Vulvar cancer : pathogenesis, molecular genetics and treatment

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CHAPTER 5

Genetic and epigenetic changes in vulvar squamous cell carcinoma and its precursor lesions: a review of the current literature

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Abstract

Vulvar cancer is a relatively rare gynecologic malignancy with an annual incidence in developed countries of approximately 2 per 100,000 women. Vulvar squamous cell carcinoma (VSCC) has two etiological pathways: a high risk human papillomavirus (HPV)-dependent route, which has usual vulvar intraepithelial neoplasia (uVIN) as a precursor lesion, and an HPV-independent route, which is associated with differentiated VIN (dVIN), lichen sclerosus, and genetic alterations, such as *TP53* mutations. Research on the molecular etiology of vulvar cancer has increased in past years, not only regarding genetic alterations, but also epigenetic changes. In genetic alterations, a mutation irreversibly changes the nucleotide sequence of the DNA, or the number of copies of chromosomes per cell is altered. In epigenetics, the nucleotide sequence remains the same but genes can be 'switched' on or off by, for example, DNA methylation or histone modification. We searched the current literature on genetic and epigenetic alterations in VSCC and its precursor lesions. Many studies have reported a higher incidence of somatic mutations in HPV-negative tumors compared to HPV-positive tumors, with *TP53* mutations being the most frequent. These somatic mutations seem to occur more often with increasing grades of dysplasia. Allelic imbalances or loss of heterozygosity are more frequently found in higher stages of dysplasia and in invasive carcinomas, but it is not exclusive to HPV-negative tumors. A limited number of studies are available on epigenetic changes in vulvar lesions, with hypermethylation of *CDKN2A* being the most frequently investigated change. For most genes, hypermethylation occurs more frequently in VSCC than in precursor lesions. As most studies have focused on HPV infection and *TP53* mutations, we suggest that more research should be performed using whole genome or next generation sequencing to determine the true landscape of genetic and epigenetic alterations in VSCC.

Introduction

Vulvar cancer is a rare malignant disease accounting for less than 5% of gynecological malignancies (1-3). The majority of these tumors are vulvar squamous cell carcinoma (VSCC). The annual incidence of VSCC in developed countries is two to three per 100,000 women and increases with age, with a peak incidence between 60 and 70 years of age (1, 4, 5).

The pathogenesis of VSCC can be subdivided into two different pathways: human papillomavirus (HPV)-dependent and HPV-independent (1-7). The HPV-dependent pathway accounts for 20-40% of VSCCs and has usual vulvar intraepithelial neoplasia (uVIN) as a precursor lesion (3, 4, 8). This pathway is more common in younger women and is associated with smoking, a higher number of sexual partners, and a compromised immune status (1, 3, 9). The incidence of VIN, especially the usual type, has increased in the last couple of years, even doubling in some countries (1, 4-6). The risk of the progression of a uVIN lesion towards VSCC seems low, occurring in 9-16% of patients who do not receive treatment and in approximately 3% of patients who have been treated (1, 6). However, some studies have reported a higher risk of progression (10, 11). The non-HPV pathway is associated with mutations in *TP53* and mainly occurs in older women (1-3, 6, 7). This pathway is associated with lichen sclerosus (LS), a chronic dermatosis associated with autoimmune diseases. Approximately 3-5% of women with LS progress towards VSCC (9, 12). Differentiated VIN (dVIN) is considered to be a precursor lesion of HPV-independent VSCC, with a higher malignant potential than uVIN (1, 6). dVIN can be difficult to diagnose for both clinicians and pathologists because of its subtle clinical and histological appearance (13). HPV-independent VSCC is associated with a worse prognosis than HPV-associated VSCC (3, 9). However, its carcinogenesis has not been fully clarified.

When diagnosed at an early stage, VSCC has a good prognosis, especially for patients without inguinofemoral lymph node metastasis at first presentation (14). Unfortunately, approximately one-third (15) of patients suffer from recurrent disease. In the latter group of patients, therapeutic options are limited due to severe morbidity associated with repeated treatment of local recurrences. Recurrent disease in inguinal lymph nodes has a very poor prognosis and is almost always fatal (16, 17). Information on genetic and epigenetic changes that play a role in the carcinogenesis of vulvar cancer may provide valuable insight into its etiology. Studies of many different types of cancer have shown that genetic and epigenetic alteration status can help predict prognosis and guide targeted therapy (18-23). For example, vemurafenib, a BRAF inhibitor, has shown clinical efficacy as targeted therapy for melanomas that harbor mutations in *BRAF* (24). In HPV-negative VSCC, mutations are often found in

the tumor suppressor gene *TP53* (1, 8, 9, 25, 26). *TP53* mutations are thought to be an early event in the development of VSCC because they are also found in dVIN and LS lesions (1, 6-8, 26). Other mutations have been described in VSCC and its precursor lesions, including mutations in the tumor suppressor genes *PTEN* and *CDKN2A* (27, 28). Other types of genetic alterations are allelic imbalances or copy number alterations, in which the number of copies of chromosomes per cell is altered. In addition to genetic mutations, epigenetic changes may also play a role in the development of VSCC. Epigenetic changes are defined as heritable changes in gene expression without changes in the DNA sequence. The best known epigenetic change is hypermethylation of CpG islands in the promoter regions of tumor suppressor genes, causing inactivation of the gene (19, 23, 29-32). In vulvar cancer, hypermethylation of the promoters of *RASSF2A*, *MGMT,* and *TSP1* has been described (30). Here, we review the current literature and summarize the current understanding of the role of genetic and epigenetic changes in VSCC and its precursor lesions.

