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Strong tumor expression of ALDH1A1 is associated with Black race, metabolic disorders, and poor breast cancer outcomes

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ABSTRACT

Racial differences in tumor biology may explain worse breast cancer outcomes in Black women relative to White women. This study provides a comparative racial analysis in Black and White women in terms of Aldehyde dehydrogenase1 member A1 (ALDH1A1) expression and its association to clinicopathological features. Expression of ALDH1A1 in both tumor and stromal cells was assessed by immunohistochemistry in tissue microarrays containing 253 breast tumors including 161 tumors from White patients and 92 from Black patients. Relationships to clinicopathological features for strong and moderate to low ALDH1A1 staining were determined using Pearson's Chi Square and an odds ratio was determined. Survival and recurrence were analyzed using Kaplan-Meier curves and Mantel Log-Rank tests. Multivariate analysis was conducted using Cox-proportional hazards tests. Black, obese, and diabetic women showed higher staining intensity in both tumor and stromal tissue. Strong tumor staining was associated with Black race, advanced stage, high grade obesity and diabetes. Strong stromal expression was associated with estrogen receptor positivity, and prediabetes/diabetes. Patients with strong tumor ALDH1A1 had shorter recurrence free and overall survival compared to those with moderate to low expression. When stratified by race, Black women with strong tumor ALDH1A1 expression had shorter recurrence free survival compared to White women. Strong tumor ALDH1A1 staining was an independent predictor of poor overall survival in both Black and White women. These findings indicate that ALDH1A1 expression is associated with poor outcomes in breast cancer, particularly in Black women, and provide the first link between tumor ALDH1A1 expression, obesity, and diabetes.

KEYWORDS: Breast cancer; ALDH1A1, Black women, metabolic disorders, obesity.

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Introduction

Breast cancer accounts for a third of all cancers diagnosed in Black women and is the leading cause of cancer death in this population (Giaquinto et al., 2022). Although incidence rates are comparable, Black women are 41% more likely to die of breast cancer than White women and are twice as likely to be diagnosed with aggressive breast cancers such as triple negative or inflammatory breast cancers (Giaquinto *et al.*, 2022; Stringer-Reasor et al., 2021). Higher mortality rates in Black women are attributable to many factors including later stage of diagnosis, limited access to high quality care, unfavorable tumor characteristics, and increased prevalence of obesity and associated comorbidities (Stringer-Reasor *et al.*, 2021). Studies examining mortality rates in Black and White women diagnosed with early stage breast cancer show that after controlling for non-biological factors such as socioeconomic status, treatment factors and tumor stage at diagnosis, Black women still had higher mortality rates than White women (Dietze et al., 2015; Hershman et al., 2005; Jatoi et al., 2003; Silber et al., 2013). These findings indicate that racial molecular and genetic differences in tumor biology may play a role in poor survival rates among Black women. However, racial differences in tumor biology and their interaction with other biological and non-biological risk factors are poorly understood.

Aldehyde dehydrogenases are detoxifying enzymes that play a critical role in metabolism of intracellular aldehydes. In particular, the ALDH1 family has been implicated in the oxidation of retinol to retinoic acid which impacts differentiation state, treatment resistance, and anti-tumor immune responses(Ayub et al., 2015; Bazewicz et al., 2019; Ginestier et al., 2007). Strong ALDH1 expressing cells are thought to be tumor initiating cells and are associated with other stem-cell markers and poor outcomes in breast cancer (Ginestier *et al.*, 2007). In triple negative breast cancer, ALDH1 expression was significantly correlated with larger tumor size, later stage and is an independent prognostic factor(Ma et al., 2017). Strong ALDH1 expression has been observed in breast cancers of Black women and is associated with basal like features in this population (Nalwoga et al., 2010).

The ALDH1 family is made up of several isoforms, with ALDH1A1 and ALDH1A3 being the most important for breast cancer progression and resistance (Marcato et al., 2011b). Although both are involved in the biosynthesis of retinoic acid, ALDH1A1 has a broader role in the metabolism of intracellular and extracellular aldehydes (Poturnajova et al., 2021). While our group and others have shown that ALDH1A3 expression correlates with prognosis, the role of ALDH1A1 has been controversial and may be dependent on cut-off levels used to score high and low (Althobiti et al., 2020; Opdenaker et al., 2014; Sjostrom et al., 2015). Strong mRNA expression of ALDH1A1 has been associated with good prognosis in triple negative breast cancer (Liu et al., 2015). However, at the protein level, strong expression in tumor cells was associated with stage, triple negativity, and poor response to neoadjuvant chemotherapy(Khoury et al., 2012), as well as worse prognosis (Sjostrom *et al.*, 2015). A recent study examining expression of ALDH1A1 in 222 breast cancers from Ghanian women reported tumor expression in 90% of tumors. Although no association with clinicopathologic features was observed with ALDH1A1 alone, significant associations with tumor stage and grade were observed in tumors with a large stem-like population indicated by strong staining of ALDH1A1 and CD44 and weak CD24 expression (Gyan et al., 2021). Outcome analysis was not performed in this study.

In addition to its possible role in breast cancer progression, expression of ALDH1A1 has been linked to lipogenic adipogenesis, accumulation of visceral fat (Reichert et al., 2011; Yasmeen et al., 2013), and altered glucose tolerance (Petrosino et al., 2014) in ALDH1A1 knock-out mouse models. Additionally, ALDH1A1 expression in adipose tissue in both mice and humans is induced by a high-fat diet (Landrier et al., 2017). Pharmacological inhibition of ALDH1A1 suppressed weight gain in a mouse model of diet-induced obesity and reduced genes involved in fatty acid synthesis in multiple tissues (Haenisch et al., 2018). As obesity is strongly associated with breast cancer risk and outcomes, particularly in Black women (Dietze et al., 2018; Micaily et al., 2021), we sought to investigate the relationship between ALDH1A1 expression, metabolic disorders and breast cancer outcomes in Black women compared to White women.

Methods

Patient samples and data

Paraffin embedded blocks of breast cancer tissue were obtained from the biorepository at the Helen F. Graham Cancer Center and Research Institute (HFGCCRI) under a protocol approved by the institutional review board of ChristianaCare, Newark, Delaware. Blocks were obtained from patients undergoing surgical resection from years 2007-2011 and were pathologically confirmed as invasive breast cancer tissue. Paraffin tissues were constructed into 4x5 tissue microarrays (TMA) with a 5 mm core size and cut as serial sections. Clinicopathologic data was taken from patient charts. Patients pathologic stage of IIA or stronger were considered to have advanced breast cancer, consistent with a recent recommendation from the Breast Cancer Surveillance study that found that this definition most accurately predicts 5 year survival (Kerlikowske et al., 2021). Obesity was determined by BMI at diagnosis. Patients were coded as obese if their BMI was greater than or equal to 30. Data regarding other co-morbidities such as blood glucose levels (normal or high (prediabetes) or a clinical diagnosis of diabetes mellitus at the time breast cancer occurrence was available in the biorepository database for a subset of patients n=130.

Immunohistochemical procedure

Paraffin-embedded slides were deparaffinized and rehydrated, and heat antigen epitope retrieval was performed for 16 hours at 60°C. Slides were stained using a 1:100 dilution of ALDH1A1 antibody (Kiefer et al., 2012) (Abcam, clone EP1933Y) using the Mouse and Rabbit Specific HRP/AEC (ABC) Detection IHC Kit (Abcam, ab93705), following the manufacturer's instructions. Negative control slides were performed with the omission of antibody. Slides were counterstained with hematoxylin. Images were captured using a Zeiss Axio microscope using a 10X objective. Segmentation of tumor and stromal cells and measurement of sum intensity per area was performed using Zen Blue.

