC	RTR	IC	RTR		
De Jong-Tieben *et al.*, 1995, The Netherlands	44 patients: SCC: 61 specs BCC: 8 specs	skin biopsies	beta		80% / -		50% / -	Nested PCR Degenerate (L1 gene)	Only beta HPV (6 new)
Shamanin *et al.*, 1996, UK,Germany	IC : SCC : 19 patients (26 specimens) BCC 9 patients (11 specimens) IS : SCC : 11 patients (20 specimens) BCC 4 patients (5 specimens)	skin biopsies	alpha (Low-risk mucosal) beta	31% / -	65% / -	36% / -	60% / -	PCR degenerate -16 different primers combination (L1 gene)	find 10 new HPV types (from HPV4 family) 20% were beta types: majority HR mucosal(16,51,54,56,61,69); cutaneous(41,60)
Boxman *et al.*, 1997, The Netherlands	25 patients (68 specimens) 9 patients (22 SCC)	skin biopsies	beta, cutaneous		91% / -			PCR degenerate (L1 gene)	New types. Most common: 1,27,57,38,23,DL40,DL267,20
De Villiers *et al.*, 1997, the UK and Germany	SCC : 9 patients (22 Specimens) BCC : 2 samples	skin biopsies	beta, cutaneous		HD primer: 50% / - AM primer: 82% / - total: 91%/-		HD primer: 0% / - AM primer: 0% / -	PCR degenerate (nested) HD primer (Shamanin 1994) AM primer (Berkhout 1995) (L1 gene)	7 new types HPV20 (21%), DL267 (18%), DL40(15%), HPV23 (9%), HPV38 (9%), HPV57 and HPV1 (6%)
Harwood *et al.*, 1999, the UK	16 SCC 6 BCC	skin biopsies (PUVA patients)	beta, cutaneous, alpha		79% / -		67% / -	PCR degenerate (nested) (L1 gene)	No type consistently found. HPV5,20,21,23,24,38
Boxman *et al.*, 2000, Australia	51 BCC 25 SCC:s	plucked hair	beta	- / 68%		- / 61%		PCR degenerate (L1 gene)	In eyebrow hairs of patients with skin cancers HPV38 (x24), X1(x22),37(x10),22,X11(x8),5,12,14,15 (x6)
Boxman *et al.*, 2000, Australia	14 BCC	skin biopsies	beta			- / 43%		PCR degenerate (L1 gene)	In skin cancers HPV38 (x24), X1(x22),37(x10),22,X11(x8),5,12,14,15 (x6)
Wieland *et al.*, 2000, Germany & Poland	61 BCC (69 specimens) Germany (n=31) Poland (n=38)	skin biopsies	beta, cutaneous, alpha (mucosal),			44% / - mainly beta: 41% / -		Nested PCR Degenerate (6 diff primers) (L1 gene)	Mainly HPV 8 and 20 (Germany); HPV38 & HPV Togawa in Poland;
Harwood *et al.*, the UK, 2000	IC: 51 patients (22SCC, 30BCC) IS: 32 patients (44SCC, 24BCC)	skin biopsies	Beta, cutaneous, alpha (mucosal)	27% / 27%	84% / 84%	37% / 37%	75% / 75%	Degenerate PCR (L1 gene)	New types Mixed infection in 57% samples Predominant beta (83%), cutaneous (54%), mucosal (15%)
Meyer *et al.*, 2000 Germany	IC: 10 patients (11 specimens) RTR: 14 patients (21 specimens)	skin biopsies	beta alpha(mucosal)	36% / 30%	57% / 50%			PCR degenerate & specific, MY09/11, MYN9/10 and CP65/70, CP66/69 (L1 gene)	Found: 5,6,8,11,14,15,16,22,26,25,36,70,
Berkhout *et al.*, 2000 The Netherlands	21 patients (351 specimens) 81 SCC , 14 BCC	skin biopsies	beta, cutaneous		78% / - beta-A: 40% beta-B: 51% beta-C : 28% A2/4: 22%		36% / - beta-A 0% beta-B: 21% beta-C: 29%	PCR degenerate (L1 gene)	beta-A: 5/8/12/14/19/20/21/25/36/47 beta-B: 9/15/17/22/23/37/38/49 beta-C: 24 16 new types
Biliris *et al.*, 2000 Greece	95 patients 3 SCC in 23 patients 22 BCC in 72 patients	skin biopsies	alpha (mucosal)	- / 13%		- / 31%		PCR degenerate & specific (L1 gene)	NMSC all HPV-DNA positive using PCR specific
De Jong Tieben *et al.*, 2000 The Netherlands	41 patients 45 SCC 6 BCC	skin biopsies	beta		73% / -		33% / -	PCR degenerate (nested) (L1 gene)	15 new types
O'connor *et al.*, 2001, Ireland	12 patients (12 SCC) 6 patients (9 SCC)	skin biopsies	beta, alpha(mucosal)	83% / 83%	88% / 83%			PCR degenerate (L1 gene)	New types.
Meyer *et al.*, 2001, Germany	IC: 13 patients IS: 15 patients (RTR, psoriasis, HIV, other treatment)	skin biopsies	beta, cutaneous, alpha(nmucosal)	54% / -	73% / -	50% / -	50% / -	PCR Degenerate (L1 gene)	Same study as 2003. In pre and malignant: beta-related (A9,A10) Higher prevalence for SCC in UV- patients vs UV+.
Boxman *et al.*, 2001 The Netherlands	SCC only: 15 BCC only: 69	plucked hairs	beta	75% / -		59% / -		PCR degenerate (nested) (L1 gene)	People with AK more likely to have skin cancers.
Struijk *et al.*, 2003, The Netherlands	64 SCC	plucked eyebrown hairs	beta: 5,8, 15,20,24,38 Genital:16 Oth:2	**All beta-HPV:** 75% **HPV5**: 23% **HPV16**: 0% **HPV8**: 17% **HPV2**: 0% **HPV15**: 17% **HPV20**: 33% **HPV24**: 25% **HPV38**: 32%				PCR degenerate (E7 gene)	
Iftner *et al.*, 2003, USA (California)	72 SCC 18BCC	skin biopsies	alpha (mucosal): 16,31,33,35, and 51 beta: 5,8,12,17,19,22, and 36 cutaneous	**All HPV:** 60% / - (with 9% beta-HPV & 21% cut. HPV)		28% / -		PCR degenerate (E1 gene)	HPV types: 33,4 (BCC: HPV16,27,33)
Meyer *et al.*, 2003 Germany	IC : 14 patients (15 specimens) RTR : 11 patients (16 specimens)	skin biopsies	beta, cutaneous, alpha (mucosal)	47% / 43%	75% / 73%			PCR Degenerate (L1 gene)	When all IS grouped: SCC: 81% Mainly HPV5 and HPV8 (mainly IS patients)

HPV: Human papillomavirus; SCC: Squamous cell carcinoma; IC: Immunocompetent patients; IS: Immunosuppressed patients; OR: Odds ratios; CI: confidence interval; RTR: renal transplant recipient; AK: actinic keratosis; NK: not known; n: number
NSE: not sun exposed; SE: sun exposed; NS: Not significant at 5% level; CIS: carcinoma in situ

