

Clinical Perspectives

Genetic and epigenetic variation in vulvar cancer: Current research and future clinical practice

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Vulvar cancer is a relatively rare gynaecological malignancy, the treatment of which is associated with significant patient morbidity. With reports that the incidence of vulvar cancer is increasing, there is a rising need for improved preventive, diagnostic and therapeutic tools. Recent advances within genetics and epigenetics present possible approaches for addressing this need, by contributing to the clarification of the aetiology of this disease, identifying screening and drug targets and introducing the potential for personalised treatments. This paper reviews the genetic and epigenetic research undertaken to date within vulvar cancer, evaluates its potential for clinical application and identifies directions for future research.

Key words: epigenetics, genetics, human papillomavirus, vulvar cancer, vulvar intraepithelial neoplasia.

Introduction

Vulvar cancer is a relatively rare malignancy worldwide, mainly affecting older women, although some reports suggest the incidence may be increasing and the age of onset decreasing.^{1,2} Among Australian women, the most recent age-standardised incidence rate is 2.3 per 100 000 (95% CI 2.0–2.6/100 000), and the mortality rate from vulvar cancer is 0.5 per 100 000 (0.4–0.6/100 000).³ The majority (>80%) of diagnoses are squamous cell carcinomas (VSCC). Two distinct aetiologies for VSCC have been posited based on the biological and clinical features of vulvar lesions.⁴ The first stems from vulvar intraepithelial neoplasia (VIN) usual type (warty, basaloid and mixed), is usually associated with human papillomavirus (HPV) infection and occurs in younger (premenopausal) women. The second occurs primarily in older (postmenopausal) women and is associated with VIN differentiated type and vulvar dystrophy, especially lichen sclerosus.

Given the relative rarity of this malignancy, research into vulvar cancer has been somewhat neglected in comparison to other gynaecological cancers. However, the extensive interest in the genetic aetiology of other, more common HPV-

associated cancers has generated an increase in research examining the genetics and epigenetics of vulvar cancer, with the aim of improving diagnosis and management.

Diagnosis and Treatment

Women diagnosed with and treated for vulvar cancer experience substantial treatment-related morbidity and negative psychosexual outcomes.⁵ Surgery remains the main treatment for vulvar cancer, with or without lymphadenectomy to the inguinofemoral region, and often with adjuvant or neoadjuvant chemotherapy or radiotherapy. HPV-related VIN is increasing, and early detection and treatment of VIN may prevent development of vulvar cancer. Additionally, early diagnosis of vulvar cancer increases survival and may facilitate vulvar conservation, as wide local excision may be possible in preference to vulvectomy. Increasing recognition of the psychosocial effects of these surgeries has given rise to recent efforts to further uncover the pathogenesis of vulvar cancer.

Elucidating the genetic mechanisms underlying the different subtypes of vulvar cancer presents the possibility of improving diagnosis and management through facilitating the identification of the following:

- Women at higher risk and in need of regular screening.
- Women at increased risk of progression from VIN to invasive cancer or more likely to develop aggressive disease.
- Alternative treatment options, such as topical chemo therapeutic agents, that are potentially less disfiguring.

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- Personalised treatment options, based on more precise diagnosis of cancer subtypes and identifying which patients are more likely to benefit from different adjuvant or neoadjuvant therapies, as well as avoiding unnecessary toxicity in patients for whom particular treatments are unsuitable.

