Pilot study of markers for high-grade anal dysplasia in a southern cohort from the Women's Interagency HIV Study (WIHS)

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ASCCP *Improving Lives Through the Prevention & Treatment of Anogenital & HPV-Related Diseases*

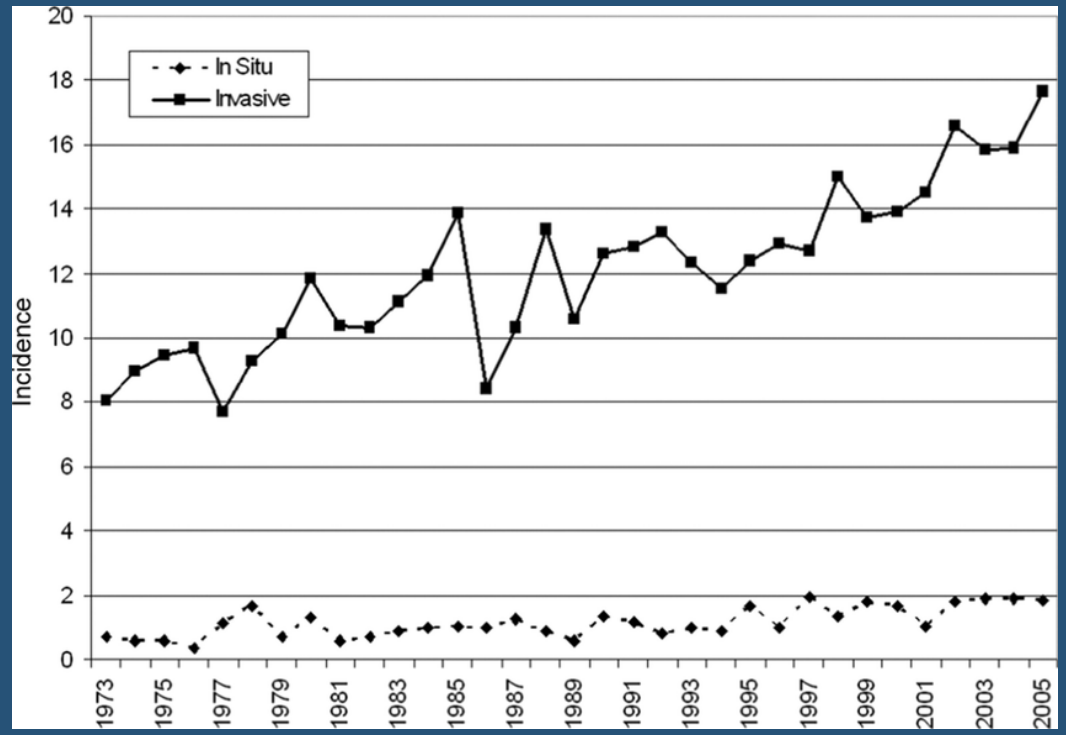
ASCCP2018 Annual Meeting

Disclosures

- No financial relationships or conflict of interest to disclose

Anal carcinoma in HIV+ women

- From 1975 to 2008, the incidence of anal cancer in women more than doubled, from 0.946 per 100,000 to 1.827 per 100,000
  - Among HIV-infected men and women, the number of anal cancers increased nearly 8-fold from 1991 to 2005.
  - HIV-infected women have a nearly 8-fold increased risk of invasive anal cancer than women in the general population



CDC, 2012, Frisch, 2000
Shvetsov, 2009

Risk for anal carcinoma and anal dysplasia

- Human papillomavirus (HPV) infection is associated with over 80% of anal carcinomas
  - High-risk (hr) HPV has been associated with high grade anal intraepithelial neoplasia (AIN) and invasive anal cancer.
- HIV-infected women have an increased risk for acquiring HPV infection and having persistent anal dysplasia

Stier, 2015

Predictors of anal dysplasia

- hrHPV
  - hr-HPV is detected at a high rate in anal samples from HIV-infected women
    - A significant fraction of precancerous lesions may be negative for HPV16/18
    - May have limited utility in screening for women at high risk of dysplasia
- Cytology
  - High prevalence of abnormal anal cytology among HIV-infected women
  - Minimal correlation with final histologic grade

- **Strategies to better identify women at risk of high-grade anal dysplasia are needed**

Epigenetics: a novel target for cancer screening?