Study, Year, Country	Number of patients and/or specimens	Sample	HPV type	Prevalence (samples/patients) SCC		Prevalence (samples/patients) BCC		Primers	Comment
				IC	RTR	IC	RTR		
Forslund *et al.*, 2003, Sweden & Australia	IC : BCC : 19 patients (19 specimens) ; SCC :11 patients (12 specs) IS: BCC: 5 patients (6 specimens); SCC: 11 patients (11 specimens)	skin biopsies	beta	33% / 33%	54% / 54%	21% / 21%	83% / -	FAP PCR (consensus) Type specific (HPV38, 92) (L1 gene)	Problem sensitivity. By type: No association B1:40% B2: 6% HPV38 mainly in AK Multiple infection IS:71% IC:60% CIS grouped with SCC.
Termorshuizen *et al.*, 2004, the Netherlands	156 SCC 320 controls	plucked eyebrown hairs	5 8 15 20 24 38	All HPV: 71% HPV5: 24% HPV8: 17% HPV15: 17% HPV20: 33% HPV24: 25% HPV38: 32%				PCR degenerate (E7 gene)	
Stockfleth *et al.*, 2004, Germany	IC : 64 patients (19 SCC, 56 BCC) RTR :18 patients (16 SCC, 8 BCC)	skin biopsies	all	37% / -	75% / -	48% / -	50% / -	PCR degenerate (nested) (L1 gene)	22 HPV types detected
Forslund *et al.*, 2004, Sweden	SCC: 31 BCC: 109	lesion swab/ lesion biopsies/ perilesional/ forhead/ buttock (swabs)	beta	58% / 19% / 81% / 94% / 81%		63% / 8% / 76% / 82% / 68%		PCR degenerate (consensus) (L1 gene)	Top of skin versus "stripped" biopsies Species 1: 89% Species 2: 41% Multiple 57% New types, subtypes
Struijk *et al.*, 2006, Australia	64 SCC	plucked hairs	5 8 15 20 24 38	HPV5: 13% HPV8: 3% HPV15: 2% HPV20: 17% HPV24: 14% HPV38: 13%				PCR degenerate (E7 gene)	
Forslund *et al.*, 2007, Sweden	SCC: 82 BCC: 126	skin biopsies	alll	**beta species1:** 15% **species2:** 17%		**beta species1:** 10% **species2:** 7%		PCR degenerate (3 laboratories) (L1 gene)	Higher HPV-DNA detection in UV-exposed skin "Stripped" surface Detection in at least 2 laboratories
Asgari *et al.*, 2008, USA	85 SCC (30 were in-situ SCC) Internal control: 72 peri-lesion 95 age-matched external controls (with two sample on from sun-exposed and one from not-sun exposed skin)	skin biopsies	all	**any:** 54% **alpha:** 8% **beta:** 49% **gamma:** 8%				PCR degenerate (L1 gene)	
Andersson *et al.*, 2008, Sweden (results on type in Forslund, 2007, Sweden)	SCC: 72 benign: 121	skin biopsies	alpha, beta, cutaneous	any: 58%		any: 39%		PCR degenerate (3 laboratories) (L1 gene)	Detection in at any laboratories
Patel *et al.*, 2008, USA	SCC: 101 BCC: 101	skin biopsies	beta	any: 84% HPV5, 8, 15, 20, 24, 36, and/or 38: 62% **Species 1:** 64% **Species 2:** 33% **Species 3,4,5:** 33%		any: 78% HPV5, 8, 15, 20, 24, 36, and/or 38: 41% **Species 1:** 47% **Species 2:** 57% **Species 3,4,5:** 30%		PCR degenerate (E1 gene)	

SCC & CIS are mixed in some study (Forslund *et al.*, 2003: Asgari *et al.*, 2008)

HPV: Human papillomavirus; SCC: Squamous cell carcinoma; IC: Immunocompetent patients; IS: Immunosuppressed patients; OR: Odds ratios; CI: confidence interval; RTR: renal transplant recipient; AK: actinic keratosis; NK: not known; n: number

NSE: not sun exposed; SE: sun exposed; NS: Not significant at 5% level; CIS: carcinoma in situ

APPENDIX C

HPV-DNA prevalence in hair follicles, normal skin, psoriasis, viral warts, actinic keratoses and carcinoma in-situ, peri-lesional samples and other disease.

PLUCKED HAIR

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Boxman *et al.*, 1997, The Netherlands	IC : 22 patients (38 specimens) RTR : 26 patients (49 specimens)	plucked hairs	beta	53% / 45%	92% / 100%	PCR degenerate (L1 gene)	New types Small sample size
Boxman *et al.*, 1999, The Netherlands.	IC :135 patients With NMSC (N=69) Without(N=66) IS :31 patients With NMSC(N=19) Without(N=12)	plucked hairs	5	With: 16% Without: 15%	With: 37% Without: 58%	PCR (nested) (HPV-5 specific) (L1 gene)	No difference with and without NMSC. Difference between IC versus IS
Boxman *et al.*, 2000, Australia	64 pairs 32 patients with BCC or SCC 32 controls	plucked hairs	beta	With: 63% / - Without: 67%		PCR degenerate (L1 gene)	NS positive association in eyebrown hairs with SCC (68% vs 56%). New types
Boxman *et al.*, 2001, Australia	276 patients with AKS 231 without Aks	plucked hairs	beta	56% 36%		PCR degenerate (L1 gene)	Unadjusted OR: 1.25 (1.55-3.28) Strata age: association only in men. HPV38 (17%)
Meyer *et al.*, 2001, Germany	63 patients (samples ?)	Plucked hairs	beta, cutaneous, alpha (mucosal)	47%/-		PCR degenerate (L1 gene)	Grouped IS and IC, Highest Prevalence after SCC & BCC
Wolf *et al.*, 2004, Austria & Germany	Gp A : PUVA + a least 1 Skin Ca. (16 patients) Gp B:PUVA + no skin Ca (35 patients) Gg C: no PUVA + no skin Ca (30 patients)	plucked hairs (in patients with psoriasis)	beta, alpha	Gp A: 73% Gp B: 69% Gp C: 36% (P<0.01 adjusted age)		PCR degenerate (L1 gene)	Mucosal/genital = 0% Majority beta: 68% Most frequent: HPV38: 15%, (not in Gp C) HPV 25: 8%, IA16 (8%) 10 types (5, 14d, 17, 24, 25, 37, 38, 51, 61, 80) & 20 new types.
De Koning *et al.*, 2006, The Netherlands	23 patients over 2 year period	plucked hairs	beta	Positive to any HPV DNA over the 2 years: 96% (22/23) and at 6 months (74%)		PM-PCR RAH (E1 gene)	Persistence of beta-HPV on healthy individuals

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; Gp, group; RHA: reverse hybridization assay; HPV: Human papillomavirus
NS: non-significant at 5% level

SKIN SWABS

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Forslund *et al.*, 1999, Sweden	4 patients (18 specimens)	normal skin	beta	39%/75%		PCR degenerate (L1 gene)	37% multiple HPV infection 12 new HPV types (mainly POS forehead) Various lesions (MM, MNSC, AK) Lesions: HPV 5,8,12,20-24 Normal: HPV12,49, new types Mixed infection 37% Different techniques = different prevalence.
Antonsson *et al.*, 2000, Sweden	IC : 80 patients (480 specimens) RTR : 52 patients (260 specimens) Dialysis: 28 patients (140 specimens)	skin swabs	beta, cutaneous alpha (mucosal)	-/ 80%	-/ 94% -/ 83%	PCR degenerate (L1 gene)	30 new types HPV DNA more common in forehead than arms and thighs
Alotaibi *et al.*, 2006, Canada	47 IC 8 RTR	skin swabs	beta	- / 87%	- / 100%	PCR degenerate (L1 gene)	
Hazard *et al.* 2007, Sweden	42 IC 31 RTR	skin swabs (forehead) 2 samples (mean time: 6.3 years)	gamma, beta and lambda types	-/69% (1st sample) - / 71% (2nd sample) (-/48% persistent infection)	-/ 71% at 1st sample - / 90% at 2nd sample (-/33% persistent infection)	PCR degenerate (L1 gene)	HPV persistence Follow-up of subject from Antonsson (2000). Many new putative HPV types. Most common prevalent: HPV20