Statistical analysis

Statistical analysis was done in graph pad PRISM 8 and IBM27 SPSS. Comparisons of staining intensity were done using student's T tests. Staining intensities were divided into quartiles, with the top quartile considered to be strong ALDH1A1 expression. Relationships for strong and moderate to low expressing slides were determined using Pearson's Chi Square and an odds ratio was determined. Survival and recurrence were analyzed using Kaplan-Meier curves and Mantel Log-Rank tests. Multivariate analysis was conducted using Cox-proportional hazards tests.

Results

Patient Characteristics

This study was comprised of a cohort of 253 patients with invasive ductal carcinoma, including

161 White patients and 92 Black patients. Patient demographics and clinicopathologic characteristics are summarized in Table 1. HER2 receptor status was not known for all patients and was not included in this analysis. Black patients were significantly more likely to have advanced stage (p=0.022) and have a BMI greater than 30 at diagnosis (p=0.013). No significant racial differences were observed in tumor grade and expression of hormone receptors in this cohort.

Table 1. Comparison of patient characteristics between White and Black Patients.

Patient characteristics	All		White		Black		p value
	number	%	number	%	number	%	
Samples	253	100	161	63.6	92	36.4	
Stage							
Early Stage	156	61.7	108	67.1	48	52.2	0.022*
Advanced	97	38.3	53	32.9	44	47.8	
Grade							
low-medium	74	29.2	54	33.5	23	25	0.164
high	179	70.8	110	68.3	69	75	
Hormone receptor expression							
HR+	75	29.6	47	29.2	28	30.4	0.887
HR-	178	70.4	114	70.8	64	69.6	
Obesity							
non-obese	131	51.8	93	57.8	38	41.3	0.013*
obese	122	48.2	68	42.2	54	58.7	
Diabetes							
normal blood glucose	99	76.10%	67	79.8	32	69.5	0.204
pre-diabetes/diabetes	31	23.80%	17	20.1	14	30.4	

Strong tumor ALDH1A1 staining is associated with race, stage, obesity, and diabetes.

Tumor microarrays were constructed from paraffin embedded surgical sections and stained with an anti-ALDH1A1 specific antibody. Tumor and stromal staining were quantified as sum intensity per area. Figure 1 shows representative samples with strong and low tumor and stromal staining. Significantly stronger staining intensities were observed in tumor tissue from Black women, obese women, and women with prediabetes/ diabetes (Figure 2A, 2C and 2E). Similarly, significantly stronger staining intensities of ALDH1A1 were found in stroma of tumors from Black women and women who were obese or had prediabetes or diabetes (Figure 2B, 2D and 2F). Strong tumor staining (specimens that were in the highest quartile) was associated with Black race (OR 1.625, CI 1.202-2.270, p=.004), advanced stage (OR 1.454, CI 1.062-1.991, p=.037), strong grade (OR 1.209, CI 1.036-1.441, p=.039),

obesity (OR 1.441, CI 1.122-1.825, p=0.009) and diabetes (OR 1.599, CI 1.206-2.119, p=.001). Although Black women in our cohort were more likely to be obese and have advance cancer, the association with race remained even when controlling for stage (Chi square 3.414, p=0.036) and obesity (Chi square 3.328, p= 0.038). Strong stromal expression was associated with Black race (OR 1.216, CI 0.843-1.624), estrogen receptor positivity (OR 1.47, CI 0.996-2.190, p=0.004), obesity (OR 1.287, CI 1.008-1.643, p=0.044), and prediabetes/diabetes (OR 1.290, CI 0.970-1.1716, p=.038). No associations between race, stage and grade were observed with strong stromal staining. (Table 2).

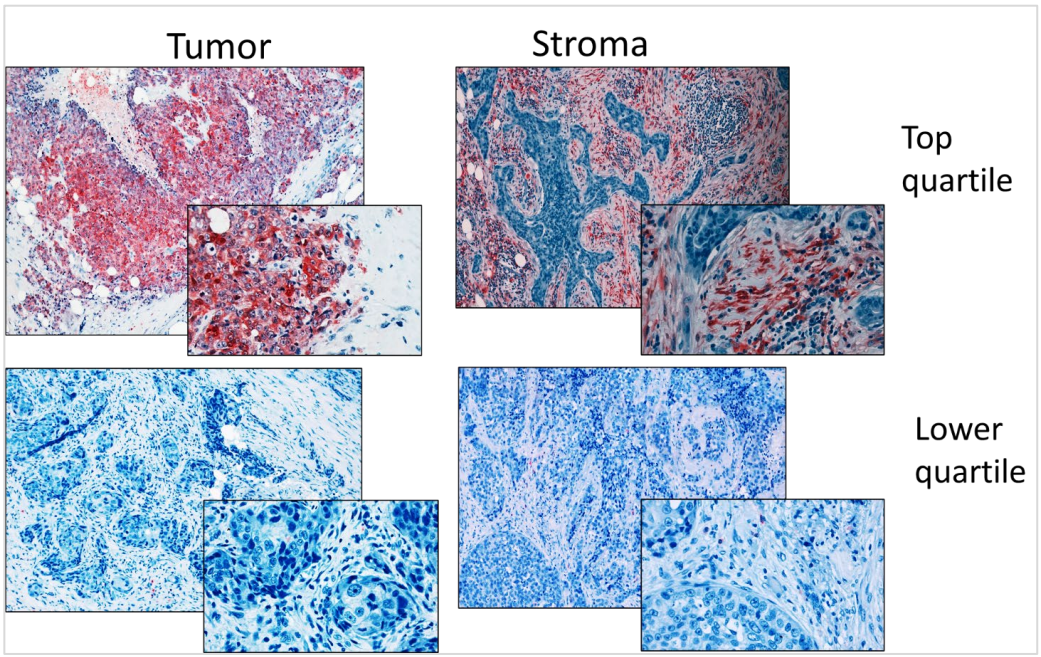
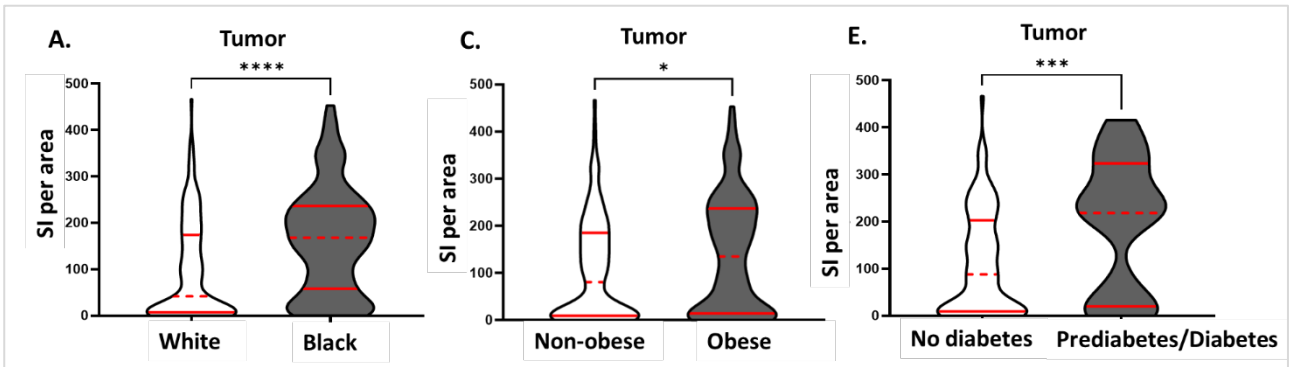


Figure 1. Immunohistochemical analysis of ALDH1A1.