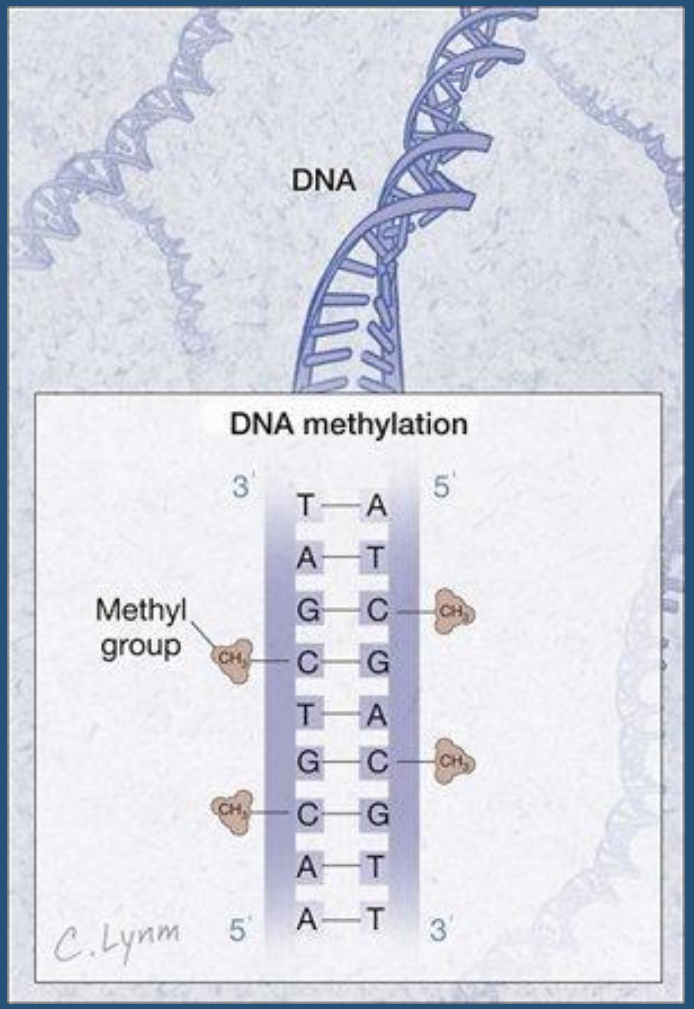
- **Epigenetics-** the study of changes in gene expression that occur independent of changes in the primary DNA sequence
  - Ex: **Methylation**, histone modification, gene silencing, etc
  - Disruption can lead to altered gene function and malignant cellular transformation
- **Methylation** has been studied as both a potential therapeutic and screening target for oncogenesis
    - Global methylation --> genomic instability, abnormal chromosomal structures, oncogene activation
    - Gene promoter methylation --> inactivation of tumor suppressor genes or DNA repair genes

Sharma, 2010

Handry, 2011

DNA Methylation

- One of the most commonly occurring epigenetic events
- Covalently adds methyl group to cystosine located 5’ to guanine in cytosine-guanine dinucleotides (CpG)
  - Occurs almost exclusively in sequence context **5'CG3**‘
- This change, though heritable, is reversible, making it a therapeutic target.



Methylation and Cervical Cancer

- Promoter hypermethylation of the genes ***FAM19A4* and/or hsa-*miR124-2*** has been detected in several human cancers and cancer cell lines, including cervical cancer and endometrial cancer
  - Increases with cervical disease severity
  - Methylation levels are particularly high in women with cervical cancer and advanced high-grade lesions
- Hypermethylation of FAM19A4 detects lesions ≥ CIN 3 with a sensitivity of 88% and specificity of 62%
  - Found to be noninferior to cytology in women ≥ age 30

- **Methylation analysis has been proposed as a valuable alternative or additive triage tool in cervical cancer screening**

De Strooper, 2014
Luttmer, 2016

Methylation and Anal Cancer

- Aberrant DNA methylation is frequent event in anal HSIL and anal carcinomas

- Differential methylation patterns found in anal carcinomas at high-risk for progression (size ≥ 5cm and/or nodal involvement) compared to low-risk tumors
  - Siegel et al examined 16CpG loci known to be involved in mammalian carcinogenesis

- **Despite evidence demonstrating high sensitivity and specificity of the targets FAM19A4 and miR124-2 to cervical cancer, no data exists on these targets in anal cancer**

1. Zhang, 2005

2. Siegel , 2014

Study Overview

- **Objective: Investigate biological markers predictive of anal high-grade squamous intraepithelial lesions (HSIL) in women**