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus

PERILESION

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Astori *et al.*, 1998, Germany & Japan	14 patients (21 specimens) 6 peri. / 15 eyelid	perilesional skin eyelid skin	beta	50% / - 27% / -		PCR degenerate (L1 gene)	New types (from 24, 8, 12 &15 family) Small sample size Main: HPV20,23,38,40 and DL267
Wieland *et al.*, 2000, Germany & Poland	perilesional to BCC N=31	perilesional	beta, cutaneous, alpha (mucosal)	26%/-		PCR degenerate (L1 gene)	New types. HPV 8,9,20,21,24, DL297,DL 298
Forslund *et al.*, 2003, Sweden & Australia	IC : 38 patients (41 specimens) IS :21patients (Peri: 23 specimens/B :21 specimens) Lesions (SCC, BCC, Aks)	perilesional/ buttock	beta	perilesional 71% Buttock 58%	perilesional 83% Buttock 67%	FAP PCR (consensus) Type specific (HPV38,92) (L1 gene)	Multiple infection: 47% of perilesional and 30% of buttocks swabs. Peri/Buttock Species 1: 69% / 59% Species 2: 11% / 10%
Forslund *et al.*, 2004 Sweden	229 specimens	perilesional/ forehead/ buttock	beta	Perilesional/ forehead/ buttock SCC: 81%/94%/81% BCC: 76%/82% 68% AK: 89%/89%/69%		PCR (consensus) (L1 gene)	Multiple types more frequently detected in swaps than biopsies Species1 : 89% Species 2: 41% Multiple 57%
Asgari *et al.*, 2008, USA	72 perilesion	skin biopsies	all	**All types:** 50% **Alpha-types:** 10% **Beta-types:** 44% **Gamma-types:** 6%		PCR degenerate (L1 gene)	

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NORMAL SKIN

Study, Year, Country	Numbers	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Astori *et al.*, 1998, Germany & Japan	15 patients (20 specimens)	normal skin	beta	35%/-		PCR degenerate (L1 gene)	New types (from 24, 8, 12 &15 family) Small sample size Main: HPV20,23,38,40 and DL267
Favre *et al.*, 1998, France & Poland	6 patients (6 specimens.)	uninvolved skin (psoriasis patients)	beta,	100%/-		PCR degenerate PCR type specific: 1,5,16,36 (L1 gene)	HPV5 (83%) HPV20,38,RTRX7
Favre *et al.*, 1998, France & Poland	15 patients (15 specimens.)	uninvolved skin (atopic dermatitis)	beta	40%/-		PCR degenerate PCR type specific: 1,5,16,36 (L1 gene)	HPV36 20% HPV15,24,ADX1,ADX2 Not HPV5 as in psoriasis
Harwood *et al.*, 1998, the UK	11 patients (12 specimens) (>=500 J PUVA per cm2)	normal skin (PUVA)	beta, cutaneous alpha (mucosa)		33%/-	PCR degenerate (nested) (L1 gene)	// renal transplant. Not types consistently found. HPV21,23,RTRX2
Forslund *et al.*, 1999, Sweden	12 patients (8 specimens)	normal skin of patients with lesions	beta	63%/-		PCR degenerate (L1 gene)	37% multiple HPV infection 12 new HPV types (mainly POS forehead) Various lesions (MM,MNSC, AKS)?? Lesions: HPV 5,8,12,20-24 Normal: HPV12,49, new types Mixed infection 37% Different techniques = different prevalence
Weissenborn *et al.*, 1999, Austria & Poland	42 patients	normal skin	beta, alpha (mucosal)	19%/-		PCR (nested, specific) (L1 gene)	HPV36 (10%)
Favre *et al.*, 2000, France & Poland	8 patients	uninvolved skin (Bullous disease)	beta	75%		PCR degenerate PCR type specific: 5 (L1 gene)	HPV 5 , 15 < X3
Favre *et al.*, 2000, France & Poland	10 patients	uninvolved skin (connective tissue disease)	beta	90%/-		PCR degenerate PCR type specific: 5 (L1 gene)	HPV 45, X4, X5, X6,X8
De Jong-Tieben *et al.*, 2000, The Netherlands	28 patients (4 with NMSC and 14 without)	normal skin	beta		11%/- (without NMSC: 0%/-) (with 13%/-)	PCR degenerate (L1 gene)	Higher Prevalence in sun exposed areas but in patients with skin cancers. HPV5,X20
Berkhout *et al.*, 2000, The Netherlands	31 patients (31 specimens)	normal skin	beta, alpha (cutaneous)		32% /32%	PCR degenerate (L1 gene)	HPV 2,5,24,29,57,X20 17 new types detected Benign lesions (39%)
Meyer *et al.*, 2001, Germany	IC : 57patients / RTR : 5 patients	normal skin	beta, cutaneous, alpha (cutaneous)	16%	20%	PCR Degenerate (L1 gene)	Same proportion in UV+/UV- patients
O'Connor *et al.*, 2001, Ireland	IC :4 patients (4 samples) RTR : 5patients (5 specimens)	normal skin (some patients with lesions; IS 3/5 and IC 3/4)	beta, cutaneous, alpha (mucosal)	50%/50%	20%/20%	PCR degenerate (L1 gene)	Mixture of IC and RTR to report results? HPV:9,15,17,23,24,37,38-rel (cutaneous: HPV7)
O'Connor *et al.*, 2001, Ireland	IC : 20 patients (20 samples)	normal skin	beta, cutaneous, alpha (mucosal)	15%/15%		PCR degenerate (L1 gene)	Mixture of IC and RTR to report results? HPV:9,15,17,23,24,37,38-rel (cutaneous: HPV7)
Iftner *et al.*, 2003, Germany & California	106 patients	normal skin	beta, cutaneous, alpha (mucosal)	5% / -		PCR degenerate (E1 gene)	
Meyer *et al.*, 2003, Germany	IC : 56 patients (56 specimens) RTR : 6 patients (6 lesions)	normal skin (punch biopsies)	beta, cutaneous, alpha (mucosal)	~13%	~15%	PCR degenerate (L1 gene)	Less types detected 13/63 normal skin taken to adjacent NMSC Higher Prevalence in perilesional.
Harwood *et al.*, 2004, UK	IC : 39 patients (57 samples) RTR : 38 patients (67 samples)	normal skin	beta, cutaneous	35%/39%	87%/87%	PCR degenerate (L1 gene)	17 new types No difference in HPV types detected in sun-exposed and not exposed areas
Alotaibi *et al.*, 2006, Canada	47 healthy skin	skin swabs	beta	- / 87%		PCR degenerate (L1 gene)	
Forslund *et al.*, 2007, Sweden	92 benign lesions Healthy skin	normal skin	Beta	**Beta species1:** 14% **Beta species2:** 8%		PCR degenerate (L1 gene)	"Stripped" surface
Asgari *et al.*, 2008, USA	95 healthy skin (sun exposed: 95 and not sun-exposed: 95)	skin biopsies	all	**Sun exposed / Not sun-exposed** **All types:** 59%/49% **Alpha-types:** 3%/4% **Beta-types:** 54%/45% **Gamma-types:** 7%/3%		PCR degenerate (L1 Gene)	

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus; UV: ultraviolet

ACTINIC KERATOSIS (AK) AND BOWEN'S DISEASE (BD or CIS)