Tumor sections showing strong (top quartile by staining intensity/area) and low/moderate (lower quartiles by staining intensity/area) tumor and stromal expression of ALDH1A1 (red stain). Nuclei were counterstained with hematoxylin (blue). Images in panels were taken with a 10X objective. Images in inserts were taken with 40X objective.



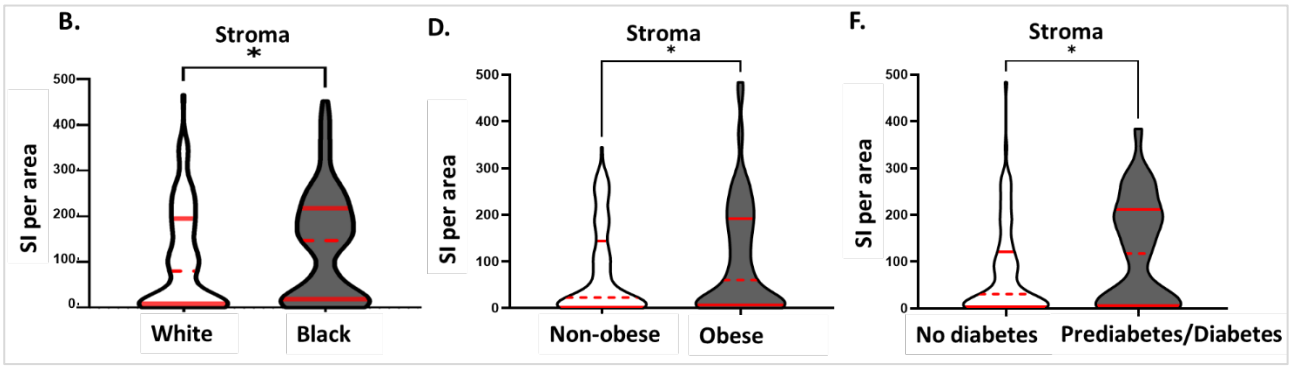


Figure 2. Differential tumor and stromal expression of ALDH1A1.

Expression of ALDH1A1 expression in white vs black tumor **(A.)** and Stroma **(B.)**, non-obese vs obese tumor **(C.)** and stroma **(D.)**, and Non-diabetic vs prediabetic/Diabetic tumors **(E.)** and stroma **(F.)** Data is reported as sum red intensity per area. Solid red lines represent quartiles, dotted red line represents mean. **** p<.0001; *** p<.001; * p<.05.

Table 2. Association of ALDH1A1 strong tumor and stromal expression with patient and tumor characteristics.

Strong expression of ALDH1A1	Tumor expression				Stromal Expression			
		OR	95% CI	p value		OR	95% CI	p value
Race	*Black*	1.625	1.202-2.270	0.004**	*Black*	1.216	0.843-1.624	0.156
	White	0.704	0.537-0.923		*White*	0.973	0.702-1.348	
Stage	*Non-advanced*	0.762	0.584-0.994	0.037*	*Non-advanced*	1.087	0.893-1.321	0.439
	Advanced	1.454	1.062-1.991		*Advanced*	0.875	0.640-1.197	
Grade	*low-medium*	0.572	0.330-03990	0.039*	*low-medium*	1.222	0.829-1.802	0.325
	high	1.209	1.036-1.411		*high*	0.921	0.786-1.079	
Estrogen receptor expression	*ER+*	0.886	0.546-1.372	0.324	*ER+*	1.472	0.996-2.190	.004**
	ER-	1.06	0.889-1.262		*ER-*	0.851	0.725-1.970	
Obesity	*Non-obese*	0.669	0.471-0.940	0.009**	*Non-obese*	0.764	0.591-0.989	.044*
	Obese	1.441	1.122-1.825		*Obese*	1.287	1.008-1.643	
Prediabetes/ Diabetes	*Normal blood glucose*	0.281	0.148-0.534	0.001**	*Normal blood glucose*	0.517	0.283-0.944	.038*
	Pre-diabetes/ diabetes	1.599	1.206-2.119		*Pre-diabetes/ diabetes*	1.29	0.970-1.716	

Strong tumor and stromal ALDH1A1 staining are associated with poor outcomes

To assess the role of strong tumor and stromal ALDH1A1 in breast cancer outcomes, Kaplan-Meyer analysis was conducted. Shorter recurrence free survival (RFS, $p<0.001$) and overall survival (OS, $p<0.001$) were observed in women whose tumors had strong expression of ALDH compared to those with moderate to low expression (Figure 3A & B).

Interestingly, of those in the top quartile, Black women had worse RFS compared to White women ($p=0.011$, Figure 4A.). No racial differences were observed in RFS between women with moderate to low tumor expression of ALDH1A1 (Figure 4B.). Race had no effect on overall survival in either expression groups (Figure 4C&D) as shorter OS was observed in both Black and White women with strong ALDH expression.

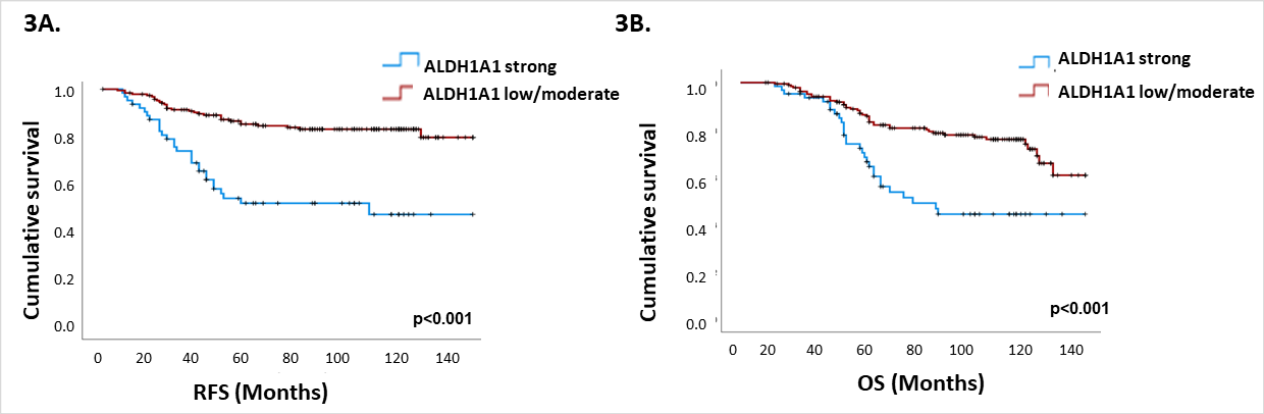
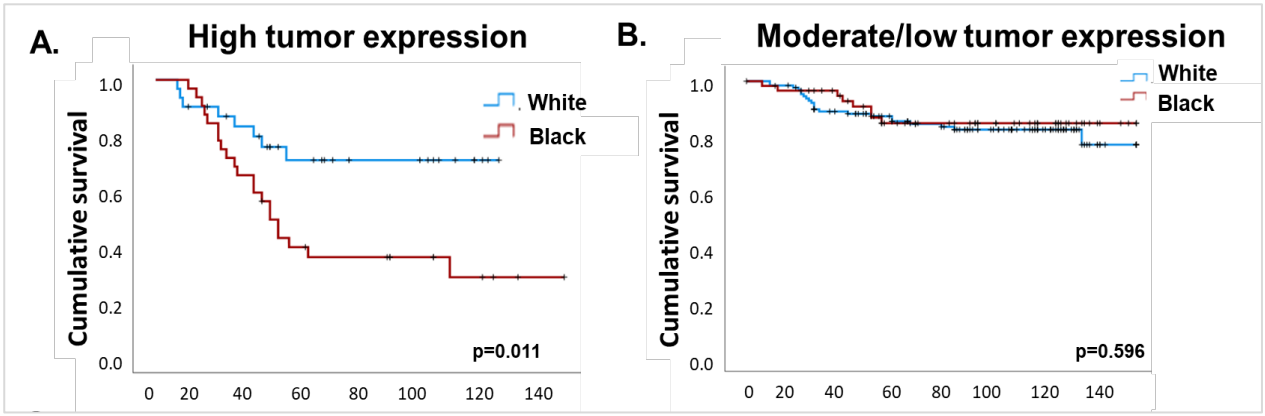