- **Study Design**: Cross-sectional cohort of HIV-positive and at-risk HIV-negative women from the Atlanta Women's HIV Interagency Study (WIHS)

Study Overview

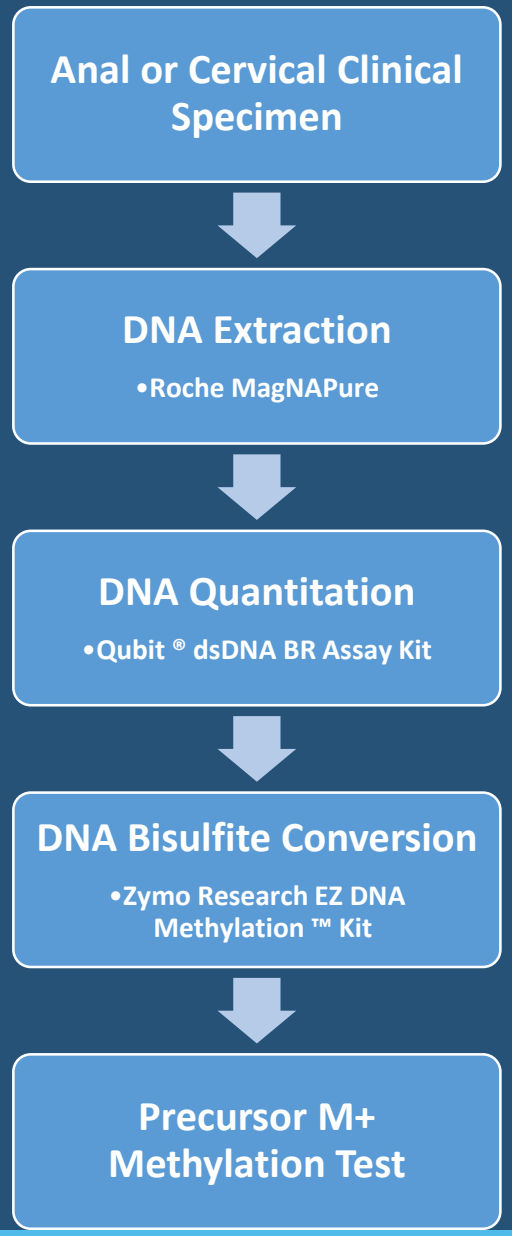
- All enrolled women underwent cervical and anal sampling for:
  - Anal cytology “pap test”
  - Anal and cervical hr-HPV genotyping
  - Anal and cervical FAM19A4 and miR124-2 gene promoter methylation

