

# Human Papillomavirus type distribution in Vulval Intraepithelial Neoplasia determined using PapilloCheck DNA microarray

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## Human Papillomavirus type distribution in Vulval Intraepithelial Neoplasia determined using PapilloCheck DNA microarray

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# SCHOLARONE<sup>™</sup> Manuscripts

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#### 20 Abstract

21	Vulval intraepithelial neoplasia is a precursor of vulval carcinoma, and is frequently
22	associated with Human Papillomavirus (HPV) infection. Estimates of HPV
23	prevalence in vulval intraepithelial neoplasia vary widely in the UK. The objective of
24	this study was to assess HPV infection in a sample of women with vulval
25	intraepithelial neoplasia, confirmed histologically, and determine the proportion of
26	disease associated with HPV types targeted by prophylactic HPV vaccines. HPV
27	infection was assessed in biopsies from 59 patients using the Greiner Bio-One
28	PapilloCheck <sup>®</sup> DNA chip assay. Valid results were obtained for 54 cases. HPV
29	infection was present in 43 of the 54 cases (79.6%: 95% CI 67.1-88.2%). The most
30	common HPV types were HPV 16 (33/54: 61.1%), HPV 33 (8/54: 14.8%), HPV 6
31	(5/54: 9.3 %) and HPV 42 (3/54: 5.6%). The mean age of HPV positive women was
32	significantly less than the mean age of HPV negative women.
33	This is the largest UK series of vulval intraepithelial neoplasia in which HPV type has
34	been investigated, and 34/54 (63.0 %, 95% CI: 49.6-78.6 %) cases were associated
35	with HPV 16/18, which are targeted by current prophylactic HPV vaccines.
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37	Key words
38	HPV, Human papillomavirus, VIN, Vulval intraepithelial neoplasia, Papillocheck

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## **39 INTRODUCTION**

40 Vulval intraepithelial neoplasia is frequently a painful, distressing condition, and a 41 precursor lesion of invasive vulval carcinoma. In recent decades the incidence of 42 vulval intraepithelial neoplasia has increased, while the mean age of patients has 43 decreased. The incidence of vulval cancer rose by 17% between 1979 and 2001 44 [MacLean, 2004], with 996 new cases registered in England in 2000 [MacLean, 45 2006]. 46 Human Papillomavirus (HPV) positivity in vulval intraepithelial neoplasia lesions is 47 reported in international meta-analyses as 84% with most cases attributable to HPV 48 type 16 [De Vuyst et al., 2009]. The most likely explanation for the increased 49 incidence of vulval intraepithelial neoplasia is the substantial rise in HPV infections 50 of the lower genital tract [Peto et al., 2004]; the lifetime risk of HPV infection being 51 estimated at up to 79% [Syrjanen et al., 1990]. The aim of this investigation was to identify HPV types present in vulval 52 53 intraepithelial neoplasia, confirmed histologically, using the Greiner Bio-One PapilloCheck<sup>®</sup> DNA chip assay and determine the proportion of vulval intraepithelial 54 55 neoplasia potentially preventable by HPV prophylactic vaccination. 56

The study population comprised women attending a specialist vulval intraepithelial

#### 57 MATERIALS AND METHODS

## 58 Study population

neoplasia clinic at the University Hospital of Wales, Cardiff, UK between 2003 and 2009, with vulval intraepithelial neoplasia confirmed histologically, who gave written informed consent for a biopsy to be taken for research purposes. The study was approved by South Wales LREC (SMKW/EL/03/5178). 59 cases were investigated, with an age range of 22-82 years, and a mean age of 45.8 years. There were six cases of vulval intraepithelial neoplasia grade 1, two of vulval intraepithelial neoplasia grade 2, and fifty one cases of vulval intraepithelial neoplasia grade 3. Sample collection and processing. Separate biopsies were taken for histology and research use. The research biopsy was immediately placed in liquid based cytology medium (which fixed cells and preserved DNA). DNA was extracted from the research biopsy using the Qiagen QIA amp kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions.

#### **Pathology review**

A recent review of vulval intraepithelial neoplasia histopathology recommended
replacement of the vulval intraepithelial neoplasia 1/2/3 subdivisions with two
categories reflecting the putative aetiology of the disease: usual vulval intraepithelial
neoplasia (HPV-associated); and differentiated vulval intraepithelial neoplasia
(associated with lichen sclerosis) [Sideri et al., 2005]. However, the existence of
differentiated vulval intraepithelial neoplasia is disputed, and in practice this diagnosis
is rarely made [McCluggage, 2009]. The histopathology of lesions in this study is