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Favre *et al.*, 1998, France & Poland	16 patients (16 specimens.)	Atopic dermatitis	beta	31%/-		PCR degenerate PCR type specific: 1,5,16,36 (L1 gene)	HPV3 0% HPV21,24,38
Harwood *et al.*, 2000, the UK	IC : 11 RTR : 17	AK and CIS	beta, cutaneous, alpha (mucosal)	55%/-	88%/-	PCR degenerate (L1 gene)	New types
Bilitis *et al.*, 2000, Greece	13 patients	AK and CIS	beta, cutaneous, alpha (mucosal)	31%/31% AK: 25% BDs:40%		PCR(consensus) -mucosal PCR (type specific): 1,2,5,8,11,16,18,33 (L1 gene)	High Prevalence in selected NMSC of HPV8,18,5 HPV18 (x3),HPV8(x2),HPV11(x1) (not present:1,2,16,33
Beckhout *et al.*, 2000 The Netherlands	11 patients with Aks (56 samples)	AK Skin biopsies	beta, cutaneous		68%/-	PCR degenerate (L1 gene)	New types Benign lesions (39%)
De Jong-Tieben *et al.*, 2000 The Netherlands	37 samples (from 75 pstients)	AK Skin biopsies	Beta		49%/-	PCR degenerate (L1 gene)	New types No difference patients with and without NMSC HPV5,X20
Lampert *et al.*, 2000, France	12 patients	Extra-genital BD	Mucosal (16) 6/11	25%/-		-PCR (consensus& type specific) Mucosal -In-situ PCR 6/11,16 (L1 gene)	BD: 6 /11: 2/3 (67%) 16: 1/3 (33%)
Derancourt *et al.*, 2001 France	28 patients	Extra-genital Bowen Disease	-6/11, 1/18,31/33/51	29%/-		In-Situ hybridation	BD: Hpv31/33/51: 2/8 (25%) hpv16:/18 8/8 (100%)
Mitsuishi *et al.*, 2001 Japan	Case report	Bowen disease of the elbow	58	100%/-		PCR consensus -Mucosal (L1 gene)	
De Villiers *et al.*, 1997, the UK and Germany	12 patients (17 specimens.)	AK & Verrucous Keratoses (different primers)	beta, cutaneous		HD primer: 24%/- AM primer: 59%/- total: 65% /-	PCR degenerate (nested) HD primer (Shamanin 1994) AM primer (Beckhout 1995) (L1 gene)	HPV 38 (12%), HPV 23 (12%), HPV 20 (12%), HPV 57 (6%)
Meyer *et al.*, 2001, Germany	Aks : IC : 51patients / RTR : 0 patients BDs : IC : 26patients / RTR : 6 patients	AK & BD	beta, cutaneous, alpha (mucosal)	22%/- 35%/-	0%/- 17%/-	PCR degenerate (L1 gene)	beta Higher proportion in UV+ patients
Meyer *et al.*, 2003, Germany	IC : 36 patients RTR : 26 patients	Bowen disease/Aks (punch biopsies)	mucosal, cutaneous	~33%	~35%	PCR degenerate (L1 gene)	44 types detected
Iftner *et al.*, 2003, Germany & California	71 patients	AK	beta, cutaneous, alpha (mucosal)	58% / -		PCR degenerate (E1 gene)	Problem of detection HPV types: 1, 2, 3, 4, 7, 27, 57.
Forslund *et al.*, 2003, Sweden & Australia	IC : 10 patients (10 specimens) IS : 6patients (6 specimens) Lesions (SCC, BCC, Aks)	AK	beta	70%	33%	FAP PCR (consensus) Type specific (HPV38,92) (L1 gene)	HPV38 (63% patients with Ak) Tested biopsies/perilesional and buttock swabs Higher Prevalence in perilesional and (buttock) swabs of patients with AK
Iftner *et al.*, 2003, Germany & California	20 patients	BD	beta, cutaneous, alpha (mucosal)	70% / -		PCR degenerate (E1 gene)	Problem of detection HPV types: 1,2,3,4,7,27,57,6,16,33,35,5,8,19,36.
Forslund *et al.*, 2004, Sweden	46 specimens	AK (different methods)	beta	Lesion swabs: 83% Lesion biopsies: 11%		PCR (consensus) (L1 gene)	Multiple types more frequently detect in swaps than biopsies B1 group: 89% B2 group: 41% Multiple 57%
Alotaibi *et al.*, 2006, Canada	12 AK	AK	beta	-/ 100% mainly beta1 and beta2		PCR degenerate (L1 gene)	
Forslund *et al.*, 2007, Sweden	49 patients	AK (biopsy)	beta	22%		PCR degenerate (L1 gene)	Higher HPV-DNA detection in UV-exposed skin
Andersson *et al.*, 2008, Sweden (results on type in Forslund, 2007, Sweden)	81 AK	AK	alpha, beta	- / 38%		PCR degenerate (L1 gene)	
Dianzani *et al.*, 2008, Italy							

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus; UV: ultraviolet
CIS: carcinoma in situ; AK: Actinic Keratosis; BD: Bowen's disease.

PSORIASIS

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Zumbotel *et al.*, 2000, France	2 patients (4 SCC)	Psoriasis patients PUVA	beta, HPV5	75%/-		-PCR (consensus& type specific) Southern blot hybridation (L1 gene)	
Rust *et al.*, 2001, USA	1 female (9 lesions: 7 SCC)	Psoriasis +high UV radiation (not PUVA)	beta	78%/- SCC:100%/-		PCR degenerate (L1 gene)	HPV5: 5/9 HPV12: 1/9 HPV17: 1/9
Favre *et al.*, 1998, France & Poland	Plaque : 28 patients (32 specimens) Guttate : 9 patients (10 specimens)	Psoriasis	beta	90%/- 80%/-		PCR degenerate PCR type specific: 1,5,16,36 (L1 gene)	HPV5 90% HPV36,20,15,38,9,15,17,24,37,21
Weissenborn *et al.*, 1999, Austria & Poland	54 patients	Psoriasis	beta, alpha (mucosal)	83%/-		PCR (nested, specific) (L1 gene)	HPV36 (62%), HPV5 (36%), HPV38 (24%) Higher Prevalence in patients with PUVA treatment 100% (4 patients) vs 73% (22 patients)
Vivianom *et al.*, 2007, Italy	38 patients with psoriasis 36 age/sex matched healthy patients	Swabs in psoriasis patients and healthier patients	beta	Psoriasis: - / 71% Controls: - / 58%		PCR degenerate (L1 gene)	No type specific HPV20 (psoriasis: 15%, control 19%) HPV 5 (2 patients with psoriasis and none from control group)
Cronin *et al.*, 2008, UK	20 patients with psoriasis (18 skin scrapes & 18 hairs) 23 controls (20 skin scrapes & 22 hairs)	Psoriasis & controls	beta	Psoriasis: 83% / - Controls: 47% / -		PCR degenerate Type specific 5, 36 , 21 (L1 gene)	P=0.03 (age & cluster adjustment) Multiple beta-infection: 36% versus 16% (P=0.3) HPV5 or 36 no difference.