Materials and methods

Relevant studies on genetic alterations (somatic mutations, allelic imbalances, loss of heterozygosity, copy number changes, and microsatellite instability) and epigenetic changes (hypomethylation and hypermethylation, microsatellite instability, and chromatin, histone, and posttranscriptional modifications) were identified from an extensive search on PubMed, Embase, Web of Science, Cochrane, and ScienceDirect. After consulting a medical librarian, a combination of Medical Subject Headings (MeSH) and free text words were formulated. Our search included the terms vulvar neoplasm, vulvar carcinoma, vulvar intraepithelial neoplasia, lichen sclerosus et atrophicus, mutation, microsatellite instability, genetic, epigenetic, hypermethylation, chromatin, histone, and posttranscriptional modifications. Research published until 31 July 2014 that studied somatic mutations and epigenetic changes in VSCC, VIN, and/or LS were included in this review. Exclusion criteria were languages other than English, Dutch, German, French, or Italian, meeting abstracts, or if the researchers only performed immunohistochemistry to evaluate protein function. Two researchers (MDT and LN) independently assessed all articles based on the title, abstract, or full article. Articles for which there was disagreement regarding inclusion or exclusion were discussed and a consensus reached. The electronic search was complemented by a manual search of bibliographies from relevant articles in order to identify additional relevant studies not encountered in the electronic search. The articles that met all inclusion criteria are described in this review.

Results

The electronic search identified 198 articles on genetic alterations in VSCC, VIN, and LS. The manual search yielded another 17 articles. 59 of these articles met the inclusion criteria and were included in this review (Tables 1 and 3). For epigenetic changes in VSCC, VIN, and LS, we found 49 articles, nine of which are included in this review (Table 4). Four articles reported on both genetic and epigenetic changes and are found in both table 1 and table 4 (28, 33-35). A flowchart illustrating the inclusion and exclusion of articles is shown in figure 1.

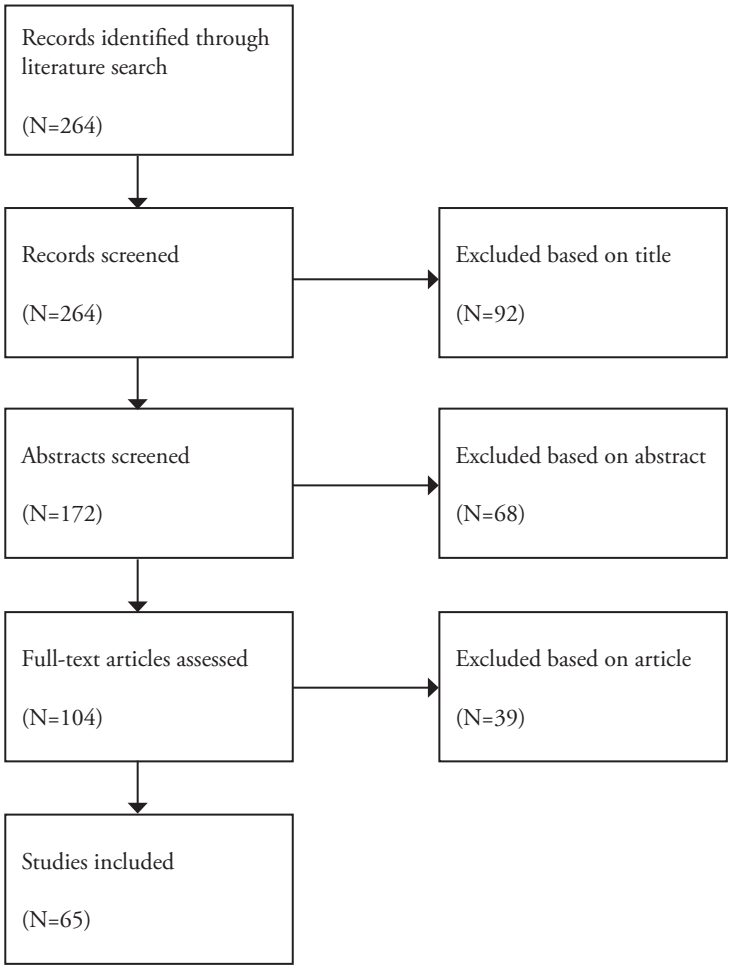

Figure 1: Inclusion and exclusion of articles

Table 1: Studies on mutations in vulvar cancer and its precursors

Author	Year	No. of patients	Diagnosis	HPV-status	Gene	Mutation %	Technique used	Remarks
Pilotti	1993	5	verrucous VC	-	*TP53*	0%	SSCP exon 5-9 + confirmation sequencing	
Kurvinen	1994	1	CIS	+	*TP53*	0%	SSCP exon 5-9 + confirmation sequencing	
		1	VIN	+	*TP53*	0%		
		2	VSCC	-	*TP53*	0%		
		7	VSCC	+	*TP53*	0%		
Lee	1994	9	VSCC	-	*TP53*	44%	SSCP exon 5-8 and part of exon 4	
		12	VSCC	+	*TP53*	8%		
Milde-Langosch	1995	12	VIN	50%*	*TP53*	33%	PCR-TGGE	* not described in association to mutations
Pilotti	1995	7	VIN*	+	*TP53*	0%	SSCP exon 5-9	*some adjacent to reported VSCC
		12	VSCC	-	*TP53*	33%		
		4	VSCC	+	*TP53*	50%		
Kim	1996	11	VSCC	-	*TP53*	36% (25% keratinising, 100% Pagets)	SSCP exon 5-8	* 11 (8 keratinising, 1 basaloid, 2 Pagets) 7 (3 keratinising, 2 basaloid, 1 Pagets, 1 warty)
		7	VSCC	+	*TP53*	0%		
Sliutz	1997	38	VSCC	not tested	*TP53*	32%	PCR-TGGE	
Wong	1997	6	VSCC	not tested	*CDKN2A* and *CDKN2B*	0%	SSCP *CDKN2A* exon 1-3 and *CDKN2B* exon 1-2	
Flowers	1999	10*	VIN	-	*TP53*	10%		* multiple samples from same patient