Genetics

Studies investigating the genetics of vulvar cancer can be broadly categorised into two groups: those focussed on genetic mutations within neoplastic tissue (Table 1) and those investigating inherited genetic variants by utilising unaffected tissue, usually peripheral blood samples (Table 2). The difference between these two approaches is that somatic variants provide insight into the pathways involved in disease causation, subtypes or progression, offering potential drug targets or prognosis information,

whereas inherited variants provide information regarding who is at greater risk of developing the disease, as well as informing disease aetiology. The heritability of VSCC has not been quantified, although familial clustering has been reported in Sweden and Australia, which is suggestive of a role for inherited genetic risk factors.^{6,7} It is also notable that heritability has been estimated to explain 27% of the variability in risk of cervical cancer, with inherited genetic variants thought to influence susceptibility to and persistence of HPV infection, and time to development of cancer.⁸

A number of studies have used peripheral blood samples to examine the effect of inherited polymorphisms within immune response genes on risk of VSCC, on the basis of their putative role in HPV persistence. This followed from the observation that patients with immunodeficient syndromes, such as HIV and idiopathic C4+ lymphopenia, were less likely to resolve HPV infection and were therefore

Table 1 Studies of somatic variants associated with VSCC and VIN

Author	Number of samples	HPV status	Genes investigated	Pathway/Role	Findings
Pinto <i>et al.</i> (2010) ¹⁸	11 dVIN+6 associated VSCC 10 associated normal epithelial tissues used as controls from same participants.	Not reported	TP53	Tumour suppressor	SNPs in TP53 associated with mutant P53 expression
Holway <i>et al.</i> (2000) ¹⁹	8 VSCC+2 carcinoma <i>in situ</i> (CIS), with additional normal tissue, dysplasia or CIS taken from the same participants.	Not reported	PTEN	Tumour suppressor	PTEN mutations found in 5/8 VSCC and in 2 patients with precursor lesions
Reddy <i>et al.</i> (2002) ²⁴	40 VSCC+matched normal vulvar epithelium and 32 VIN.	Not reported	CHK2	Tumour suppressor	No loss of CHK2 expression, but 2/40 VSCC showed mutations in CHK2 and also expressed mutant P53
Choschzick <i>et al.</i> (2011) ¹⁵	142 VSCC screened via tissue microarray. Of these, 21 positive and 18 negative tumours were examined.	18 positive 21 negative	TP53	Tumour suppressor	TP53 mutations associated with TP53 overexpression and negative HPV status mutations linked to cellular oxidative stress, that is, resulting from lichen sclerosis
Lee <i>et al.</i> (1994) ¹⁷	21 VSCC	12 positive 9 negative	TP53	Tumour suppressor	1/12 HPV-positive samples had a missense mutation of p53, 4/9 HPV negative also had point mutations of TP53
Brooks <i>et al.</i> (2000) ¹⁴	36 VSCC with matched normal tissue.	13 positive 23 negative	TP53	Tumour suppressor	Preferential loss of heterozygosity in the 72P allele of TP53 occurred independently of HPV status
Chulvis do Val <i>et al.</i> (2004) ¹⁶	20 VIN	8 positive 3 negative 9 unknown	TP53	Tumour suppressor	TP53 mutations in exon 7 were associated with a high risk of progression from VIN to VSCC
Woelber <i>et al.</i> (2012) ¹³	183 VSCC	43 positive 111 negative 29 unknown	EGFR	Epidermal growth factor/oncogene	Increases in EGFR copy number were associated with advanced stage and metastases, independent of HPV status

HPV, human papillomavirus; VIN, vulvar intra-epithelial neoplasia; VSCC, vulvar squamous cell carcinoma; SNP, single nucleotide polymorphism.