SCC: Squamous cell carcinoma; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus; UV: ultraviolet

VIRAL WARTS

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Meyer *et al.*, 2003, Germany	IC : 51 patients RTR : 22 patients	Viral warts (punch biopsies)	cutaneous, alpha (mucosal)	~94%	~92%	PCR degenerate (L1 gene)	A2,A4,B2,E1 Mmainly HPV: 1,2,3,4,10,27,57,7,40,72
Berkhout *et al.*, 2000, The Netherlands	- patients (12 specimens)	Viral warts	beta, cutaneous		75%/-	PCR degenerate (L1 gene)	HPV 2,5,24,29,57,X20 17 new types detected Benign lesions (39%)
O'Connor *et al.*, 2001, Ireland	IC : 5 patients (8 samples) RTR : 9 patients (11 specimens)	Viral warts	beta, cutaneous, alpha (mucosal)	100%/100%	100%/100%	PCR degenerate (L1 gene)	Mixture of IC and RTR to report results? HPV 9,15,17,23,24,37,38-rel (Cutaneous: HPV7)
Iftner *et al.*, 2003, Germany & California	209 patients	Viral warts	beta, cutaneous, alpha (mucosal)	91% / -		PCR degenerate (E1 gene)	Problem of detection HPV types: 1,27,57,2,10,4,65,16,31,33
Harwood *et al.*, 1999, the UK	23 patients (51 warts)	Viral warts	beta, cutaneous alpha (mucosal)		cutaneous: 84% mucosal: 27% beta: 80%	PCR nested degenerate (L1 gene)	HPV 27 more frequent Cutaneous: 84% Mucosal: 27% beta: 80%
Harwood *et al.*, 1998, the UK	4 patients (5 specimens)	Viral warts (PUVA)	beta, cutaneous, alpha (mucosal)		80%/-	PCR degenerate (nested) (L1 gene)	// renal transplant. Not types consistently found.
De Villiers *et al.*, 1997, the UK and Germany	8 patients (15 specimens.)	Viral warts (different primers)	beta, cutaneous		HD primer: 93%/- AM primer: 100%/- total: 100% /-	PCR degenerate (nested) HD primer (Shamanin 1994) AM primer (Berkhout 1995) (L1 gene)	HPV 57 (27%), HPV 27 (20%), HPV 1 (20%), HPV 23 (13%)
Meyer *et al.*, 2001, Germany	IC : 39 patients RTR : 20 patients	Viral warts	beta, cutaneous, alpha (mucosal)	92%	90%	PCR degenerate (L1 gene)	A,A2, A4 (cutaneous types: HPV2,3,7,10,27,57)

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus

OTHER DISEASES

Study, Year, Country	Number	Sample	HPV type	Prevalence (samples/patients) IC	Prevalence (samples/patients) IS	PCR detection methods	Comment
Schell *et al.*, 2000 Greece	2 patients	Verrucous carcinoma of the foot _SCC	Alpha (mucosal: 16)	100%/-		PCR (consensus& type specific) (L1 gene)	
Forslund *et al.*, 2000, Sweden	Uterine cervix (39 patients/ samples) Up. Limbs (40 patients/43 samples) Fingers (15 patients/samples)	UC Up.limbs Fingers (SCC/BD)	HPV 16/ 18/33/70/73	UC: 49%/- Fing: 67%/- Up.limbs: 7%/- HPV16: UC: 41%/- Fing: 53%/- Up.Limbs: 5%/-		PCR degenerate (L1 gene)	
Baldursson *et al.*, 2000, Sweden	58 patients (31 lesions of patients with SCC, 35 without)	Venous Ulcer SCC	beta, alpha (mucosal)	Leg ulcer SCC: 0%/- Leg ulcer without: 28%/-		PCR degenerate (L1 gene)	No HPV in any skin cancers No beta in any samples
Weber *et al.*, 2000 Germany/Austria	3 patients	Netherton's syndrome	beta, alpha (mucosal)	SCC: 60% (3/5) BCC: 33% (1/3)		PCR degenerate (L1 gene)	Beta only found in NMSC
Favre *et al.*, 2000, France & Poland	19 patients	Bullous disease	beta	74%/-		PCR degenerate PCR type specific: 5 (L1 gene)	HPV9, 5, 36, X1, X2
Favre *et al.*, 2000, France & Poland	7 patients	Connective TISue disease	beta	100%/-		PCR degenerate PCR type specific: 5 (L1 gene)	HPV25, 36,X4,X5,X6,X7

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; NMSC: non-melanoma skin cancer; IC: Immunocompetent patients; IS: Immunosuppressed patients; HPV: Human papillomavirus

APPENDIX D

Questionnaire

QUESTIONNAIRE

STUDY: Skin cancer among organ transplant recipients

Thank you for taking part in this study. Please complete this questionnaire prior to your scheduled appointment time. Please use black ink and capital letter. If you have any difficulties, you can discuss them with our nurse at your appointment.

All information that you supply to us will remain strictly confidential

Study ID_____ (for office use)

Date of questionnaire completion __/__/____

Hospital _____ (for office use)

Hospital number _____ (for office use)

Interviewer _____ (for office use)

Surname_____

Forename_____

Address _____

_____

_____

_____

POST CODE_____

Date of birth ___/___/_____

Sex: (please circle one) **MALE / FEMALE**

Country of birth_____

Name of GP_____

Address of GP _____

_____

_____

_____

POST CODE_____

Q1 **How many brothers and sisters do you have?** _____

Q2 **In your family, were you the first, second, third, etc born?** _____

Q3 **Did you regularly share a bedroom as a child?** (circle one) YES / NO

Q4 **Did you regularly share a bed as a child?** (circle one) YES / NO

Q5 **Are you currently married or living with a partner?** (circle one) YES / NO

Q6 **How many children do you have?** _____

Q7 **How many people usually live together in your household?** _____

Q8 **What is the highest technical, professional or academic qualification you have completed?** (tick one)

"O" level or equivalent	
"A" level or equivalent	
college/university degree	
clerical or commercial qualifications (eg secretarial, apprenticeships)	
None of these, other	

(a) If other, please specify: _____

(b) At what age did you leave school? _____**years**

Q9 **Do you or have you ever regularly smoked cigarettes?** (tick one)

NO	
YES I am a **current** smoker	
YES I am an **ex**-smoker	

If your answer is "NO" please go to Question 10 (Q10)

(a) On average, how many cigarettes do you smoke or did you used to smoke each day? (circle one)

Less than 5 5-9 10-19 More than 20

(b) At what age did you start smoking? _____

(c) If you no longer smoke, how long ago did you stop? _____

Q10 During the last year, about how often have you drunk alcohol? (Tick one)

Never	
Former drinker, but now stopped	
< 1 day per week	
1-3 days per week	
3-6 days per week	
Daily	

If your answer is "Never" please go to Question 11 (Q11)

(a) On days when you drink, about how many glasses of alcohol do you have? (*a "glass" is equivalent to a small bottle of beer; a glass of wine; a single measure of spirit*)

Beer_____
Wine_____
Spirits_____

(b) On days when you drink, when do you usually take alcohol? (circle one)

With a meal Not with a meal No regular pattern

Q11 How often do you exercise enough to make you sweat or get out of breath? (circle one)

Never/rarely less than once a week once a week 2-3 times a week most days

Q12 Current height_____

Q13 Current weight_____

Q14 Ethnicity: (circle one)

White Asian Oriental Black Other_____

Q15 What is your current occupation? _____

Q16 Have you ever had an outdoor occupation? (circle one) YES / NO

(a) If yes, which one? _____

and for how many years?_____ years

Q17 Have you ever had any outdoor hobbies? (circle one) YES / NO

(a) If yes, which one(s)? _____

and for how many years?_____ years

Q18 Have you ever lived abroad? (circle one) YES / NO

If your answer is "NO" please go to Question 19 (Q19)

Could you please fill in all places you have lived, for at least 6 months, starting from when you were born?

From (Age)	To (Age)	Town	Country

Q19 In the last year, about how many times did you visit a renal doctor? (circle one)

never once twice three times four or more times

Q20 In the last year, about how many times did you visit a skin doctor? (circle one)

never once twice three times four or more times

Q21 Have you ever been given advice on how to protect your skin from sunlight from any of the following?

Renal doctor: Yes / No / Can't remember (circle one)

Skin doctor: Yes / No / Can't remember (circle one)

Renal nurse: Yes / No / Can't remember (circle one)

GP: Yes / No / Can't remember (circle one)

Media: Yes / No / Can't remember (circle one)

Other person: Yes / No / Can't remember (circle one)
If yes, from who? _____

(a) When did you get this advice?