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82	reported using both the vulval intraepithelial neoplasia 1/2/3 and usual/differentiated
83	classification. Pathology review was undertaken blind to HPV status by an
84	experienced Consultant Histopathologist with a special interest in gynaecological
85	pathology (GR).
86	
87	PapilloCheck <sup>®</sup> assay
88	HPV infection was identified using the Greiner Bio-One Papillocheck® assay (Greiner
89	Bio-One GmbH, Frickenhausen, Germany), which enables simultaneous detection and
90	typing of 24 different HPV types in a single reaction (HPV 16, 18, 31, 33, 35, 39, 45,
91	51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, 6, 11, 40, 42, 43 & 44). The assay uses
92	multiplex PCR with fluorescent primers to amplify a 350bp fragment of the E1 gene.
93	The PCR product is then hybridised to a PapilloCheck <sup>®</sup> DNA array, comprising 28
94	probes, each in 5 replicate spots. To identify false negatives the assay includes
95	amplification of the human ADAT1 gene. Hybridization is assessed using the
96	CheckScanner <sup>™</sup> array reader. Amplification, hybridization, detection and
97	interpretation were performed according to the manufacturer's instructions. A valid
98	result was defined as one which passed all of the PapilloCheck <sup>®</sup> internal controls
99	(including DNA adequacy, sample inhibition, and hybridisation).
100	Previous studies have confirmed the analytical [Dalstein et al., 2009; Jones et al.,
101	2009] and clinical validity [Halfon et al., 2009; Hesselink et al., 2009] of this assay in
102	large sample sets as compared to GP5+/6+, HC2 and Roche Linear Array. The assay
103	is certified within the European Union as an in vitro diagnostic for qualitative
104	detection of HPV in clinical specimens.
105	

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**RESULTS** 

#### 108 HPV type distribution

109 HPV type distribution, stratified by histology, is shown in Table I. Valid results were

110 obtained for 54 of the 59 cases. HPV infection was present in 43/54 cases (79.6%:

111 95% CI 67.1-88.2%). HPV 16 and/or 18 were present in 34/54 cases (63.0 %, 95%

112 CI: 49.6-78.6%). HPV 16 and/or 18 were the only types present in 26/54 cases (48%,

113 95% CI: 35.4-61.2%).

114 High Risk (HR) HPV types were present in 38 cases and Low Risk (LR) types in 9

115 cases; 4 cases contained both HR and LR types. Multiple HPV types were present in

116 10 cases; in these cases the distribution of types was: one vulval intraepithelial

117 neoplasia grade 1 sample contained HPV 18, 42 and 44/55; all other cases with

118 multiple types were vulval intraepithelial neoplasia 3 and the combinations of types

119 were: 3 cases with HPV 16 and 33; 2 cases with HPV 16 and 6; and one case each of

120 (16,18), (33, 42), (16, 31, 33), (16, 51). In the 5 cases where only LR types were

121 present, 3 were HPV 6 (histology: two cases of vulval intraepithelial neoplasia grade

122 3, and one vulval intraepithelial neoplasia grade 3 with well-differentiated carcinoma

123 and viral warts), 1 was HPV 40 (vulval intraepithelial neoplasia 1) and 1 contained

124 HPV 42 (vulval intraepithelial neoplasia 1).

126 HPV and age

HPV positive cases were aged 22-81 with a mean of 43.4 years. HPV negative cases
were aged 33-82 years with a mean of 54.9 years. The means of these groups were

129 significantly different (t test p = 0.019).

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## 131 HPV and histology

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132	There was no significant correlation between HPV positivity and grade of lesion. Of
133	the HPV positive cases, 40/43 (93.0%) showed vulval intraepithelial neoplasia grade 2
134	or worse. This was slightly lower in HPV negative lesions, where 9/11 (81.8%)
135	showed vulval intraepithelial neoplasia grade 2 or worse, but this difference was not
136	significant (Fisher's $P = 0.266$ ).
137	There was no significant correlation between HPV type and histological grade;
138	however it was noticeable that all of the HPV 16 positive cases were associated with
139	vulval intraepithelial neoplasia 2 or worse, while a high risk type (HPV 18) was
140	identified in only 1/5 vulval intraepithelial neoplasia grade 1 cases with valid HPV
141	results.
142	Slides were available for pathology review for 54/59 cases. 52/54 cases were "Usual
143	vulval intraepithelial neoplasia"; 2/54 cases were "Probable Differentiated vulval
144	intraepithelial neoplasia". Both cases of probable Differentiated vulval intraepithelial
145	neoplasia were in HPV negative patients: one an 82 year old woman with vulval
146	intraepithelial neoplasia 3, the other a 61 year old woman with vulval intraepithelial
147	neoplasia 1 (but with a history of vulval cancer). Among the cases with valid HPV
148	results, 42/49 cases of usual vulval intraepithelial neoplasia were HPV positive
149	(85.7% 95%CI: 73.3-92.9%).

This is the largest UK series of vulval intraepithelial neoplasia in which HPV type has been determined. The main limitation of this study was the use of a self selected sample, i.e. women attending a specialist vulval intraepithelial neoplasia clinic. This might bias the sample towards women with persistent or recurrent disease, which might possibly result in some over-representation of more persistent types (HPV 16). A further limitation was the use of separate biopsies for pathology and HPV analysis; in practice both biopsies were immediately adjacent and cases were only included if both biopsies were part of the same macroscopically visible lesion. It is however theoretically possible that the research biopsy may not be representative of the diagnostic biopsy.