		11*	VIN	+	*TP53*	9%		
		15	VSCC	-	*TP53*	29% KSC, 0% basaloid		
		15	VSCC	+	*TP53*	33% KSC, 8% basaloid		
Ngan	1999	25	VSCC	-	*TP53*	20%	SSCP exon 5-8 + confirmation sequencing	
		23	VSCC	+	*TP53*	22%		
Brooks	2000	23	VSCC	-	*TP53*	74%	SSCP exon 4-9	codon 72P/R same cohort as Marin 2000 and O'Nion 2001
		13	VSCC	+	*TP53*	31%		
Holway	2000	2*	VIN	not tested	*PTEN*	100%	SSCP exon 5-8	* same patients as VSCC
		10	VSCC	not tested	*PTEN*	60%		1 patient had PTEN mutation in VIN but not in adjacent VSCC. In 3 patients different mutations were found in VIN and VSCC
Marin	2000	36	VSCC	not tested	*TP53*	58%	SSCP exon 4-9 + confirmation sequencing	
		10	LS	-	*TP53*	70%		
		29 (3 basaloid, 26 squamous)	VC	-	*TP53*	55%		
		11 (3 basaloid, 8 squamous)	VC	+	*TP53*	45%		
Wada	2000	1	VIN	+	*TP53* + *KRAS*	0% *TP53*, 0% *KRAS*	SSCP *TP53* exon 5-8, KRAS exon 1	
O'Nions	2001	23	VSCC	-	*TP53* + *CDKN2A*	74% *TP53*, 13% *CDKN2A*	SSCP CDKN2A exon 1α + 2, TP53 exon 7-9	
		13	VSCC	+	*TP53* + *CDKN2A*	31% *TP53*, 0% *CDKN2A*		

Gasco	2002	23	VSCC	-	*CDKN2A* + *Stratifin* +*TP53*	13% *CDKN2A*, 0% *Stratifin*, 73,9 % *TP53*		
		20	VIN	-	*CDKN2A* + *Stratifin* +*TP53*	0% *CDKN2A*, 0% *Stratifin*, 0% *TP53*		*CDKN2A* and *stratifin* were tested on 11 patients
		12	VIN	+	*CDKN2A* + *Stratifin* + *TP53*	0% *CDKN2A*, 0% *Stratifin*, 0% *TP53*		*CDKN2A* and *stratifin* were tested on 11 patients
		13	VSCC	+	*CDKN2A* + *Stratifin* +*TP53*	0% *CDKN2A*, 0% *Stratifin*, 30,8% *TP53*		
Rampone	2002	8	LS	not tested	*TP53*	63%	Sanger sequencing exon 5-9	
		10	LSC	not tested	*TP53*	0%		
Reddy	2002	32	VIN	not tested	*CHK2*	0% *CHK2*		
		40	VSCC	not tested	*CHK2* + *TP53*	5 % *CHK2*, 100% *TP53* *	SSCP *CHK2* exon 1a, 1b, 2-14, TP53	* only tested in *CHK2* mutated samples
Vanin	2002	62*	LS	-	*TP53*	5%	Sanger sequencing exon 5-8	* 25 with VSCC, 37 without VSCC
		29	VSCC	-	*TP53*	28%		
Rolfe	2003	12	LS	not tested	*TP53*	58%	Sanger sequencing exon 5-8	
		27	VSCC	not tested	*TP53*	81%		
Almeida	2004	2	undifferentiated VIN	-	*TP53*	50%	SCCP exon 5-8	
		6	undifferentiated VIN	+	*TP53*	17%		
Chulvis do Val	2004	13	undifferentiated VIN	64%*	*TP53*	38%	SSCP exon 5-8	* not described in association to mutations
Olawaiye	2007	2	VSCC	not tested	*EGFR*	0%	Sanger sequencing exon 18-24	
Osakabe	2007	16	VSCC	-	*TP53*	63%	SCCP exon 5-8	

		5	VSCC	+	*TP53*	20%		
		7	Bowenoid early invasion and 1 invasive SCC	+	*TP53*	0%		
Soufir	2007	21	LS	not tested (not for all)	*CDKN2A* + *TP53*	0% *CDKN2A*, 0% *TP53*	SSCP *CDKN2A* exon 1α, 1β and 2, *TP53* exon 4-9	
		2	VIN	not tested (not for all)	*CDKN2A* + *TP53*	0% *CDKN2A*, 0% *TP53*		
		5	VSCC	not tested (not for all)	*CDKN2A* + *TP53*	20% *CDKN2A*, 60% *TP53*		
Tapp	2007	224	LS	not tested	*TP53* + *KRAS* (2+1 hotspot codons only)	0% had a single mutant population that exceeded 20 per 10^6	PCR/RE/LCR	reports SBS single base instability (not somatic mutations, but 1 in a million errors) and only looked at 2 hotspots in *TP53* (codon 248 and 273) and 1 in *KRAS* (codon 12)
Aulman	2008	12	VIN (7 uVIN, 5 dVIN)	-	*TP53*	17%	SSCP exon 4-10	
		20	uVIN	+	*TP53*	0%		
		24	VSCC	-	*TP53*	17%		
		4	VSCC	+	*TP53*	0%		
Growdon	2008	19	VSCC	-	*EGFR*	0%	Sanger sequencing exon 18-21	
		22	VSCC	+	*EGFR*	0%		
		5*	CIS	not tested	*PTEN*	60%		
Pinto	2010	11	VIN	-	*TP53*	60%	Sanger sequencing	
		5	VSCC	-	*TP53*	80%		