Before my first transplant: Yes / No / Can't remember (circle one)

After my first transplant: Yes / No / Can't remember (circle one)

(b) How many times have you been given this advice? (circle one)

Never Only once A few times Often Can't remember

(c) Have you ever received written advice (eg a leaflet) about protecting yourself from sunlight? (circle one)

Yes / No / Can't remember

Q22 Skin type: (tick one)

Never tans, always burns	
Rarely tans, usually burns	
Usually tans, can burn	
Always tans, rarely burns	
Asian/Middle Eastern	
African/Afro-Caribbean	

Q23 Eye colour: (tick one)

dark brown	
hazel	
green	
blue	

Q24 What was your NATURAL hair colour at 18 years of age? (Tick one)

Black	
Dark brown	
Light brown	
Blonde	
Red	

Q25 Do you use suncream? (circle one)

Never Sometimes Usually Always

If your answer is "Never" please go to Question 26 (Q26)

(a) How do you use it? (circle one)

Only when it is sunny Daily all year Daily for part of year

(b) If you use it daily, which months would you use it in the UK? (please circle each relevant month)

January February March April May June

July August September October November December

(c) What factor suncream do you mainly use? (circle one)

2-5 8-10 15-25 more than 25

(d) Approximately how many tubes of suncream do you use each year? (circle one)

None 1 2 3 4 more than 4

(e) Where do you apply suncream? (circle one)

Face only Face and hands All exposed areas

Q26 During the summer, do you specifically try and avoid being directly exposed to the sun? (circle one)

Never Sometimes Usually Always (circle one)

If your answer is "Never" please go to Question 27 (Q27)

(a) If you try and avoid being directly exposed to the sun, which times would you do this? (please circle all relevant times)

9am 10am 11am 12pm 1pm 2pm 3pm 4pm 5pm 6pm

Q27 Do you dress to protect yourself against the sun (eg wear a hat)? (circle one)

Never Sometimes Usually Always

Q28 Do you ever go on holiday to sunny countries? (circle one) YES/NO

If yes, how often? (circle one)

Once every 2-3 years or less Once a year More than once a year

Q29 What do you understand are the main reason(s) why transplant patients should take extra precautions in the sun? (please answer in one or two sentences)

.....

.....

.....

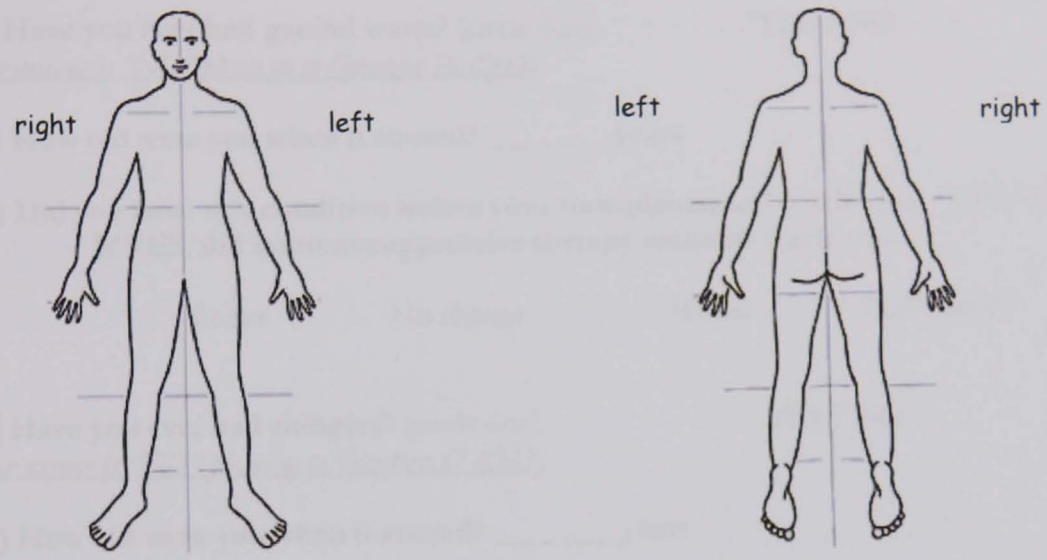
Q30 Have you ever had skin cancer diagnosed? (circle one) YES / NO

If your answer is "NO" please go to Question 31 (Q31)

If yes, what year(s) was it first diagnosed? _____

How many skin cancers have you had diagnosed? _____

Draw on the figure below the position of your skin cancer; *(if you have had more than one, please draw these on the figure also, as well as the year they were diagnosed)*



Q31 Have you ever had any other cancer before? (circle one) YES / NO

(a) If yes, what type(s)? _____

(b) And what year(s) was it diagnosed? _____

Q32 Has anyone in your family had SKIN CANCER before? (circle one)

YES / NO / DON'T KNOW

If yes, was it your…? (circle one or more)

Mother/Father Brother/Sister Son/Daughter Aunt/Uncle Other_____

Q33 Has anyone in your family had any OTHER CANCER before? (circle one)

YES / NO / DON'T KNOW

If yes, was it your…? (circle one or more)

Mother/Father Brother/Sister Son/Daughter Aunt/Uncle Other_____

If yes, what cancer(s)_____

Q34 Have you ever had skin warts? (circle one) YES / NO
If your answer is "NO" please go to Question 35 (Q35)

a) How old were you when it started? _____years

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q35 Have you ever had genital warts? (circle one) YES / NO
If your answer is "NO" please go to Question 36 (Q36)

a) How old were you when it started? _____years

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q36 Have you ever had shingles? (circle one) YES / NO
If your answer is "NO" please go to Question 37 (Q37)

a) How old were you when it started? _____years

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q37 Have you ever had acne? (circle one) YES / NO

If your answer is "NO" please go to Question 38 (Q38)

a) How old were you when it started? _____years

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q38 Have you ever had eczema? (circle one) YES / NO / DON'T KNOW

If your answer is "No" please go to Question 39 (Q39).

a) How old were you when it started? _____years

b) If you no longer have eczema, how long ago did it stop? _____

c) Please give details of relapses or flares in disease: .....
.....

.....

d) What is your current treatment?_____

e) What was your previous treatment? (circle one)

hospitalisation/uv treatment/tablets/topical/other_____

f) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

g) Do you have a family history of eczema? (circle one) YES / NO

If YES, who in the family is affected?

Mother/Father Brother/Sister Son/Daughter Aunt/Uncle Other_____

h) Do any of the following make your eczema worse? (circle one or more)

Wool Cat Household dust Grass pollen Tree pollen Drugs Others

If "others" or "drugs", please specify: _____

Q39 Have you ever had asthma? (circle one) YES / NO

If your answer is "NO" please go to Question 40 (Q40)

a) How old were you when it started? _____**years**

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

c) Do any of the following make your asthma worse? (circle one or more)

Wool Cat Household dust Grass pollen Tree pollen Drugs Others

If "others" or "drugs", please specify: _____

Q40 Have you ever had hayfever? (circle one) YES / NO

If your answer is "NO" please go to Question 41 (Q41)

a) How old were you when it started? _____**years**

b) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

c) Do any of the following make your hayfever worse? (circle one or more)

Wool Cat Household dust Grass pollen Tree pollen Drugs Others

If "others" or "drugs", please specify: _____

Q41 Have you ever had nettle rash/urticaria? (circle one) YES / NO

If your answer is "NO" please go to Question 42 (Q42)

a) How old were you when it started? _____**years**

b) Do any of the following make it worse? (circle one or more)