Figure 3. Tumor ALDH1A1 expression is associated with breast cancer outcomes.
Kaplan-Meier survival curves showing shorter recurrence free survival (A.) and overall survival (B.) in patients with high expression of ALDH1A1 (top quartile). Black tick marks represent censured patients. A sample was considered to have strong staining if its staining index was in the top quartile. Lower quartiles were considered to have low to moderate staining.



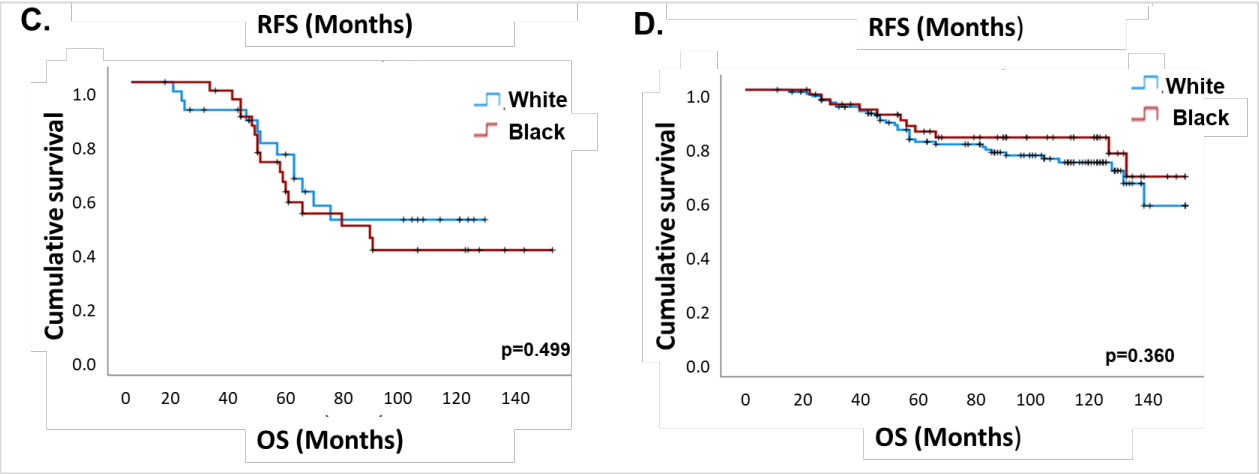


Figure 4. High ALDH1A1 is associated with shorter RFS in Black women compared to White women. (A.). When stratified by race shorter RFS is observed in black women with strong tumor (staining intensity in top quartile) ALDH1A1 expression compared to white women. (B.) No difference in RFS was observed between black and white women with moderate to low tumor expression of ALDH1A1 (staining intensity in lower 3 quartiles) (C.) No significant racial differences were observed in OS in women with high or (D.) low to moderate tumor expression of ALDH1A1. Black tick marks represent censured patients.

Although a trend of shorter RFS and OS was observed in women with strong ALDH1A1 stromal expression compared to those with moderate to low stromal expression, this was not significant (Figure 5A.&B.). No racial differences in outcomes were observed between Black and White women with strong stromal ALDH1A1 expression (Figure 6A.-D.). Multivariate analysis revealed that advanced stage, hormone receptor negativity, and strong tumor ALDH1A1 expression were independently associated with worse survival outcomes. (Table 3).

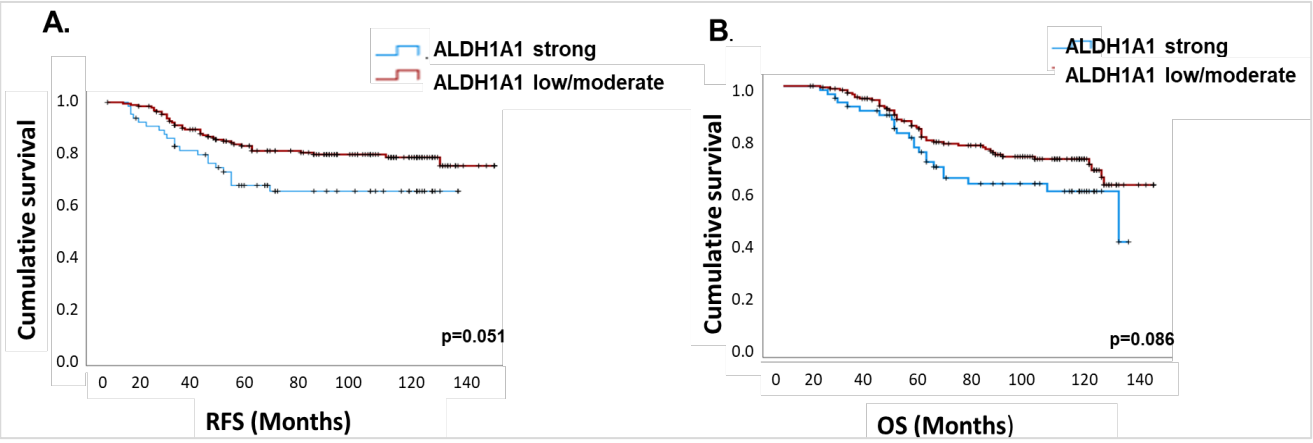


Figure 5. Stromal ALDH1A1 expression is not significantly associated with breast cancer outcomes. Kaplan-Meier survival curves showing shorter recurrence free survival (A.) and overall survival (B.) in patients with high expression of ALDH1A1 (staining intensity in top quartile). Black tick marks represent censured patients.