Choschzick	2011	21	VSCC	-	*TP53*	77%	Sanger sequencing exon 5-8	
		18	VSCC	+	*TP53*	24%		
Janku	2011	2	VSCC	not tested	*PIK3CA*	0%	Sanger sequencing c532-554 of exon 9 and c1011-1062 of exon 20	
Horowitz	2012	17	VSCC	not tested	*EGFR*	0%	Sanger sequencing	
Gambichler	2013	10	LS	not tested	*TP53, NRAS, KRAS, IDH1, IDH2, TET2*	0%	Sanger sequencing *IDH1* exon 4, *IDH2* exon 4, *TET2* exon 3 + 11, *TP53* exon 4,6,7, *KRAS* codon 12, *HRAS* exon 3, *NRAS* exon 2-3	
		5	CIS	-	*EGFR*	0%		
		5	CIS	+	*EGFR*	0%		
Trietsch	2014	89	VSCC*	-	*BRAF*, *CDKN2A*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, *PTEN*, and *TP53*	0% *BRAF*, 16% *CDKN2A*, 0% *CTNNB1*, 0% *FBXW7*, 0% *FGFR2*, 0% *FGFR3*, 0% *FOXL2*, 11% *HRAS*, 1% *KRAS*, 0% *NRAS*, 8% *PIK3CA*, 3% *PPP2R1A*, 1% *PTEN*, 62% *TP53*	Hot spot mass spectrometry, Sanger sequencing *TP53 exon 5-9*	*Partial overlap in VSCC patients reported in a recent article by Spaans et al. (1)

		18	VSCC*	+	*BRAF*, *CDKN2A*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, *PTEN*, and *TP53*	0% *BRAF*, 0% *CDKN2A*, 0% *CTNNB1*, 0% *FBXW7*, 0% *FGFR2*, 0% *FGFR3*, 0% *FOXL2*, 0% *HRAS*, 0% *KRAS*, 0% *NRAS*, 0% *PIK3CA*, 0% *PPP2R1A*, 0% *PTEN*, 17% *TP53*

HPV: human papillomavirus
N: number
LS: lichen sclerosus
LSC: lichen sclerosus chronicans
VSCC: vulvar squamous cell carcinoma
VIN: vulvar intraepithelial neoplasia
uVIN: usual vulvar intraepithelial neoplasia
dVIN: differentiated vulvar intraepithelial neoplasia
CIS: carcinoma in situ
SCCP: single strand confirmation polymorphism
PCR: polymerase chain reaction
TGGE: temperature gradient gel electrophoresis
KSC: keratinizing squamous carcinoma
LCR: ligand chain reaction
RE: restriction endonuclease
Nb. HPV status was interpreted as unknown if it was not specified for all genes tested for mutations

Table 2: Overall mutation frequencies

	LS				VIN				VSCC							
	HPV neg		HPV unknown		HPV pos		HPV neg		HPV unknown		HPV pos		HPV neg		HPV unknown	
TP53	10/72	14%	12/285	4%	2/66	3%	10/47	21%	11/29	38%	28/171	16%	109/361	30%	28/108	26%
PTEN									2/2	100%	0/18	0%	1/89	1%	6/10	60%
EGFR											0/22	0%	0/19	0%	0/19	0%
BRAF											0/18	0%	0/89	0%		
HRAS											0/18	0%	10/89	11%		
KRAS			0/10	0%							0/18	0%	1/89	1%		
NRAS			0/10	0%							0/18	0%	0/89	0%		
CDKN2A			0/21	0%	0/4	0%	0/2	0%	0/2	0%	0/44	0%	20/135	15%	1/11	9%
CTNNB1											0/18	0%	0/89	0%		
PPP2R1A											0/18	0%	3/89	3%		
FBXW7											0/18	0%	0/89	0%		
PIK3CA											0/18	0%	7/89	8%	0/2	0%
IDH1			0/10	0%												
IDH2			0/10	0%												
TET2			0/10	0%												
CHK2									0/32	0%					2/40	5%
FGFR2											0/18	0%	0/89	0%		
FGFR3											0/18	0%	0/89	0%		
FOXL2											0/18	0%	0/89	0%		
Stratifin					0/4	0%	0/2	0%			0/13	0%	0/23	0%		

LS: lichen sclerosus
VIN: vulvar intraepithelial hyperplasia
VSCC: vulvar squamous cell carcinoma
HPV: human papillomavirus
Nb. HPV status was interpreted as unknown if it was not specified for all genes tested for mutations

Somatic mutations

A total of 34 articles were included that described somatic mutations (Table 1) (8, 25-28, 33-61). Mutations were most often studied and detected in *TP53*, with frequencies of up to 70% for LS, 60% for VIN, and 81% for vulvar cancer. *CDKN2A* mutations were not detected in LS or VIN, but occurred in 0-60% of VSCCs. Table 2 shows the overall frequencies of mutations for all included studies. HPV-negative tumors harbored more mutations than HPV-positive tumors, and the percentage of mutated samples gradually increased with higher stages of (pre)cancerous lesions.

Allelic imbalances, loss of heterozygosity, and copy number changes

A total of 24 articles were included that reported allelic imbalances or copy number changes in vulvar cancer and its precursors (Table 3) (36, 45, 47-49, 51, 52, 55, 56, 58, 60, 62-73). Allelic imbalances occurred most often on chromosomes 3, 8, 11, 13, and 17. Three studies focused on the total DNA index, and each found high percentages of aneuploidy and tetraploidy (62-64). Bryndorf was the only one to test HPV infection and found the highest percentage of aneuploidy and tetraploidy in HPV-negative VSCC. Allelic imbalances were more frequently observed in higher stages of both precancerous and cancerous lesions (63).