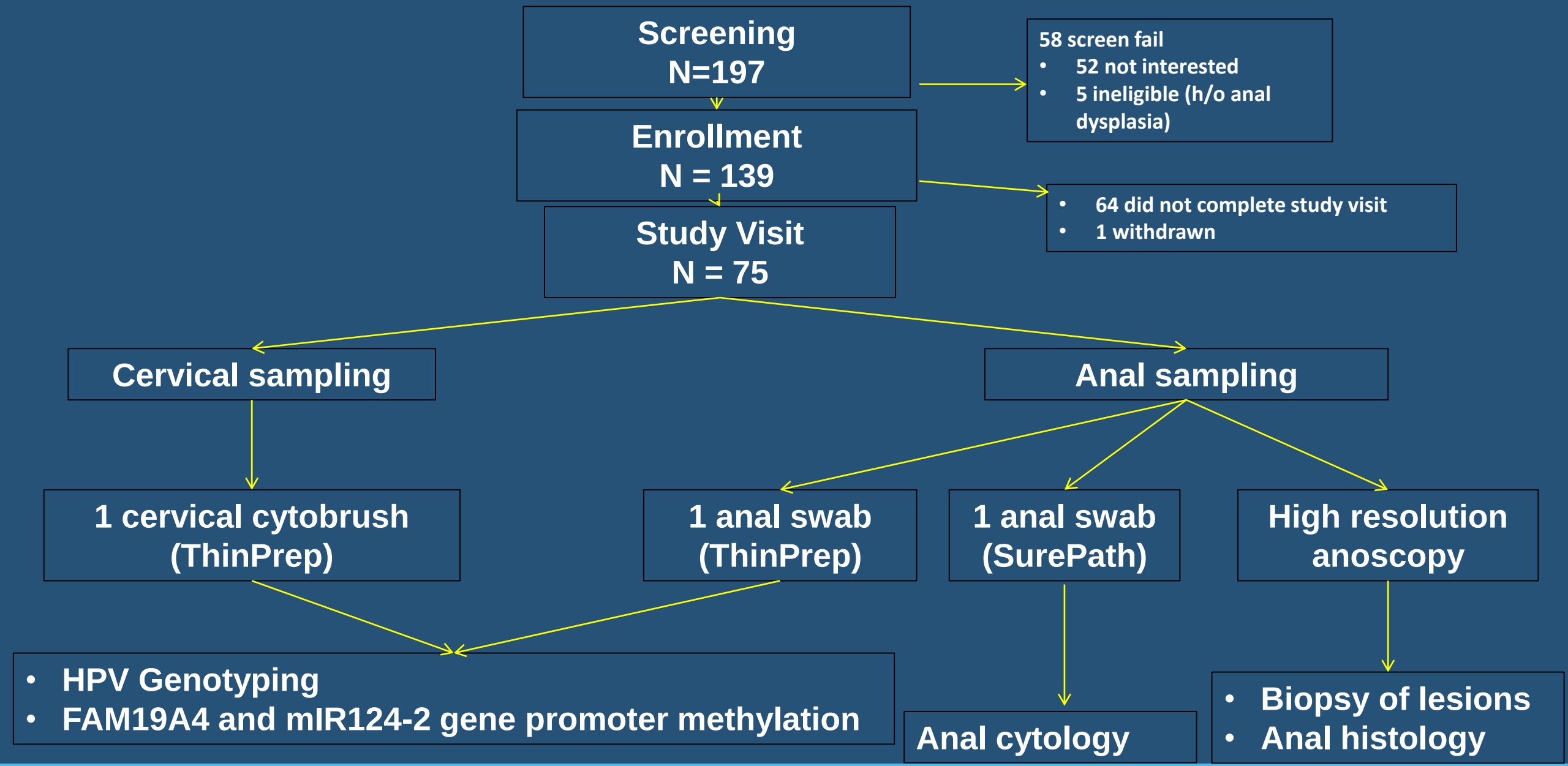
- Women simultaneously underwent high resolution anoscopy with biopsy of suspicious lesions

- Previously collected data on cervical cytology and histology within 12 months of study visit abstracted from WIHS database

Methylation Analysis

- Extracted DNA subjected to bisulfite treatment
- DNA methylation analysis performed by qMSP, using primers and probes specific for methylated DNA of *FAM19A4* and *hsa miR-124-2*
  - methylation-independent β-actin (ACTB) as sample quality control
- Cycle threshold (CT) value set to represent number of PCR cycles necessary for detection of signal above background
  - ΔCT value is calculated as the difference between the CT value of the FAM19A4 or hsa-miR124-2 targets and the CT value of the reference (ACTB).
- Normalized by subtracting the ΔCT value of ACTB from ΔCT of the targets, resulting in **a ΔΔCT value**
  - A sample was considered hypermethylated if ΔΔCT ratio was lower than preset threshold of the respective target




Screening
N=197
58 screen fail
• 52 not interested
• 5 ineligible (h/o anal dysplasia)
Enrollment
N = 139
• 64 did not complete study visit
• 1 withdrawn
Study Visit
N = 75
Cervical sampling
Anal sampling
1 cervical cytobrush (ThinPrep)
1 anal swab (ThinPrep)
1 anal swab (SurePath)
High resolution anoscopy
• HPV Genotyping
• FAM19A4 and mIR124-2 gene promoter methylation
Anal cytology
• Biopsy of lesions
• Anal histology

Results: Demographic & Clinical Characteristics

Variable n(%) or mean (SD)	Overall n = 75	HIV+ n = 52	HIV- n= 23
Age in years	48.7 (9)	49.1 (8)	47.7 (10)
Race/ethnicity			
Black	64 (87)	46 (89)	18 (82)
White	4 (5)	3 (6)	1 (5)
Hispanic	3 (4)	2 (4)	1 (45)
Other/unknown	3 (4)	1 (2)	2 (9)
Health insurance*	63 (85)	50 (96)	13 (59)
Unemployed	54 (73)	41 (79)	13 (59)
≤ HS Education	52 (70)	37 (71)	15 (68)
Drinks per week			
Abstainer	30 (40)	22 (42)	4 (18)
0-7	35 (47)	25 (48)	9 (41)
>7	9 (12))	5 (10)	13 (59)
Current Smoker	43 (58)	30 (58)	13 (59)