Table 2 Studies of inherited variants associated with VSCC and VIN

Study	Number of samples	HPV status	Genes investigated	Pathway/Role	Findings
Hussain <i>et al.</i> (2008) ¹²	486 VSCC	285 positive 35 negative 27 unclear 139 not tested	IL2	Immune Response to HPV	Polymorphisms within IL2 interacted with cigarette smoking to increase the risk of developing VSCC
Hussain <i>et al.</i> (2013) ¹¹	53 parent case triads, 473 VSCC and 1111 controls.	358 positive 42 negative 126 not tested	IL10, IL12A, IL12B, IL10RA, IL10RB, IL12RB1, IL12RB2		Minor allele of rs3181224 in IL12B associated with reduced risk of VSCC
Chen <i>et al.</i> (1999) ²⁵	137 VSCC (120 <i>in situ</i> and 17 invasive) and 248 controls.	68 positive 68 negative 1 unknown	GSTM1	Facilitates the excretion of toxins and carcinogens	GSTM null allele was not associated with increased risk of VSCC in smokers
Riener <i>et al.</i> (2004) ²⁶	68 VSCC and 227 controls.	Not reported	NOS3	Generates nitric oxide (NO), a mediator of malignant growth.	Allelic variation on intron 4 influences the length of disease free survival
Bodelon <i>et al.</i> (2012) ²⁷	517 VSCC and 1100 controls.	350 positive 79 negative 88 unknown	CD83	Immune response pathway, marker of dendritic cell maturation	No association with risk of VSCC
Bodelon <i>et al.</i> (2014) ¹⁰	517 VSCC and 1100 controls.	350 positive 79 negative 88 unknown	32 candidate genes: A2I2, IKBKE, IRAK1, IRAK4, IRF3, LST1, LTA, LTB, MAP3K1, MAP3K7, NCR3, NFKB1, NFKB2, RELA, RELB, TANK, TBK1, TICAM1, TICAM2, TIRAP, TLR3, TLR4, TLR7, TLR9, TNF, TNFRSF1A, TNFRSF1B, TOLLIP, TRAF3, T RAF6, VISA, ZBP1	Immune response pathway	Variants in genes associated with TNF regulation (LST1, LTA, LTB, NCR3 and TNF) were significantly associated with VSCC. In particular one variant in the LTA (lymphotoxin alpha) gene increased the risk of VSCC by 51%

HPV, human papillomavirus; VIN, vulvar intra-epithelial neoplasia; VSCC, vulvar squamous cell carcinoma.

at increased risk of progression to HPV-associated lesions and neoplasia.⁹ Most notably, a variant within the *LTA* gene was found to increase risk of vulvar cancer by 51% (CI 1.30–1.75).¹⁰ This gene is part of the tumour necrosis factor superfamily and is involved in influencing cytokine response. Within the inflammatory response pathway, there is some evidence to support the hypothesis that genetic variants in the interleukin genes may also affect immune response to HPV infection, especially among cigarette smokers.^{11,12} While these findings provide some clues as to the aetiology of VSCC, especially HPV-dependent types, it remains an incomplete picture. Furthermore, the susceptibility variants identified to date are insufficient to sensitively and specifically identify women at increased risk of developing vulvar cancer, and much work remains in this area.

Conversely, an example of a somatic study is Woelber and colleagues' investigation of the oncogene *EGFR*.¹³ Increases in the copy number of *EGFR* were found to be associated with advanced stage and metastases in HPV-independent VSCC, suggesting that *EGFR* inhibitors

could prove useful in the treatment of this subset of patients, similar to the use of trastuzumab (Herceptin) in HER2+ breast cancer or cetuximab (Erbix) in colorectal or head and neck cancers. However, the majority of somatic studies have focussed on clarifying the role of *TP53*, a gene well recognised for its role in tumour suppression and the most common location for somatic mutations in human cancer.^{14–18}

Nevertheless, genetic studies have contributed to our understanding of this disease. There is, for instance, genetic evidence that corroborates the division of VSCC into two subtypes. In HPV-dependent VSCC, HPV oncogenic proteins E6 and E7 contribute to the degradation of the tumour suppressors p53 and pRb. In HPV-independent VSCC, on the other hand, somatic mutations have been identified in *TP53* in tumour tissue, which result in a mutant form of p53 being overexpressed in these cancers.^{15,18,19} Thus, a key difference between HPV-dependent and HPV-independent VSCC is found in the different means by which tumour suppression is dysregulated. These findings underscore the importance of

ascertaining HPV status in vulvar cancer studies, a practice which has not been consistently applied in previous studies.