Wool Cat Household dust Grass pollen Tree pollen Drugs Others

If "others" or "drugs", please specify: _____

c) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q42 Do you suffer from psoriasis? (circle one) YES / NO

If your answer is "NO" please go to Question 43 (Q43)

a) How old were you when it started? _____**years**

b) How many patches do you currently have? (circle one)

1-2 3-4 5-9 10+

c) Did you have this condition before your transplantation? (circle one) YES / NO
If YES, did immunosuppressive therapy make it? (circle one)

Better No change Worse Don't know

Q43 **What is your renal diagnosis?**_____

Q44 **In what year was your renal disease first diagnosed?**_____

Q45 **Have you ever been on dialysis?** (circle one) YES / NO

(a) If yes, how many years in total were you on dialysis?_____

(b) What type of dialysis did you have? (circle one)

Haemodialysis / Peritoneal dialysis

Q46 **In what year did you first have a renal transplant?** _____

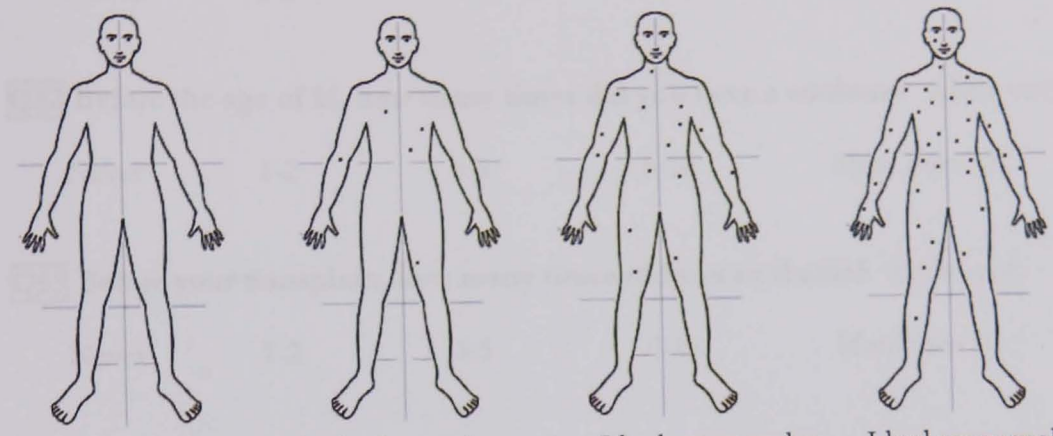
Q47 **How many transplants have you had?** _____

Q48 **Prior to transplantation, for how many years were you on dialysis?** _____

IN THE PERIOD **BEFORE** YOUR TRANSPLANT

Q49 About how many MOLES did you have on your skin?

Match your answers with the pictures below (*Moles are small brown, black or pink, either raised or flat skin markings that do not change after sun exposure*) *Please tick one box*

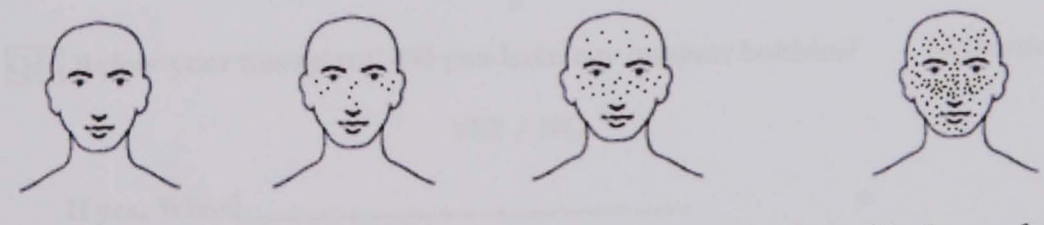


I had no moles | I had a few moles | I had some moles | I had many moles

Q50 About how many FRECKLES did you have?

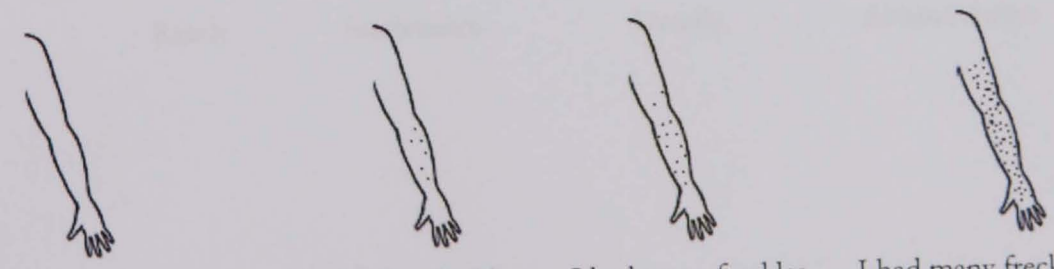
Match your answers with the pictures below for face and arms

- **FACE** (Tick one)



I had no freckles | I had a few freckles | I had some freckles | I had many freckles

- **ARMS** (Tick one)



I had no freckles | I had a few freckles | I had some freckles | I had many freckles

IN THE PERIOD **BEFORE** YOUR TRANSPLANT

Q51 Before your transplant, how many times in your life did you have a sunburn? *A sunburn means when your skin is red and painful You may blister and peel* (circle one)

Never 1-2 3-5 6-10 More than 10

Q52 Before the age of 18, how many times did you have a sunburn? (circle one)

Never 1-2 3-5 6-10 More than 10

Q53 Before your transplant, how many times did you sunbathe? (circle one)

Never 1-2 3-5 6-10 More than 10

Q54 Before your transplant, how many times in your life have you had a sunny holiday abroad?

Never 1-2 3-5 6-10 More than 10 (circle one)

Q55 Before your transplant, how many times in your life have you used a sun-bed?

Never 1-2 3-5 6-10 More than 10 (circle one)

Q56 Before your transplant, did you have any outdoor hobbies? (circle one)

YES / NO

If yes, What?_____

Q57 Before your transplant, did you ever use sunscreens? (circle one) YES / NO

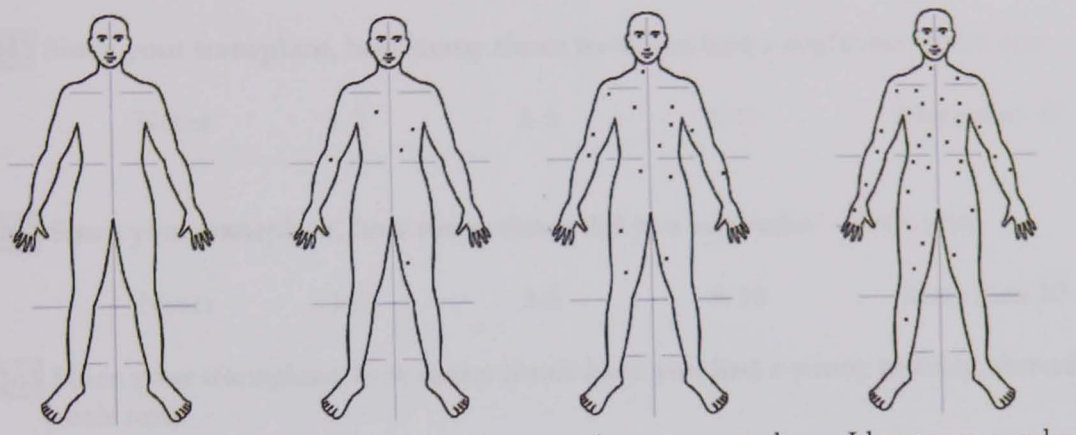
If yes, how often did you apply sunscreen when you were in the sun? (circle one)

Rarely Sometimes Usually Almost always

CURRENTLY

Q58 About how many MOLES do you have on your skin now?