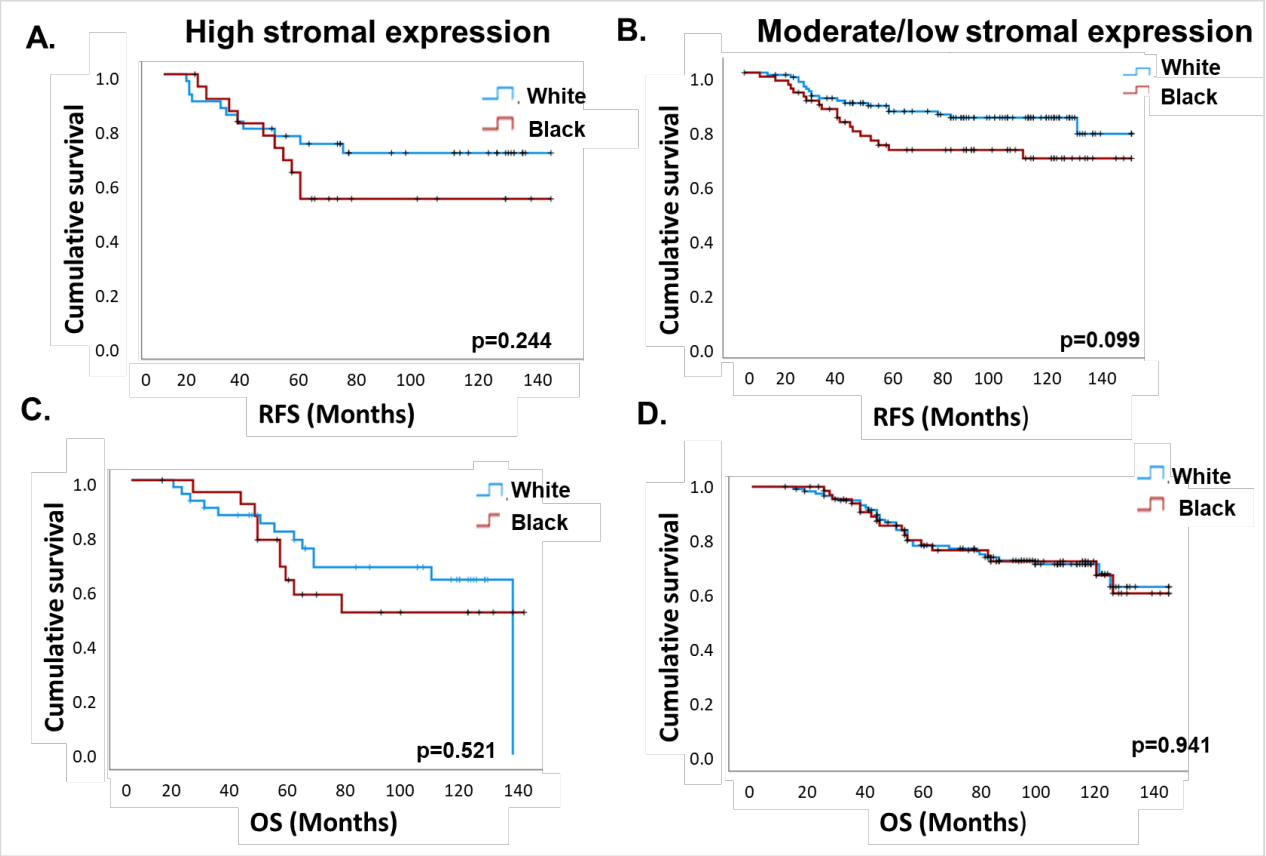


Figure 6. Stromal expression is not associated with breast cancer outcomes when stratified by race.
(A.). When stratified by race shorter RFS in black women with high stromal (staining intensity in top quartile) ALDH1A1 expression compared to white women. (B.) No difference in RFS was observed between black and white women with moderate to low stromal expression of ALDH1A1 (staining intensity in lower 3 quartiles) (C.) No significant racial differences were observed in OS in women with high or (D.) low to moderate stromal expression of ALDH1A1. Black tick marks represent censured patients.

Discussion

Considerable data indicates that tumor biology may be a contributing factor to racial disparities in breast cancer. Previous studies have reported increased expression of ALDH1A1 in tumors of women with African Ancestry (Jiagge et al., 2018b). Our findings confirm that expression of ALDH1A1 is increased in breast tumor tissue from Black women compared to those from White women. Strong tumor expression of ALD1A1 was associated with Black race, high grade, advanced cancer stage, obesity, and diabetes. In contrast to previous studies, we did not find an association of between strong tumor ALDH1A1 expression and estrogen receptor negativity.

Although ALDH1A1 activity has been linked to metastatic behavior and treatment resistance (Croker et al., 2017), the association between tumor ALDH1A1 expression and survival is controversial. We observed poor RFS and OS in women with strong tumor expression of ALDH1A1. A multivariate analysis revealed that tumor, but not stromal, ALDH1A1 expression is an independently associated with cancer outcomes. Analyses stratified by race revealed worse overall survival in both Black and White women with strong tumor ALDH1A1

compared to those with moderate to low expression. However, among women with strong ALDH1A1 staining, Black women had shorter recurrence free survival compared to White women.

Members of the ALDH1 family, including ALDH1A1. have been associated with stemness in normal and malignant breast cells (Douville et al., 2009; Ginestier *et al.*, 2007; Ginestier et al., 2009; Honeth et al., 2014; Marcato et al., 2011a; Schwartz et al., 2013). However, research on stemness in tumors of Black women is limited (Jiagge et al., 2018a). One study found that the overall number of cells expressing the stem cell markers CD44+/CD24- cells was elevated in tumors from Black women (Nakshatri et al., 2015). This study also found that Black but not White tumors contained a distinct population of CD44+/CD24- cells that showed differential gene expression which was like that of mammary stem cells and included upregulation of TGFβ/Wnt and CTNBB1/NF-κB pathways and downregulation of the p53 and ER pathway. Additionally, Gyan et al found that expression of the CD44+CD24- population together with ALDH1A is associated with aggressive tumor characteristics including high tumor grade and clinical prognostic staging in Black women (Gyan *et al.*, 2021). Another study found that tumor cells from Black women exhibited a cancer stem like phenotype including increased growth and migration compared to tumor cells from White women (Siddharth et al., 2021).In this study, tumor cells from Black women also showed different gene expression patterns than those from White tumors, including elevated expression of stemness genes Gli-1 and Notch1.

Stromal expression of ALDH1A1 has been investigated in only a few studies (Bednarz-Knoll et al., 2015; Sjostrom *et al.*, 2015) which report that expression of ALDH1A in tumor stromal may be associated with improved outcomes in breast cancer. We found no association between strong stromal staining and outcomes in our study. However, we found that strong ALDH1A1 stromal expression is associated with estrogen receptor positivity, obesity, and diabetes. Although no associations between stromal staining and breast cancer subtypes have been reported, a positive association between strong stromal ALDH1A1 expression and decreased basal markers in tumor cells has previously been observed (Bednarz-Knoll *et al.*, 2015). Further investigation of the prognostic significance of ALDH1A1 stromal expression by breast cancer subtype is needed.

Metabolic disorders are associated with breast cancer risk and outcomes, particularly in Black women(Jones et al., 2003; Zhao et al., 2020). Previous studies have shown that in addition to its role in stemness and oxidative stress, ALDH1A1 plays a key role in glucose tolerance and abdominal fat formation (Ma et al., 2020; Petrosino *et al.*, 2014). Further, increased expression of ALDH1A1 has been linked to accumulation of visceral fat in murine models and mediates inflammatory responses to a high-fat diet in females (Yasmeen *et al.*, 2013). Our findings are the first to show an association between metabolic disorders and ALDH1A1 expression in tumors.