Microsatellite instability

We included three articles that reported on microsatellite instability (MSI) (65, 74, 75), a condition in which repetitive DNA sequences are susceptible to errors because the Mismatch Repair system is not functioning properly (Table 4). The articles by Bujko and Lin looked at MSI in HPV-positive and negative VSCC. Bujko et al. found no MSI in the 44 patients they investigated (29 HPV-negative and 15 HPV-positive) (74). Lin reported MSI in locus 3.1 in one of two patients with HPV-positive VSCC (65). Pinto et al. focused on MSI and allelic imbalances in uVIN, dVIN and LS, and found that MSI was confined exclusively to HPV-negative dVIN and LS lesions, but did not occur in the 15 uVINs they studied (75). The data by Pinto suggest that these molecular changes are possibly early events in the HPV-independent route of vulvar carcinogenesis, and that MSI may play a role in the malignant potential of LS. However, in a small cohort of 4 patients with VSCC described by Lin et al., 2 patients with HPV-positive tumors displayed MSI as well. These data indicate that the exact role of MSI in vulvar carcinogenesis needs to be elucidated.

Table 3: Studies on allelic imbalances in vulvar cancer and its precursors

Author	Year	No. of patients	Diagnosis	HPV-status	Gene/locus	AI %	loss or gain	Technique used	Remarks
Wong	1997	6	VSCC	not tested	*CDKN2A* and *CDKN2B*	50% *CDKN2A*, 50% *CDKN2B*	loss	LOH	
Lin	1998	2	VIN	-		0% 1.2, 0% 2.3, 50% 2.4, 0% 3.1, 0% 3.4, 0% 4.1, 50% 5.2, 50% 5.3, 0% 8.2, 0% 21.1	loss	LOH	
		2	VIN	+		0% 1.2, 50% 2.3, 50% 2.4, 0%3.1, 50% 3.4, 0% 4.1, 0% 5.2, 0% 5.3, 50% 8.2, 0% 21.1	loss		
		2	VSCC	-		0% 1.2, 100% 2.3, 100% 2.4, 50% 3.1, 50% 3.4, 50% 4.1, 100% 5.2, 50% 5.3, 50% 8.2, 50% 21.1	loss		

		2	VSCC	+		50% 1.2, 0% 2.3, 100% 2.4, 0% 3.1, 0% 3.4, 0% 4.1, 0% 5.2, 100% 5.3, 50% 8.2, 0% 21.1	loss		
Flowers	1999	10*	VIN	-	3p chromosomal regions (3p 12, 3pl 4.2, 3pl 4.3-21.1, 3p2l.3, 3p22-24, 3p24.3, 3p25), 13q14 (*RB*) and 17pl3.1 (*TP53*) loci	54% 3p, 14% 13q (*RB*), 9% 17p (*TP53*)	loss	LOH	* multiple samples from same patients
		10*	VIN	+	3p chromosomal regions (3p 12, 3pl 4.2, 3pl 4.3-21.1, 3p2l.3, 3p22-24, 3p24.3, 3p25), 13q14 (*RB*) and 17pl3.1 (*TP53*) loci	16% 3p, 6% 13q (*RB*), 0% 17p (*TP53*)	loss		
		15	VSCC	-	3p chromosomal regions (3p 12, 3pl 4.2, 3pl 4.3-21.1, 3p2l.3, 3p22-24, 3p24.3, 3p25), 13q14 (*RB*) and 17pl3.1 (*TP53*) loci	93% 3p, 27% 13q (*RB*), 62% 17p (*TP53*)	loss		
		15	VSCC	+	3p chromosomal regions (3p 12, 3pl 4.2, 3pl 4.3-21.1, 3p2l.3, 3p22-24, 3p24.3, 3p25), 13q14 (*RB*) and 17pl3.1 (*TP53*) loci	67% 3p, 31% 13q (*RB*), 15% 17p (*TP53*)	loss		

Scheistroen	1999	167	VSCC	not tested	77 % diploid, 23% aneuploid		FACS
Pinto	1999	8	VSCC	-	Overall 36% LOH. Most frequent: 83% 5q, 100% 10p, 29% 1p, 25% 2q, 50% 3p, 63% 8p, 63% 8q, 60% 10q, 50% 11q, 29% 15q, 80% 17p, 50% 21q, 60% 22q.	loss	LOH
		8	VSCC	+	Overall 30% LOH. Most frequent: 13% 5q, 17% 10q, 33% 1p, 0% 2q, 50% 3p, 13% 5q, 33% 8p, 50% 8q, 17% 10p, 25% 11q, 43% 15q, 43% 17p, 67% 21q, 20% 22q	loss	

Pinto	2000	16	VIN (5 uVIN, 11 dVIN)	-	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	15%*	both	LOH	*scoring informative (heterozygous) loci
		14	VIN (10 uVIN, 4 dVIN)	+	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	25%*	both		*scoring informative (heterozygous) loci
		17	LS	-	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	10%*	both		*scoring informative (heterozygous) loci
Brooks	2000	23	VSCC	-	*TP53*	61%	loss	LOH	codon 72P/R same cohort as Marin 2000 and O'Nion 2001
		13	VSCC	+	*TP53*	54%	loss		
Carlson	2000	12	LS	not tested	chr 17	chr 17 aneusomy:100%. DNA index aneuploidy: 58%		FISH	
		3	VIN	not tested	chr 17	chr 17 aneusomy: 100% DNA index aneuploidy: 67%			
		14*	VSCC	not tested	chr 17	chr 17 aneusomy: 93%. DNA index aneuploidy: 86%			* 10 SCC, 4 SCCIS
Marin	2000	36	VSCC	not tested	*TP53*	54%	loss	LOH	
Wada	2000	1	VIN	+	3p14.2, 3p, 9p21, 9p23, 13q22, 17p12	0%	loss	LOH	