The studies undertaken to date have selected candidate genes for investigation based on their putative role in vulvar cancer, most frequently within the tumour suppression or immune response pathways. The lack of genomewide studies, small sample sizes and inconsistent reporting of HPV status all contribute to the currently incomplete picture of the genetics of vulvar cancer. Further, the distinction between tumour and germline variants, while useful, is only a generalisation; variants found within tumour tissue may be either acquired or inherited, and many somatic variants will not be causative, but rather the result of acquired genomic instability, for example disruption to the cell cycle and repair mechanisms. Associations between genetic variants and disease are insufficient to determine causality, and functional studies are required to clarify the role of a particular variant in disease causation or progression.

Epigenetics

Epigenetics pertains to heritable changes to gene expression without modification of the underlying DNA architecture, such as DNA methylation, histone modifications and

miRNA regulation. Of these, the most widely examined is DNA methylation, and this is reflected in the vulvar cancer epigenetic literature (Table 3). Methylation occurs at cytosine–guanine dinucleotides, aggregated in CpG islands, which are typically located in the promoter regions of genes. DNA from tumour cells is typically globally hypomethylated compared with normal tissue, although specific hypermethylation causing inactivation of selected tumour suppressor genes has been reported in many human cancers.²⁰

Several studies have attempted to clarify the complexities of DNA methylation in VSCC, with variable results (Table 3). Most have employed methylation-specific PCR to examine either a single gene or panel of candidate genes, rather than genomewide array techniques, and most have focussed on the differentiated VIN pathway. The study with the widest scope examined the methylation status of 22 tumour suppressor genes and found that nine of these were aberrantly methylated at the promoter, the most frequent of which was *TP73* in nine of 13 cell lines.²¹ However, the HPV status was not known for all of the cell lines used in this study, and only 13 cell lines from 12 patients were used, rendering extrapolation from these suggestive findings difficult.

Attempts have also been made to develop models for detecting lymph node metastases in vulvar cancer using

Table 3 Studies of DNA methylation associated with VSCC and LS

Study	Number of samples	HPV status	Genes investigated	Pathway/Role	Findings
Aidé <i>et al.</i> (2010) ²⁸	15 Lichen Sclerosus (LS)	Not reported	DAPK and p16	Tumour suppressor genes	DAPK promoter methylation was observed in 2 of 13 and p16 in 7 of 15 samples. P16 was methylated in both DAPK methylated samples. Methylation may play a role in progression to VSCC
Oonk <i>et al.</i> (2012) ²²	20 participants with primary, lymph node negative and lymph node positive VSCC samples.	Not reported	P16INK4a, MGMT, TWIST, CADM1, TERT, TFPI2	Multiple pathways involved	Methylation of 3 genes: P16INK4a, TERT and TFPI2 detected lymph node metastases with a sensitivity of 67% and a specificity of 100%
Guerrero <i>et al.</i> (2011) ²⁹	30 VSCC 12 LS adjoining VSCC 12 NORMAL adjoining LS and 21 non associated LS.	5 positive 25 negative	RASSF1A, RASSF2A, p16, TSP-1, MGMT	Cell signalling, control, repair, neovascularisation	TSP1 methylation was associated with poor prognosis. MGMT and RASSF2A methylation were associated with VSCC and LS together but not isolated LS
Stephen <i>et al.</i> (2009) ²¹	13 cell lines from 12 patients with VSCC	1 positive 9 negative 3 unknown	35 genes 22 tumour suppressor genes of which 9 showed aberrant methylation TP73, FHIT, VHL, APC, ESR1, CDKN2B, DAPK1, GSTP1, GSF4.	Tumour suppressor	9 of 22 genes showed aberrant methylation TP73, FHIT, VHL, APC, ESR1, CDKN2B, DAPK1, GSTP1, IGSF4, confirmed by a decrease in mRNA expression of TP73 and IGSF4

HPV, human papillomavirus; VSCC, vulvar squamous cell carcinoma. LS, lichen sclerosis.