The mechanisms driving increased expression of ALDH1A1 in tumor and stroma cells, particularly in Black women, have yet to be fully understood. Functional polymorphisms in ALDH1A1 have been identified and studied in the context of alcohol dependence (Agarwal et al., 1981).The frequency of these alterations varies across different ethnic groups and several variants have strong prevalence among Black individuals (Crawford et al., 2014; Ji et al., 2019; Liu et al., 2011; Spence et al., 2003). A three base-pair insertion with prevalence exclusively to Black people was shown to increase expression of ALDH1A1 (Spence *et al.*, 2003). To date, the role of

ALDH1A1 polymorphisms in breast cancer has not been well studied. Additionally, ALDH1A1 is regulated by factors that are also associated with breast cancer risk including decreased estrogen (Petrosino, 2014), alcohol-associated inflammation (Wang et al., 2017) and high fat diets (He et al., 2020; Srinivasan et al., 2019). Moreover, we and other groups have shown that tumor expression of ALDH1A1 can be induced by activation of the IL-6-STAT3 pathway (Arnold et al., 2020; Hellsten et al., 2011) and may be associated with chronic inflammation.

Our study has several limitations including a small sample size. Although our findings show that high ALDH1A1 expression is correlated with race and metabolic disorders including obesity and diabetes, these findings need to be confirmed in larger studies. We were able to obtain information on diabetes and blood glucose levels on a subset of patients; however, this information was missing in our biorepository database for many patients and warrants further study. Due to the smaller sample size, we were not able to further stratify patients to compare differences among race in prediabetes and diabetes groups.

Although our analysis included stratification by receptor subtype, we were not able to classify patients according to molecular subtyping. Racial differences have been reported in PAM50 molecular subtypes, with Black women having increased luminal B subtypes (Troester et al., 2018). As ALDH1A1 expression is associated to both luminal B and TNBC subtypes (Althobiti *et al.*, 2020) this could be a confounding factor. Although our study investigated the association of stromal expression to race, comorbidities and outcomes, our study focused on staining index of the total stroma and did not determine differences by individual cell type. We did observe strong stromal staining in multiple cell types, which warrants further investigation.

In conclusion, this study shows that ALDHA1A1 expression in breast tumor and stromal cells is associated with Black race, obesity, and diabetes. When stratified by race, Black women whose tumors express high levels of ALDH1A1 have significantly shorter recurrence times than White women with strong ALDH1a1 expression. Moreover, ALDH1A1 expression was found to be an independent driver of outcomes. These findings indicate that ALDH1A1 is associated to disparities in breast cancer outcomes and provide the first link between tumor ALDH1A1 expression, obesity, and diabetes. Further study is needed to understand the mechanisms driving high ALDH1A1 expression in these cohorts.

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Conflicts of interest

The authors report no conficlts of interest

Authors' contributions

Kindly add. S.M.: experimental procedures and contributed to the writing of the draft, L.F.: performed experimetnal procedures and manuscript reveiw., S.D.S.: conceptualiztion, manuscript review and editing, Z.S. conceptualization, manuscript review and editing, J.S.M.: conceptualization, statistical analysis, writing of the draft and review and editing of manuscript.

References

Agarwal, D.P., Harada, S., and Goedde, H.W. (1981). Racial differences in biological sensitivity to ethanol: the role of alcohol dehydrogenase and aldehyde dehydrogenase isozymes. Alcohol Clin Exp Res *5*, 12-16. https://www.ncbi.nlm.nih.gov/pubmed/7013538.

Althobiti, M., El Ansari, R., Aleskandarany, M., Joseph, C., Toss, M.S., Green, A.R., and Rakha, E.A. (2020). The prognostic significance of ALDH1A1 expression in early invasive breast cancer. Histopathology *77*, 437-448. https://www.ncbi.nlm.nih.gov/pubmed/32369651.

Arnold, K.M., Opdenaker, L.M., Flynn, N.J., Appeah, D.K., and Sims-Mourtada, J. (2020). Radiation induces an inflammatory response that results in STAT3-dependent changes in cellular plasticity and radioresistance of breast cancer stem-like cells. Int J Radiat Biol *96*, 434-447. https://www.ncbi.nlm.nih.gov/pubmed/31850822.

Ayub, T.H., Keyver-Paik, M.D., Debald, M., Rostamzadeh, B., Thiesler, T., Schroder, L., Barchet, W., Abramian, A., Kaiser, C., Kristiansen, G., et al. (2015). Accumulation of ALDH1-positive cells after neoadjuvant chemotherapy predicts treatment resistance and prognosticates poor outcome in ovarian cancer. Oncotarget *6*, 16437-16448. https://www.ncbi.nlm.nih.gov/pubmed/25999351.

Bazewicz, C.G., Dinavahi, S.S., Schell, T.D., and Robertson, G.P. (2019). Aldehyde dehydrogenase in regulatory T-cell development, immunity and cancer. Immunology *156*, 47-55. https://www.ncbi.nlm.nih.gov/pubmed/30387499.

Bednarz-Knoll, N., Nastaly, P., Zaczek, A., Stoupiec, M.G., Riethdorf, S., Wikman, H., Muller, V., Skokowski, J., Szade, J., Sejda, A., et al. (2015). Stromal expression of ALDH1 in human breast carcinomas indicates reduced tumor progression. Oncotarget *6*, 26789-26803. https://www.ncbi.nlm.nih.gov/pubmed/26305673.

Crawford, A., Dalvie, S., Lewis, S., King, A., Liberzon, I., Fein, G., Koenen, K., Ramesar, R., and Stein, D.J. (2014). Haplotype-based study of the association of alcohol and acetaldehyde-metabolising genes with alcohol dependence (with or without comorbid anxiety symptoms) in a Cape Mixed Ancestry population. Metab Brain Dis *29*, 333-340. https://www.ncbi.nlm.nih.gov/pubmed/24567230.

Croker, A.K., Rodriguez-Torres, M., Xia, Y., Pardhan, S., Leong, H.S., Lewis, J.D., and Allan, A.L. (2017). Differential Functional Roles of ALDH1A1 and ALDH1A3 in Mediating Metastatic Behavior and Therapy Resistance of Human Breast Cancer Cells. Int J Mol Sci *18*. https://www.ncbi.nlm.nih.gov/pubmed/28937653.

Dietze, E.C., Chavez, T.A., and Seewaldt, V.L. (2018). Obesity and Triple-Negative Breast Cancer: Disparities, Controversies, and Biology. Am J Pathol *188*, 280-290. https://www.ncbi.nlm.nih.gov/pubmed/29128565.

Dietze, E.C., Sistrunk, C., Miranda-Carboni, G., O'Regan, R., and Seewaldt, V.L. (2015). Triple-negative breast cancer in African-American women: disparities versus biology. Nat Rev Cancer *15*, 248-254. https://www.ncbi.nlm.nih.gov/pubmed/25673085.

Douville, J., Beaulieu, R., and Balicki, D. (2009). ALDH1 as a functional marker of cancer stem and progenitor cells. Stem Cells Dev *18*, 17-25. https://www.ncbi.nlm.nih.gov/pubmed/18573038.

Giaquinto, A.N., Miller, K.D., Tossas, K.Y., Winn, R.A., Jemal, A., and Siegel, R.L. (2022). Cancer statistics for African American/Black People 2022. CA Cancer J Clin *72*, 202-229. https://www.ncbi.nlm.nih.gov/pubmed/35143040.