Jee	2001	10	VSCC	not tested	DNA copy number changes in 80%. Loss: 50% 4p13-pter, 40% 3p, 10% 5q14-q23, 10% 6q11-q16, 10% 11q21-qter, 10% 13q14-q32. Gain: 40% 3q, 30% 8q, 10% 9p, 10% 14, 10% 17, 10% 20q	both	CGH
Rosenthal	2001	13	VSCC	-	LOH of 48% 17p, 40% 9p, 48% 3p, 44% 4q, 43% 5p, 44% 11p	loss	
		54	VSCC	+	LOH of 48% 17p, 40% 9p, 48% 3p, 44% 4q, 43% 5p, 44% 11p	loss	
Allen	2002	8	VSCC	-	Most common: 75% 8q gain, 0% 3q gain, 13% 3p loss, 50% 11q loss	both	CGH

		10	VSCC	+		Most common 20% 8q gain, 50% 3q gain, 40% 3p loss, 40% 11q loss	both		
Reddy	2002	32	VIN	not tested	*CHK2*	0%*			
		40	VSCC	not tested	*CHK2*	2%*	loss	direct sequencing of RT-PCR product	* only tested in *CHK2* mutated samples
Vanin	2002	62*	LS	-	*TP53*	0%	loss	LOH	* 25 with VSCC, 37 without VSCC
		29	VSCC	-	*TP53*	74%	loss		
Bryndorf	2004	4	condyloma	-		0 chromosomal abberations	both	hrCGH and FACS	
		2	VIN	-		100% diploid. Most common gain of: 0% chr 1, 0% 3q, 0% 20q, 0% 20p, 0% 3q, 0% 8q. Loss of 0% 3p, 0% 8p	both		

	9	VIN	+	40% diploid, 30% aneuploid, 30% tetraploid. Most common gain of: 60% chr 1, 50% 3q, 50% 20q, 40% 20p, 30% 8q. Loss of 20% 3p, 0% 8p	both
	6	VSCC	-	25% diploid, 75% aneuploid. Most common gain of: 0% chr 1, 75% 3q, 50% 20q, 50% 20p, 100% 8q. Loss of 50% 3p, 50% 8p	both
	4	VSCC	+	50% diploid, 50% tetraploid. Most common gain of: 0% chr 1, 66% 3q, 17% 20q, 17% 20p, 33% 8q. Loss of 83% 3p, 33% 8p	both

Huang	2005	8	VSCC	75%*		gains of 1q 13%, 3q 38%, 5p 38%, 8q 75%. Losses 3p 38%, 4p 13%, 11p 13%	both	CGH	* not described in association to genetic changes
Olawaiye	2007	2	VSCC	not tested	*EGFR*	0%		q rtPCR	
Osakabe	2007	16	VSCC	-		LOH of 44% 3p14.2 (*FHIT*), 38% 3p26 (*VHL*), 38% 5q31 (*APC*), 63% 9q21 (*p16*), 67% 9q22.3 (*PTECH*), 38% 10p15 (*PAHX*), 30% 13q14.3-21.1 (*Rb*), 40% 17p13 (*TP53*), 44% 18q21 (*DCC*). Fractional allelic loss 43%	loss	LOH	
		5	VSCC	+		LOH of 50% 3p14.2 (*FHIT*), 100% 9q21 (*p16*), 50% 9q22.3 (*PTCH*), Fractional allelic loss 18%	loss		
Yangling	2007	10	VSCC	-	3q, 3p, 4p, 8q, 12q	Gain: 10% 3q, 70% 8q 0% 12q, Loss: 40% 3p, 50% 4p	both	CGH	

		11	VSCC	+	3q, 3p, 4p, 8q, 12q	Gain: 73% 3q, 64% 12q, 9% 8q. Loss: 46% 3p, 55% 4p	both		
Growdon	2008	19	VSCC	-	*EGFR* + *HER2*	32% *EGFR*, 0% *HER2*, 16% polysomy chr 7	gene amplification	FISH	
		22	VSCC	+	*EGFR* + *HER2*	0% *EGFR*, 0% *HER2*			
		5	CIS	-	*EGFR* + *HER2*	0% *EGFR*, 0% *HER2*			
		5	CIS	+	*EGFR* + *HER2*	0% *EGFR*, 0% *HER2*			
Aulman	2008	12	VIN (7 uVIN, 5 dVIN)	-	3q26	73%	gain	FISH	
		20	uVIN	+	3q26	50%	gain		
		24	VSCC	-	3q26	83%	gain		
		4	VSCC	+	3q26	75%	gain		
Horowitz	2012	17	VSCC	not tested	*EGFR*	12%	gene amplification	FISH	
Lavorato-Rocha	2013	139	VSCC	33%*	*TP53*	65% normal gene / chr copy number, 19% polysomy, 9% monosomy, 6% deletion	both	FISH	* not described in association to genetic changes

Micci	2013	14	VSCC	not tested	Amongst others *FHIT*, *PTPRD*	70% aneuploid, 20% tetraploid, 10% diploid. 90% array-CGH imbalances. Loss of a region of 64% 8p23.1, 57% 8p21.3, 57% 8p12, 50% 3p14.2, 50% 3p13, 50% 8p23.3-p23.1, 50% 8p23.1-p11.23, 50% 8p11.22-p11.1, 50% 8q23.3, 50% 8q24.12-q24.22, 50% 9p23. Homozygous deletion of 29% p23 (*PTPRD*). No common amplified region.	both	arrayCGH + rtPCR + karyotyping