methylation markers, with moderate success. Oonk and colleagues used methylation-specific PCR on a panel of six genes and identified three that could predict lymph node metastases with a specificity of 100%, but only a sensitivity of 67%, limiting its clinical utility.²²

As in other cancers, epigenetic disruption is likely to be an important mechanism in VSCC and one that is potentially modifiable. Several epigenetic drugs, such as decitabine (Dacogen) and azacitidine (Vidaza), have been shown to be effective in treating haematological malignancies, but less progress has been made with solid tumours.²⁰ Elucidating the role of epigenetic factors in VIN and VSCC will determine the potential clinical value of DNA methylation inhibitors or histone deacetylase inhibitors in this disease. To achieve this, substantial work remains to be performed, not only with methylation, but also regarding the role of histone modifications and miRNA regulation.

Future Directions

Early findings suggest that genetic and epigenetic variants are important in the aetiology of vulvar cancer and offer promising diagnostic and therapeutic targets. Less work has focussed on HPV-dependent VSCC than on HPV-independent cancer, although it is expected that diagnoses of HPV-dependent malignancies will decline as vaccination against HPV, including HPV16, becomes increasingly widespread. Furthermore, research to date has focussed on specific candidate genes and has been limited by small sample sizes and inconsistent reporting of HPV infection status.

Currently, the first genomewide study of HPV-dependent VSCC is being undertaken in Australia, investigating a vulvar cancer cluster among young Aboriginal women resident in Arnhem Land.²³ This cluster, in which the incidence rate among women aged <50 years is more than 70 times the national rate for the same age group, is likely to identify population-specific risk variants, although these findings will provide clues as to which genes may be important in other populations. There remains a need for adequately powered, prospective, multicentre studies employing genomewide methodologies in both genetics and epigenetics to fill in the substantial gaps in our understanding of this complex cancer.

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References

- 1 Hampl M, Deckers-Figiel S, Hampl JA *et al.* New aspects of vulvar cancer: changes in localization and age of onset. *Gynecol Oncol* 2008; **109**: 340–345.
- 2 Joura EA, Losch A, Haider-Angeler M-G *et al.* Trends in vulvar neoplasia: increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med* 2000; **45**: 613–615.
- 3 AIHW, Cancer Australia. Gynaecological Cancers in Australia: An Overview. Vol. Cancer series no. 70. Canberra: Australian Institute of Health and Welfare, 2012.
- 4 Sideri M, Jones RW, Wilkinson EJ *et al.* Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med* 2005; **50**: 807–810.
- 5 Wills A, Obermair A. A review of complications associated with the surgical treatment of vulvar cancer. *Gynecol Oncol* 2013; **131**: 467–479.
- 6 Hussain SK, Sundquist J, Hemminki K. Familial clustering of cancer at human papillomavirus-associated sites according to the Swedish Family-Cancer Database. *Int J Cancer* 2008; **122**: 1873–1878.
- 7 McWhirter RE, Thomson RJ, Marthick JR *et al.* Runs of homozygosity and a cluster of vulvar cancer in young Australian Aboriginal women. *Gynecol Oncol* 2014; **133**: 421–426.
- 8 Magnusson PK, Lichtenstein P, Gyllenstein UB. Heritability of cervical tumours. *Int J Cancer* 2000; **88**: 698–701.
- 9 Jamieson DJ, Paramsothy P, Cu-Uvin S, Duerr A. Vulvar, vaginal, and perianal intraepithelial neoplasia in women with or at risk for human immunodeficiency virus. *Obstet Gynecol* 2006; **107**: 1023–1028.
- 10 Bodelon C, Madeleine MM, Johnson LG *et al.* Genetic variation in the TLR and NF- κ B pathways and cervical and vulvar cancer risk: a population-based case-control study. *Int J Cancer* 2014; **134**: 437–444.
- 11 Hussain SK, Madeleine MM, Johnson LG *et al.* Nucleotide variation in IL-10 and IL-12 and their receptors and cervical and vulvar cancer risk: a hybrid case-parent triad and case-control study. *Int J Cancer* 2013; **133**: 201–213.
- 12 Hussain SK, Madeleine MM, Johnson LG *et al.* Cervical and vulvar cancer risk in relation to the joint effects of cigarette smoking and genetic variation in interleukin 2. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1790–1799.
- 13 Woelber L, Hess S, Bohlken H *et al.* EGFR gene copy number increase in vulvar carcinomas is linked with poor clinical outcome. *J Clin Pathol* 2012; **65**: 133–139.
- 14 Brooks LA, Tidy JA, Gusterson B *et al.* Preferential retention of codon 72 arginine p53 in squamous cell carcinomas of the vulva occurs in cancers positive and negative for human papillomavirus. *Cancer Res* 2000; **60**: 6875–6877.
- 15 Choschzick M, Hantaredja W, Tennstedt P *et al.* Role of TP53 mutations in vulvar carcinomas. *Int J Gynecol Pathol* 2011; **30**: 497–504.
- 16 Chulvis do Val I, Almeida Filho G, Valiante PM *et al.* Vulvar intraepithelial neoplasia p53 expression, p53 gene mutation and HPV in recurrent/progressive cases. *J Reprod Med* 2004; **49**: 868–874.
- 17 Lee YY, Wilczynski SP, Chumakov A *et al.* Carcinoma of the vulva: HPV and p53 mutations. *Oncogene* 1994; **9**: 1655–1659.
- 18 Pinto AP, Miron A, Yassin Y *et al.* Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol* 2010; **23**: 404–412.