Ginestier, C., Hur, M.H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., Jacquemier, J., Viens, P., Kleer, C.G., Liu, S., et al. (2007). ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell *1*, 555-567. https://www.ncbi.nlm.nih.gov/pubmed/18371393.

Ginestier, C., Wicinski, J., Cervera, N., Monville, F., Finetti, P., Bertucci, F., Wicha, M.S., Birnbaum, D., and Charafe-Jauffret, E. (2009). Retinoid signaling regulates breast cancer stem cell differentiation. Cell Cycle *8*, 3297-3302. https://www.ncbi.nlm.nih.gov/pubmed/19806016.

Gyan, E., Green, A., Ahenkorah-Fondjo, L., Jackson, A., Toss, M.S., Akakpo, P.K., Derkyi-Kwarteng, L., Rahman, G.A., and Owiredu, W. (2021). The role of ALDH1A1 in contributing to breast tumour aggressiveness: A study conducted in an African population. Ann Diagn Pathol *51*, 151696. https://www.ncbi.nlm.nih.gov/pubmed/33460998.

Haenisch, M., Treuting, P.M., Brabb, T., Goldstein, A.S., Berkseth, K., Amory, J.K., and Paik, J. (2018). Pharmacological inhibition of ALDH1A enzymes suppresses weight gain in a mouse model of diet-induced obesity. Obes Res Clin Pract *12*, 93-101. https://www.ncbi.nlm.nih.gov/pubmed/28919001.

He, Y., Yang, T., Du, Y., Qin, L., Ma, F., Wu, Z., Ling, H., Yang, L., Wang, Z., Zhou, Q., et al. (2020). High fat diet significantly changed the global gene expression profile involved in hepatic drug metabolism and pharmacokinetic system in mice. Nutr Metab (Lond) *17*, 37. https://www.ncbi.nlm.nih.gov/pubmed/32489392.

Hellsten, R., Johansson, M., Dahlman, A., Sterner, O., and Bjartell, A. (2011). Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. PLoS One *6*, e22118. https://www.ncbi.nlm.nih.gov/pubmed/21779382.

Hershman, D., McBride, R., Jacobson, J.S., Lamerato, L., Roberts, K., Grann, V.R., and Neugut, A.I. (2005). Racial disparities in treatment and survival among women with

early-stage breast cancer. J Clin Oncol *23*, 6639-6646. https://www.ncbi.nlm.nih.gov/pubmed/16170171.

Honeth, G., Lombardi, S., Ginestier, C., Hur, M., Marlow, R., Buchupalli, B., Shinomiya, I., Gazinska, P., Bombelli, S., Ramalingam, V., et al. (2014). Aldehyde dehydrogenase and estrogen receptor define a hierarchy of cellular differentiation in the normal human mammary epithelium. Breast Cancer Res *16*, R52. https://www.ncbi.nlm.nih.gov/pubmed/24887554.

Jatoi, I., Becher, H., and Leake, C.R. (2003). Widening disparity in survival between white and African-American patients with breast carcinoma treated in the U. S. Department of Defense Healthcare system. Cancer *98*, 894-899. https://www.ncbi.nlm.nih.gov/pubmed/12942554.

Ji, Z., He, L., Regev, A., and Struhl, K. (2019). Inflammatory regulatory network mediated by the joint action of NF-kB, STAT3, and AP-1 factors is involved in many human cancers. Proc Natl Acad Sci U S A *116*, 9453-9462. https://www.ncbi.nlm.nih.gov/pubmed/30910960.

Jiagge, E., Chitale, D., and Newman, L.A. (2018a). Triple-Negative Breast Cancer, Stem Cells, and African Ancestry. Am J Pathol *188*, 271-279. https://www.ncbi.nlm.nih.gov/pubmed/29137951.

Jiagge, E., Jibril, A.S., Davis, M., Murga-Zamalloa, C., Kleer, C.G., Gyan, K., Divine, G., Hoenerhoff, M., Bensenhave, J., Awuah, B., et al. (2018b). Androgen Receptor and ALDH1 Expression Among Internationally Diverse Patient Populations. J Glob Oncol *4*, 1-8. https://www.ncbi.nlm.nih.gov/pubmed/30307804.

Jones, L.A., Brawley, O., Johnson-Thompson, M., Lythcott, N., and Newman, L. (2003). Overview of the summit meeting evaluating research in African-American women. Cancer *97*, 207-210. https://www.ncbi.nlm.nih.gov/pubmed/12491483.

Kerlikowske, K., Bissell, M.C.S., Sprague, B.L., Buist, D.S.M., Henderson, L.M., Lee, J.M., and Miglioretti, D.L. (2021). Advanced Breast Cancer Definitions by Staging System Examined in the Breast Cancer Surveillance Consortium. J Natl Cancer Inst *113*, 909-916. https://www.ncbi.nlm.nih.gov/pubmed/33169794.

Khoury, T., Ademuyiwa, F.O., Chandrasekhar, R., Jabbour, M., Deleo, A., Ferrone, S., Wang, Y., and Wang, X. (2012). Aldehyde dehydrogenase 1A1 expression in breast cancer is associated with stage, triple negativity, and outcome to neoadjuvant chemotherapy. Mod Pathol *25*, 388-397. https://www.ncbi.nlm.nih.gov/pubmed/22080062.

Kiefer, F.W., Vernochet, C., O'Brien, P., Spoerl, S., Brown, J.D., Nallamshetty, S., Zeyda, M., Stulnig, T.M., Cohen, D.E., Kahn, C.R., and Plutzky, J. (2012). Retinaldehyde dehydrogenase 1 regulates a thermogenic program in white adipose tissue. Nat Med *18*, 918-925. https://www.ncbi.nlm.nih.gov/pubmed/22561685.

Landrier, J.F., Kasiri, E., Karkeni, E., Mihaly, J., Beke, G., Weiss, K., Lucas, R., Aydemir, G., Salles, J., Walrand, S., et al. (2017). Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue. FASEB J *31*, 203-211. https://www.ncbi.nlm.nih.gov/pubmed/27729412.

Liu, J., Zhou, Z., Hodgkinson, C.A., Yuan, Q., Shen, P.H., Mulligan, C.J., Wang, A., Gray, R.R., Roy, A., Virkkunen, M., et al. (2011). Haplotype-based study of the association of alcohol-metabolizing genes with alcohol dependence in four independent populations. Alcohol Clin Exp Res *35*, 304-316. https://www.ncbi.nlm.nih.gov/pubmed/21083667.

Liu, Y., Baglia, M., Zheng, Y., Blot, W., Bao, P.P., Cai, H., Nechuta, S., Zheng, W., Cai, Q., and Shu, X.O. (2015). ALDH1A1 mRNA expression in association with prognosis of triple-negative breast cancer. Oncotarget *6*, 41360-41369. https://www.ncbi.nlm.nih.gov/pubmed/26462023.

Ma, F., Li, H., Li, Y., Ding, X., Wang, H., Fan, Y., Lin, C., Qian, H., and Xu, B. (2017). Aldehyde dehydrogenase 1 (ALDH1) expression is an independent prognostic factor in triple negative breast cancer (TNBC). Medicine (Baltimore) *96*, e6561. https://www.ncbi.nlm.nih.gov/pubmed/28383433.

Ma, Z., Jiang, L., Li, G., Liang, D., Li, L., Liu, L., and Jiang, C. (2020). Design, synthesis of 1,3-dimethylpyrimidine-2,4-diones as potent and selective aldehyde dehydrogenase 1A1 (ALDH1A1) inhibitors with glucose consumption improving activity. Bioorg Chem *101*, 103971. https://www.ncbi.nlm.nih.gov/pubmed/32480173.