HPV: human papillomavirus
N: number
LS: lichen sclerosus
LSC: lichen sclerosus chronicans
SCC: vulvar squamous cell carcinoma
VIN: vulvar intraepithelial neoplasia
AI: allelic imbalance
LOH: loss of heterozygosity
FISH: fluorescence in situ hybridization
RT-PCR: real time polymerase chain reaction
(hr)CGH: (high resolution) comparative genomic hybridization
FACS: fluorescence-activated cell sorting
SCCIS: squamous cell carcinoma in situ
Nb. HPV status was interpreted as unknown if it was not specified for all genes tested for allelic imbalances

Table 4: Studies on microsatellite instability (MSI) in vulvar cancer and its precursors

Author	Year	No. of patients	Diag-nosis	HPV-status	Locus	% MSI	Technique used
Lin	1998	2	VSCC	-	3.1	0%	PCR
		2	VSCC	+	3.1	50%	
Bujko	2012	29	VSCC	-		0%	PCR
		15	VSCC	+		0%	
Pinto	2000	5	uVIN	-	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	0%	PCR
		10	uVIN	+	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	0%	
		11	dVIN	-	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	27%	
		4	dVIN	+	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	0%	
		17	LS	-	3p, 5q, 8p, 8q, 10p, 10q, 11q, 17p, 18q, 21q, 22q	12%	

HPV: human papillomavirus
LS: lichen sclerosus
VSCC: vulvar squamous cell carcinoma
VIN: vulvar intraepithelial neoplasia
PCR: polymerase chain reaction

Epigenetic alterations

Nine articles were included that reported on epigenetic alterations in VSCC or its precursors (Table 5) (28-30, 33, 34, 76-79). *CDKN2A* was studied most often (28-30, 33, 34, 76, 78, 79). *CDKN2A* is more frequently hypermethylated in VSCC (up to 68%) and VIN (up to 72%) than in LS (up to 47%), but there is great variability in the reported frequencies. An overview of all genes tested for hypermethylation and the percentage of hypermethylation is shown in table 6. When HPV status was not specified for all genes tested for hypermethylation, HPV status was interpreted as unknown.

Table 5: Studies on hypermethylation in vulvar cancer and its precursors

Author	Year	No. of patients	Diag-nosis	HPV-status	Gene	% Hypermethylation	Technique used	Remarks
O'Nions	2001	13	VSCC	HPV 16 +	*CDKN2A*	15,4%	msPCR	
		23	VSCC	HPV 16 -	*CDKN2A*	47,8%	msPCR	
Gasco	2002	0	VIN 1	HPV 16 +	*Stratifin, CDKN2A*	0% *Stratifin*, 0% *CDKN2A*	msPCR	
		4	VIN 1	HPV 16 -	*Stratifin, CDKN2A*	0% *Stratifin*, 0% *CDKN2A*	msPCR	
		1	VIN 2	HPV 16 +	*Stratifin, CDKN2A*	0% *Stratifin*, 0% *CDKN2A*	msPCR	
		5	VIN 2	HPV 16 -	*Stratifin, CDKN2A*	40% *Stratifin*, 40% *CDKN2A*	msPCR	
		11	VIN 3	HPV 16 +	*Stratifin, CDKN2A*	45,5% *Stratifin*, 9,1% *CDKN2A*	msPCR	
		11	VIN 3	HPV 16 -	*Stratifin, CDKN2A*	72,7% *Stratifin*, 72,7% *CDKN2A*	msPCR	
		13	VSCC	HPV 16 +	*Stratifin, CDKN2A*	53,8% *Stratifin*, 15,4% *CDKN2A*	msPCR	
		23	VSCC	HPV 16 -	*Stratifin, CDKN2A*	56,5% *Stratifin*, 47,8% *CDKN2A*	msPCR	
Lerma	2002	21	LS	not tested	*CDKN2A*	42,8%	ms-PCR	
		13	9 uVIN, 4 dVIN	not tested	*CDKN2A*	69,2%	ms-PCR	
		38	VSCC	not tested	*CDKN2A*	68%	ms-PCR	

Soufir	2007	2	LS	HPV 16 +	*CDKN2A, p14*	0% *CDKN2A*, 0% *p14*	ms-PCR	
		8	LS	HPV 16 -	*CDKN2A, p14*	12,5% *CDKN2A*, 0% *p14*	ms-PCR	
		2	VIN3	HPV 16 +	*CDKN2A, p14*	0% *CDKN2A*, 0% *p14*	ms-PCR	
		2	VSCC	HPV 16 +	*CDKN2A, p14*	0% *CDKN2A*, 0% *p14*	ms-PCR	
		2	VSCC	HPV 16 -	*CDKN2A, p14*	0% *CDKN2A*, 0% *p14*	ms-PCR	
Aide	2010	15	LS	not tested	*DAPK* + *CDKN2A*	13% *DAPK*, 47% *CDKN2A*	ms-PCR	
Guerrero	2011	21	LS not associated with VSCC	HPV + 25%	*RASSF1A, RASSF2A, CDKN2A, TSP-1* and *MGMT*	52,4% *RASSF1A*, 0% *RASSF2A*, 19% *CDKN2A*, 52,4% *TSP-1*, 0% *MGMT*	ms-PCR	25% HPV positive, but HPV status not specified per gene investigated for hypermethylation
		12	LS associated with VSCC	not tested	*RASSF1A, RASSF2A, CDKN2A, TSP-1* and *MGMT*	33,3% *RASSF1A*, 8,3% *RASSF2A*, 16,6% *CDKN2A*, 50% *TSP-1*, 41,7% *MGMT*	ms-PCR	
		1	VSCC	HPV +	*RASSF1A, RASSF2A, CDKN2A, TSP-1* and *MGMT*	0% *RASSF1A*, 0% *RASSF2A*, 0% *CDKN2A*, 20% *TSP-1*, 0% *MGMT*	ms-PCR	TSP-1 hypermethylation was tested on 5 patients
		11	VSCC	HPV -	*RASSF1A, RASSF2A, CDKN2A, TSP-1* and *MGMT*	45,5% *RASSF1A*, 72,7% *RASSF2A*, 54,5% *CDKN2A*, 40% *TSP-1*, 72,7% *MGMT*	ms-PCR	TSP-1 hypermethylation was tested on 25 patients