- 19 Holway AH, Rieger-Christ KM, Miner WR *et al.* Somatic mutation of PTEN in vulvar cancer. *Clin Cancer Res* 2000; **6**: 3228–3235.
- 20 Esteller M. Epigenetics in cancer. *New Engl J Med* 2008; **358**: 1148–1159.
- 21 Stephen JK, Chen KM, Raitanen M *et al.* DNA hypermethylation profiles in squamous cell carcinoma of the vulva. *Int J Gynecol Pathol* 2009; **28**: 63–75.
- 22 Oonk MH, Eijssink JJ, Volders HH *et al.* Identification of inguinofemoral lymph node metastases by methylation markers in vulvar cancer. *Gynecol Oncol* 2012; **125**: 352–357.
- 23 McWhirter RE, Mununggirritj D, Marika D *et al.* Ethical genetic research in Indigenous communities: challenges and successful approaches. *Trends Mol Med* 2012; **18**: 702–708.
- 24 Reddy A, Yuille M, Sullivan A *et al.* Analysis of CHK2 in vulvar neoplasia. *Br J Cancer* 2002; **86**: 756–760.
- 25 Chen C, Cook LS, Li XY *et al.* CYP2D6 genotype and the incidence of anal and vulvar cancer. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 317–321.
- 26 Riener EK, Hefler LA, Grimm C *et al.* Polymorphisms of the endothelial nitric oxide synthase gene in women with vulvar cancer. *Gynecol Oncol* 2004; **93**: 686–690.
- 27 Bodelon C, Madeleine MM, Johnson LG *et al.* Genetic variation in CD83 and risks of cervical and vulvar cancers: a population-based case-control study. *Gynecol Oncol* 2012; **124**: 525–528.
- 28 Aidé S, Lattario F, Almeida G *et al.* Promoter hypermethylation patterns of death-associated protein kinase and p16 genes in vulvar lichen sclerosis. *J Low Genit Tract Dis* 2010; **14**: 282–286.
- 29 Guerrero D, Guarch R, Ojer A *et al.* Differential hypermethylation of genes in vulvar cancer and lichen sclerosis coexisting or not with vulvar cancer. *Int J Cancer* 2011; **128**: 2853–2864.