Marcato, P., Dean, C.A., Giacomantonio, C.A., and Lee, P.W. (2011a). Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform. Cell Cycle *10*, 1378-1384. https://www.ncbi.nlm.nih.gov/pubmed/21552008.

Marcato, P., Dean, C.A., Pan, D., Araslanova, R., Gillis, M., Joshi, M., Helyer, L., Pan, L., Leidal, A., Gujar, S., et al. (2011b). Aldehyde dehydrogenase activity of breast cancer stem cells is primarily due to isoform ALDH1A3 and its expression is predictive of metastasis. Stem Cells *29*, 32-45. https://www.ncbi.nlm.nih.gov/pubmed/21280157.

Micaily, I., Hackbart, H., Butryn, M., and Abu-Khalaf, M.M. (2021). Obesity in early onset breast cancer in African American patients. Breast J *27*, 603-607. https://www.ncbi.nlm.nih.gov/pubmed/34117672.

Nakshatri, H., Anjanappa, M., and Bhat-Nakshatri, P. (2015). Ethnicity-Dependent and -Independent Heterogeneity in Healthy Normal Breast Hierarchy Impacts Tumor Characterization. Sci Rep *5*, 13526. https://www.ncbi.nlm.nih.gov/pubmed/26311223.

Nalwoga, H., Arnes, J.B., Wabinga, H., and Akslen, L.A. (2010). Expression of aldehyde dehydrogenase 1 (ALDH1) is associated with basal-like markers and features of aggressive tumours in African breast cancer. Br J Cancer *102*, 369-375. https://www.ncbi.nlm.nih.gov/pubmed/20010944.

Opdenaker, L.M., Arnold, K.M., Pohlig, R.T., Padmanabhan, J.S., Flynn, D.C., and Sims-Mourtada, J. (2014). Immunohistochemical analysis of aldehyde dehydrogenase isoforms and their association with estrogen-receptor status and disease progression in breast cancer. Breast Cancer (Dove Med Press) *6*, 205-209. https://www.ncbi.nlm.nih.gov/pubmed/25540596.

Petrosino, J.M., Disilvestro, D., and Ziouzenkova, O. (2014). Aldehyde dehydrogenase 1A1: friend or foe to female metabolism? Nutrients *6*, 950-973. https://www.ncbi.nlm.nih.gov/pubmed/24594504.

Poturnajova, M., Kozovska, Z., and Matuskova, M. (2021). Aldehyde dehydrogenase 1A1 and 1A3 isoforms - mechanism of activation and regulation in cancer. Cell Signal *87*, 110120. https://www.ncbi.nlm.nih.gov/pubmed/34428540.

Reichert, B., Yasmeen, R., Jeyakumar, S.M., Yang, F., Thomou, T., Alder, H., Duester, G., Maiseyeu, A., Mihai, G., Harrison, E.H., et al. (2011). Concerted action of aldehyde dehydrogenases influences depot-specific fat formation. Mol Endocrinol *25*, 799-809. https://www.ncbi.nlm.nih.gov/pubmed/21436255.

Schwartz, T., Stark, A., Pang, J., Awuah, B., Kleer, C.G., Quayson, S., Kingman, S., Aitpillah, F., Abantanga, F., Jiagge, E., et al. (2013). Expression of aldehyde dehydrogenase 1 as a marker of mammary stem cells in benign and malignant breast lesions of Ghanaian women. Cancer *119*, 488-494. https://www.ncbi.nlm.nih.gov/pubmed/22930220.

Siddharth, S., Parida, S., Muniraj, N., Hercules, S., Lim, D., Nagalingam, A., Wang, C., Gyorffy, B., Daniel, J.M., and Sharma, D. (2021). Concomitant activation of GLI1 and Notch1 contributes to racial disparity of human triple negative breast cancer progression. Elife *10*. https://www.ncbi.nlm.nih.gov/pubmed/34889737.

Silber, J.H., Rosenbaum, P.R., Clark, A.S., Giantonio, B.J., Ross, R.N., Teng, Y., Wang, M., Niknam, B.A., Ludwig, J.M., Wang, W., et al. (2013). Characteristics associated with differences in survival among black and white women with breast cancer. JAMA *310*, 389-397. https://www.ncbi.nlm.nih.gov/pubmed/23917289.

Sjostrom, M., Hartman, L., Honeth, G., Grabau, D., Malmstrom, P., Hegardt, C., Ferno, M., and Nimeus, E. (2015). Stem cell biomarker ALDH1A1 in breast cancer shows an association with prognosis and clinicopathological variables that is highly cut-off dependent. J Clin Pathol *68*, 1012-1019. https://www.ncbi.nlm.nih.gov/pubmed/26175266.

Spence, J.P., Liang, T., Eriksson, C.J., Taylor, R.E., Wall, T.L., Ehlers, C.L., and Carr, L.G. (2003). Evaluation of aldehyde dehydrogenase 1 promoter polymorphisms identified in human populations. Alcohol Clin Exp Res *27*, 1389-1394. https://www.ncbi.nlm.nih.gov/pubmed/14506398.

Srinivasan, S., Dubey, K.K., and Singhal, R.S. (2019). Influence of food commodities on hangover based on alcohol dehydrogenase and aldehyde dehydrogenase activities. Curr Res Food Sci *1*, 8-16. https://www.ncbi.nlm.nih.gov/pubmed/32914100.

Stringer-Reasor, E.M., Elkhanany, A., Khoury, K., Simon, M.A., and Newman, L.A. (2021). Disparities in Breast Cancer Associated With African American Identity. Am Soc Clin Oncol Educ Book *41*, e29-e46. https://www.ncbi.nlm.nih.gov/pubmed/34161138.

Troester, M.A., Sun, X., Allott, E.H., Geradts, J., Cohen, S.M., Tse, C.K., Kirk, E.L., Thorne, L.B., Mathews, M., Li, Y., et al. (2018). Racial Differences in PAM50 Subtypes in the Carolina Breast Cancer Study. J Natl Cancer Inst *110*. https://www.ncbi.nlm.nih.gov/pubmed/28859290.

Wang, B., Wang, Z., de Avila, J.M., Zhu, M.J., Zhang, F., Gomez, N.A., Zhao, L., Tian, Q., Zhao, J., Maricelli, J., et al. (2017). Moderate alcohol intake induces thermogenic brown/beige adipocyte formation via elevating retinoic acid signaling. FASEB J *31*, 4612-4622. https://www.ncbi.nlm.nih.gov/pubmed/28679528.

Yasmeen, R., Reichert, B., Deiuliis, J., Yang, F., Lynch, A., Meyers, J., Sharlach, M., Shin, S., Volz, K.S., Green, K.B., et al. (2013). Autocrine function of aldehyde dehydrogenase 1 as a determinant of diet- and sex-specific differences in visceral adiposity. Diabetes *62*, 124-136. https://www.ncbi.nlm.nih.gov/pubmed/22933113.

Zhao, P., Xia, N., Zhang, H., and Deng, T. (2020). The Metabolic Syndrome Is a Risk Factor for Breast Cancer: A Systematic Review and Meta-Analysis. Obes Facts *13*, 384-396. https://www.ncbi.nlm.nih.gov/pubmed/32698183.