Aide	2012	23	LS	not tested	*DAPK* + *CDKN2A*	17% *DAPK*, 35% *CDKN2A*	ms-PCR	
Oonk	2012	20	VSCC	not tested	*CDKN2A, MGMT, TWIST1, CADM1, TERT* and *TFPI2*	65% *CDKN2A*, 45% *MGMT*, 35% *TWIST 1*, 55% *CADM1*, 100% *TERT*, 60% *TFPI2*	msPCR	
Guerrero	2013	21	LS	HPV + 25%	*TSLC-1*	25% *TSLC-1*	ms-PCR	25% HPV positive, but HPV status not specified per gene investigated for hypermethylation
		30	VSCC	16,7% +	*TSLC-1*	44,4% *TSLC-1*	ms-PCR	Same cohort as Guerrero 2011. Only new results are described here.

HPV: human papillomavirus
LS: lichen sclerosus
LSC: lichen sclerosus chronicans
VSCC: vulvar squamous cell carcinoma
VIN: vulvar intraepithelial neoplasia
msPCR: methylation-specific polymerase chain reaction
Nb. HPV status was interpreted as unknown if it was not specified for all genes tested for hypermethylation

Table 6: Overall hypermethylation frequencies

	LS						VIN						VSCC					
	HPV pos		HPV neg		HPV unknown		HPV pos		HPV neg		HPV unknown		HPV pos		HPV neg		HPV unknown	
CDKN2A	0/2	0%	1/8	12,5%	26/92	28,3%	1/14	7,1%	10/20	50%	9/13	69,2%	4/29	13,8%	28/59	47,5%	39/58	67,2%
p14	0/2	0%	0/8	0%			0/2	0%					0/2	0%	0/2	0%		
DAPK					6/38	15,8%												
MGMT					0/33	0%											9/20	45%
TWIST1																	7/20	35%
CADM1																	11/20	55%
TERT																	20/20	100%
TFPI2																	12/20	60%
RASSF1A					15/33	45,5%												
RASSF2A					1/33	3,0%												
TSP-1					17/33	51,5%												
Stratifin							5/12	41,7%	10/20	50%			7/13	53,8%	11/23	56,5%		
TSLC-1					9/21	42,9%											11/30	44,4%

LS: lichen sclerosus
VIN: vulvar intraepithelial hyperplasia
VSCC: vulvar squamous cell carcinoma
HPV: human papillomavirus
Nb. HPV status was interpreted as unknown if it was not specified for all genes tested for hypermethylation

Discussion

A growing body of research has focused on genetic and epigenetic changes in vulvar cancer. The combined results of the currently available literature on genetic and epigenetic changes confirm the hypothesis that HPV and *TP53* mutations play almost separate, but key roles in the carcinogenesis of VSCC (Table 5). Patients infected with HPV are less likely to carry somatic mutations than patients without HPV, but allelic imbalances seem to occur in both groups. The cumulative number of genetic changes increases with increasing grade of dysplasia and cancer stage. Although only a few studies have sufficient numbers of patients to perform survival analysis related to genetic and epigenetic changes, the findings suggest that tumors harboring a mutation, which are most often HPV-independent VSCC, have a worse prognosis than VSCC without (epi) genetic changes (36, 43, 50, 54, 58, 62, 73, 80).

The frequencies of detected mutations vary between studies. These differences can be explained, in part, by the composition of the cohorts. The included cohorts may vary in terms of age and ethnic background or tumor stage, which is known to be related to genetic alterations. Also, differences in the techniques used and coverage of the screened exons may play a role. Detection methods have improved over the last few decades, which is reflected in an overall increase in the number of detected *TP53* mutations within HPV-negative tumor samples.

The amount of research on epigenetic changes in VSCC and its precursors is limited, but studies in other types of cancer have shown the importance of these tumor characteristics in the development of targeted therapy (81). We only found articles on hypermethylation. In our literature search we did not find any articles on other possible epigenetic changes in VSCC or its precursors, such as chromatin remodeling or histone modifications. Most research on hypermethylation has studied different genes so a comparison cannot be made. Only *CDKN2A* has been investigated by more than one group. The hypermethylation frequencies that were found differ greatly between LS, VIN, and VSCC. The trend appears to be more hypermethylation in VSCC, but with the limited data it is difficult to draw any conclusions. With the fast development of research techniques focusing on epigenetic alterations in tumors, and the knowledge already gained on targeted therapy for epigenetically altered tumors, future research on this topic is promising.

In conclusion, genetic and epigenetic changes are detected more often with increasing precursor and tumor stage, and are more frequently found in HPV-negative patients than HPV-positive patients. However, compared to other types of cancer, studies on genetic and epigenetic changes in vulvar cancer and its precursors is relatively few and,

therefore, our knowledge on this subject is still limited. Most genetic studies focus on HPV infection and TP53 mutations, , the latter being the most frequent genetic change found in human cancers so far. Recent studies provide evidence that somatic mutations often do occur in other genes, such as CDKN2A and HRAS. Of all premalignant and malignant vulvar lesions, HPV-independent VSCC represents the largest group of patients with the worst prognosis and most difficulties in the diagnosis and treatment of progressive tumors. The upcoming availability of screening methods for somatic mutations that provide information on the complete or very large parts of the genome, such as next generation sequencing, may provide us with more insight into the mutational and epigenetic landscape and the etiology of vulvar cancer. Hopefully, these advances will increase future treatment possibilities and improve prognosis.

Conflict of interest statement

There are no conflicts of interest.

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