*p <0.05

Anal characteristics by HIV status

Variable n(%) or mean (SD)	Overall n = 75	HIV+ n = 52	HIV- n= 23
Anal Histology			
Normal/no biopsies	37 (50)	26 (52)	11 (48)
LSIL/condyloma	23 (32)	16 (32)	7 (30)
HSIL	13 (18)	8 (16)	5 (22)
Anal Cytology			
Normal	26 (35)	19 (37)	7 (30)
ASCUS/LSIL	45 (60)	30 (58)	15 (65)
ASC-H	4 (5)	3 (6)	1 (4)
Anal HPV			
Any	57 (77)	42 (82)	15 (65)
hr HPV	36 (49)	28 (55)	8 (35)
16/18*	13 (18)	12 (24)	1 (4)

*p <0.05

Cervical characteristics by HIV status

Variable n(%) or mean (SD)	Overall n = 75	HIV+ n = 52	HIV- n= 23
Cervical cytology			
Normal	64 (85)	42 (82)	22 (96)
ASCUS	7 (9)	6 (12)	1 (4)
LSIL	2 (3)	2 (4)	0 (0)
ASC-H/HSIL	2 (3)	2 (4)	0 (0)
Cervical histology*			
None	49 (65)	31 (60)	18 (78)
Normal	13 (17)	9 (17	4 (17)
LSIL	9 (13)	9 (17)	0 (0)
HSIL	1 (1)	1 (2)	0 (0)
Cervical HPV			
Any	49 (65)	36 (69)	13 (57)
hr HPV	20 (27)	16 (31)	4 (17)
16/18	3 (4)	3 (6)	0 (0)

*p <0.05

Anal and cervical hypermethylation by HIV status

Variable n(%) or mean (SD)	Overall n = 75	HIV+ n = 52	HIV- n= 23
Anal Hypermethylation			
Any (FAM19A4 or miR124-2)	69 (95)	49 (98)	20 (87)
FAM19A4 only	69 (95)	36 (72)	16 (70)
miR124-2 only	52 (71)	49 (98)	20 (87)
Cervical Hypermethylation			
Any	19 (26)	13 (26)	6 (29)
FAM19A4 only	19 (26)	13 (26)	6 (29)
miR124-2 only	5 (7)	4 (8)	1 (5)

Univariable results: Anal HSIL vs Other

Variable, n (%) or mean (SD)	Anal HSIL, n =13	Other, n = 62	P value
Age in years	48.5 (9)	48.6 (8)	1.0
Race/Ethnicity[e] Black White Hispanic Other	 11 (85) 1 (8) 0 (0) 1 (8)	 52 (88) 3 (5) 3 (5) 1 (2)	0.41
EtOH drinks/week Abstainer 0-7 >7	 1 (8) 8 (61) 4 (31)	 28 (47) 26 (44) 5 (9)	0.01
Current smoker	10 (77)	31 (52)	0.13
HIV positive	8 (62)	42 (70)	0.53

Univariable results, cont: Anal HSIL vs Other

Variable, n (%) or mean (SD)	Anal HSIL, n =13	Other, n = 62	P value
Anal Cytology			0.20
Normal	2 (15)	23 (38)	
ASCUS/LSIL/ASC-H	11 (85)	37 (62)	
Anal HPV			
hrHPV	11 (85)	25 (42)	0.01
16/18	5 (48)	8 (14)	0.05
Cervical Cytology			0.007
Normal	8 (62)	54 (90)	
ASCUS	3 (23)	4 (7)	
LSIL	2 (15)	0 (0)	
ASC-H/HSIL	0 (0)	2 (3)	

Univariable results, cont: Anal HSIL vs Other

Variable, n (%) or mean (SD)	Anal HSIL, n =13	Other, n = 62	P value
Anal Hypermethylation	11 (85)	57 (97)	0.15
Cervical Histology			0.05
None	5 (39)	43 (72)	
Normal	4 (31)	8 (13)	
LSIL/condyloma	4 (31)	5 (8)	
HSIL	0 (0)	1 (2)	
Indicated but missed	0 (0)	3 (5)	
Cervical HPV			
hrHPV	6 (46)	14 (23)	0.17
16/18	2 (15)	1 (2)	0.08
Cervical Hypermethylation	8 (62)	11 (19)	0.004