Several studies have investigated HPV prevalence in vulval intraepithelial neoplasia in the UK and estimate HPV positivity at 37.5 to 100%, with the proportion of HPV positive cases containing HPV 16 varying from 37.5 to 93.3% [Abdel-Hady et al., 2001; Baldwin et al., 2003; Daayana et al., 2010; Fiander et al., 2006; Gasco et al., 2002; Todd et al., 2002; Tristram and Fiander, 2005; Woo et al., 2007]. The sample size in these studies ranged from 8-29 cases. The sample size for the current study is larger, and the findings are more consistent with the overall results of a recent international meta-analysis, in which 84.0% of vulval intraepithelial neoplasia cases tested HPV positive (67.5% were HPV 16, 7.7% were HPV 33, and 4.6% were HPV 18) [De Vuyst et al., 2009]. The corresponding proportions in the current study were overall HPV positivity of 79.6% (61.1 % were positive for HPV 16, 14.8% for HPV 33, and 3.7% for HPV 18). These are also similar to a separate meta-analysis which

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174 indicated an overall HPV positivity of 80.4% for vulval intraepithelial neoplasia 2/3 175 (HPV 16 present in 71.2%, HPV33 in 7.7% and HPV 18 in 5.5% [Smith et al., 2009]). 176 Patients who tested positive for HPV infection were significantly younger (by over 11 177 years on average) than those testing negative. This is consistent with HPV associated disease being more common in younger women and vulval intraepithelial neoplasia 178 179 associated with lichen sclerosis being more common in older patients [Bonvicini et 180 al., 2005]. 181 The finding that 85.7% of usual vulval intraepithelial neoplasia were HPV positive is 182 in accord with data previously reviewed [van de Nieuwenhof et al., 2008]. The 7 HPV 183 negative usual vulval intraepithelial neoplasia patients represent an interesting group; 184 it is not clear whether these patients are accounted for by mis-diagnosis of 185 differentiated vulval intraepithelial neoplasia (possibly due to sampling error), or 186 whether there is a subset of usual vulval intraepithelial neoplasia that is truly HPV negative. 187 188 Three cases showing vulval intraepithelial neoplasia grade 2 or worse were associated 189 190 with a low risk HPV type alone (HPV6). One of these cases showed vulval 191 intraepithelial neoplasia grade 3 with well differentiated squamous cell carcinoma and 192 viral warts; it is possible that the warts were associated with the HPV 6 but the vulval 193 intraepithelial neoplasia and carcinoma were not. This apparent association between 194 high grade vulval intraepithelial neoplasia and LR HPV types runs contrary to the 195 suggestion that LR types cause only warts and low grade lesions. However an 196 association between LR HPV types and high grade disease was observed in the De 197 Vuyst meta analysis [De Vuyst et al., 2009] which reported approximately 5% of vulval intraepithelial neoplasia 2/3 as linked to HPV 6. 198

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200	HPV associated vulval intraepithelial neoplasia is most common in women in their
201	30's and 40's [Hart, 2001]. The incidence of VIN is approximately 5 per 100,000
202	women per year and is increasing [Joura, 2002]. HPV infection is endemic in the UK
203	with almost 30% of women in the 20-25 year age range now infected with a high risk
204	anogenital HPV type [Hibbitts et al., 2008]. Hence, in the short term, the number of
205	women affected by vulval intraepithelial neoplasia is likely to continue increasing.
206	HPV vaccination has been shown to be 100% effective in prevention of vulval
207	epithelial neoplasia 2/3 associated with HPV 16/18 in a per protocol population [Joura
208	et al., 2007]. In the UK vaccination of 12-13 year old girls against HPV 16/18
209	infection began in 2008. Hence it is likely to be 20 to 30 years before the effects of
210	this intervention become apparent in reduced incidence of vulval intraepithelial
211	neoplasia. Ultimately however, as HPV 16/18 were present in 63.0% of vulval
212	intraepithelial neoplasia, our data suggest that greater than half of vulval
213	intraepithelial neoplasia cases could be potentially prevented by vaccination.
214	

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	HPV Type								
Histology	16	18	31	33	51	6	40	42	44/55*
VIN 1	0	1	0	0	0	0	1	2	1
VIN 2	1	0	0	0	0	1	0	0	0
VIN 3	32	1	1	8	1	4	0	1	0
Total	33	2	1	8	1	5	1	3	1
%	61.1	3.7	1.9	14.8	1.9	9.3	1.9	5.6	1.9

Table I. HPV type distribution within histological grade.

There were 10 cases showing multiple infection, hence the number of types identified is greater that the number of cases. Percentages indicate the proportion of cases that tested positive for a given type.

i type. -hybridiz. \*the HPV 44 probe cross-hybridizes to HPV 55, hence these types are reported together.