Match your answers with the pictures below (*Moles are small brown, black or pink, either raised or flat skin markings that do not change after sun exposure*) *Please tick one box*

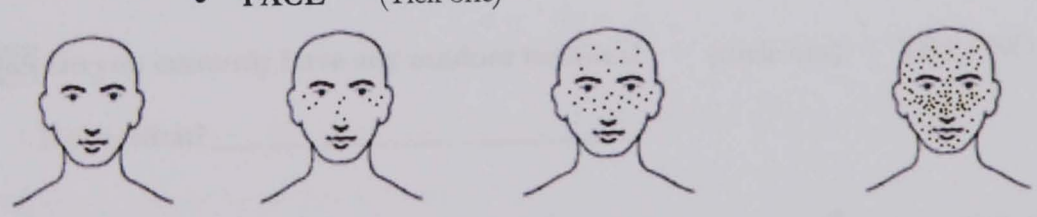


I have no moles | I have a few moles | I have some moles | I have many moles

Q59 About how many FRECKLES do you have now?

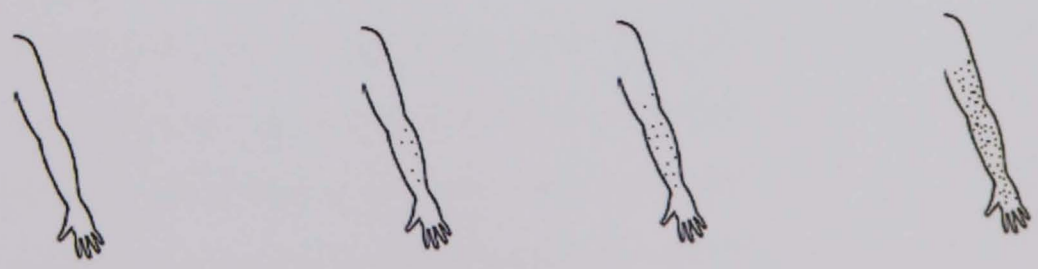
Match your answers with the pictures below for face and arms

- **FACE** (Tick one)



I have no freckles | I have a few freckles | I have some freckles | I have many freckles

- **ARMS** (Tick one)



I have no freckles | I have a few freckles | I have some freckles | I have many freckles

CURRENTLY

Q60 **Since your transplant, have you been advised to stay out of the sun?** (circle one)

YES / NO

Q61 **Since your transplant, how many times have you had a sunburn?** (circle one)

Never 1-2 3-5 6-10 More than 10

Q62 **Since your transplant, how many times did you sunbathe?** (circle one)

Never 1-2 3-5 6-10 More than 10

Q63 **Since your transplant, how many times have you had a sunny holiday abroad?** (circle one)

Never 1-2 3-5 6-10 More than 10

Q64 **Since your transplant, how many times have you used a sun-bed?** (circle one)

Never 1-2 3-5 6-10 More than 10

Q65 **Do you currently have any outdoor hobbies?** (circle one) YES / NO

If yes, what?_____

Q66 **Since your transplant, do you ever use sunscreens?** (circle one) YES / NO

If yes, how often do you apply sunscreen when you were in the sun? (circle one)

Rarely Sometimes Usually Almost always

ADDITIONAL QUESTIONS FOR WOMEN ONLY

Q67 If you have had children, in what year was each of your children born?

1st Child	_____	6th Child	_____
2nd Child	_____	7th Child	_____
3rd Child	_____	8th Child	_____
4th Child	_____	9th Child	_____
5th Child	_____	10th Child	_____

Q68 Have you ever had an abnormal smear test? (circle one) YES / NO

If yes, in what year?_____

Q69 Do you still menstruate? (circle one) YES /NO

If your periods have stopped, how old were you when this happened?_____**years**

Q70 Have you ever had a hysterectomy? (circle one) YES / NO

If yes, in what year?_____

Q71 Have you ever used the oral contraceptive pill? (circle one) YES / NO

If your answer is "NO" please go to Question 66 (Q66)

If yes, about how old were you when you first used the pill? _____**years**

Are you currently using the pill? (circle one) YES / NO

If not, about how old were you when you last came off the pill? _____**years**

For how many years in total did you use the pill?_____**years**

Q72 Have you ever used hormone replacement therapy (HRT)? (circle one) YES / NO

If yes, about how old were you when you first used HRT? _____**years**

Had your period stopped before you started using HRT? (circle one) YES / NO

For about how many years in total have you used HRT?_____**years**

Are you now using HRT? (circle one) YES / NO

What is the name of the most recent type of HRT you have used?_____

SECTION FOR THE DOCTOR/RESEARCH NURSE TO COMPLETE

(Information from patient notes)

Patient IDNUM:_____

CURRENT MEDICATION:

_____ _____ _____
_____ _____ _____
_____ _____ _____
_____ _____ _____
_____ _____ _____
_____ _____ _____
_____ _____ _____

HAS THIS PATIENT HAD:

Cutaneous squamous cell carcinoma YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

What stage? _____

Basal cell carcinoma YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

Malignant melanoma YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

What stage? _____

Actinic Keratosis YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

Carcinoma *in situ* (Bowen's disease) YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

Lymphoma / PTLD YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

Cytomegalovirus YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

Herpes simplex YES / NO / DON'T KNOW (circle one)

If yes, date first diagnosed_____

SECTION FOR THE DOCTOR/RESEARCH NURSE TO COMPLETE

Questions about skin cancer, if the patient has had one diagnosed

On how many separate occasions has a skin cancer been diagnosed?_____

FOR THE FIRST TUMOUR DIAGNOSED:

What was the year of diagnosis?_____

What was the histology?_____ Was it? (circle one) invasive or *in situ*

What was the location of the tumour?

Head Back Chest Abdomen Leg Arm

FOR THE SECOND TUMOUR DIAGNOSED:

What was the year of diagnosis?_____

What was the histology?_____ Was it? (circle one) invasive or *in situ*

What was the location of the tumour?

Head Back Chest Abdomen Leg Arm

FOR THE THIRD TUMOUR DIAGNOSED:

What was the year of diagnosis?_____

What was the histology?_____ Was it? (circle one) invasive or *in situ*

What was the location of the tumour?

Head Back Chest Abdomen Leg Arm

Have you taken a blood sample? (circle one) YES / NO

Have you taken a hair? (circle one) YES / NO

APPENDIX E

CD

The CD includes:

Appendix E1: Adjusted odds ratio (OR) by risk factors from the questionnaire for each single HPV type, among Caucasian OTR controls from London and Oxford (N=425)

Appendix E2: Adjusted count ratio (CR) for multiple HPV seropositivity by risk factors from the questionnaire, among transplant Caucasian controls from London and Oxford (N=425)

Appendix E3: Adjusted count ratio (CR) for multiple HPV seropositivity by risk factors from the questionnaire, among transplant Caucasian controls from Oxford (N=182)

Appendix E4: Adjusted count ratio (CR) for multiple HPV seropositivity by risk factors from the questionnaire, among transplant Caucasian controls from London (N=243)

Appendix E5: Seroprevalence for single HPV type between OTR with skin type V and VI and between Asian and Black individuals (the 2 largest groups of non-Caucasian patients) of Oxford and London

Appendix E6: Seroprevalence for multiple HPV types between OTR with skin type V and VI and between Asian and Black individuals (the 2 largest groups of non-Caucasian patients) of Oxford and London

Appendix E7: Multiple HPV seroprevalence by genus and mean number (SD) of multiple HPV seropositivity per Caucasian transplant, IC and dialysis patients across genus

Appendix E8: Multiple HPV seroprevalence by genus and mean number (SD) of multiple HPV seropositivity per non-Caucasian transplant and dialysis patients across genus

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