Multivariable logistic regression model for anal HSIL

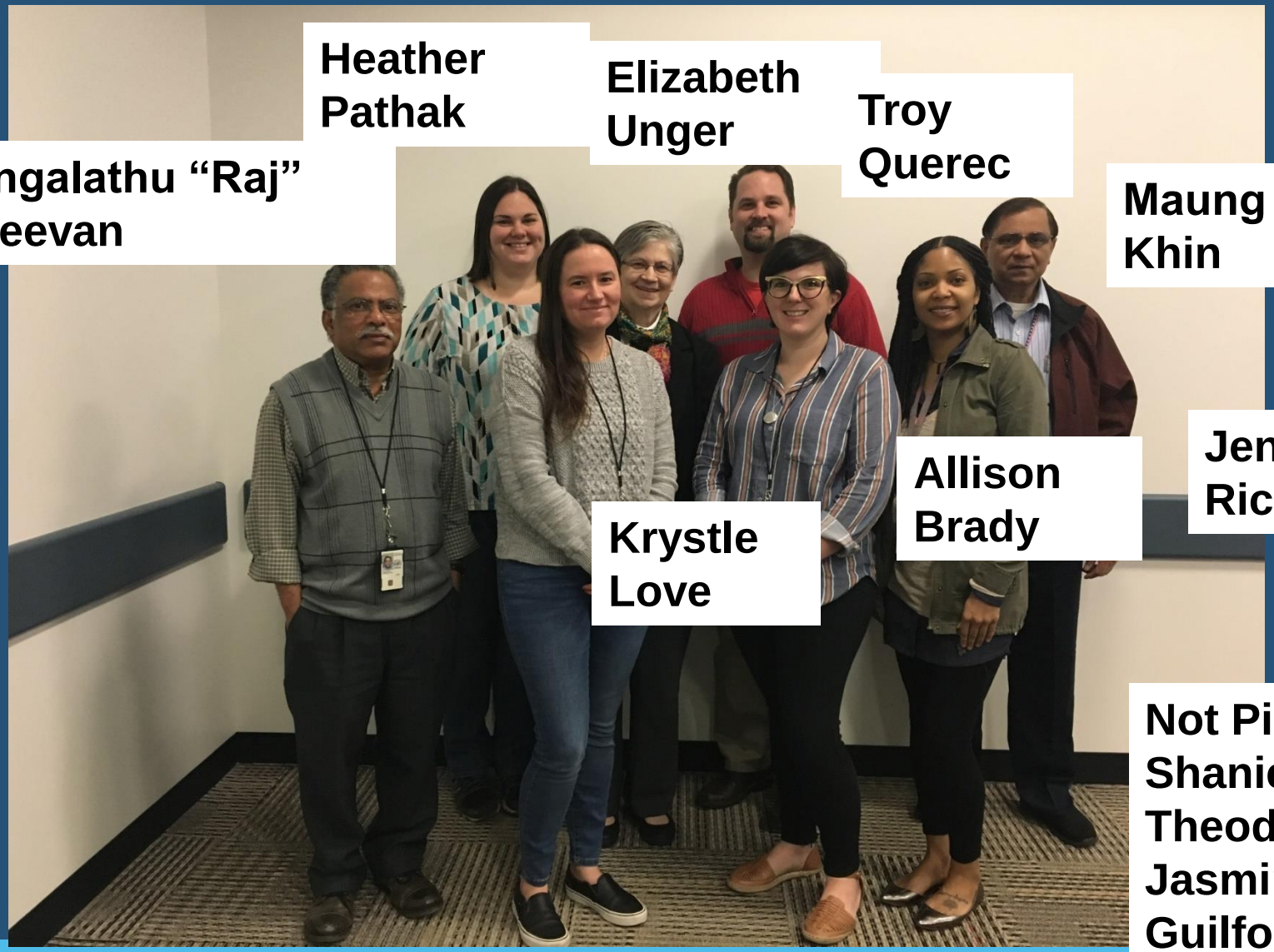
Variable	Estimate	SE	p-value	OR	OR 95%CI
Anal hrHPV (ref: No)	1.80	0.80	0.0242	**6.08**	1.27-29.18
Cervical hypermethylation (ref: No)	1.87	0.69	0.0071	**6.49**	1.66-25.35

Discussion

- Model of best-fit to predict anal HSIL in women included:
  - **Cervical hypermethylation**
  - **Anal hr-HPV**

- **Anal hypermethylation of FAM19A4 and/or mIR 124-was NOT associated with anal HSIL**

CDC Research Team



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Sources of funding:
NIH/NIAID: K23AI124913; KL2TR000455; UL1TR000454 (CDL)
Atlanta WIHS: U-01 AI103408
Emory CFAR: P30 AI050409

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