



Metabolomic approaches to polyamines including acetylated derivatives in lung tissue of mice with asthma

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Abstract

Introduction Recently, the relationship between polyamine (PA) metabolism and asthma has been studied in severe asthmatic therapy, but systematic PA metabolism including their acetylated derivatives was not fully understood.

Objectives Profiling analysis of polyamines (PAs) was performed to understand the biochemical events and monitor altered PA metabolism in lung tissue of mice with asthma.

Methods Polyamine profiling of lung tissue of mice with asthma was performed without derivatization by liquid chromatography–tandem mass spectrometry (LC–MS/MS) combined with star pattern recognition analysis. The PA levels between control and asthma groups were evaluated by multivariate analysis.

Results In mouse lung tissue, seven PAs were determined by LC–MS/MS in multiple reaction monitoring (MRM) mode. Their levels were normalized to the corresponding mean levels of the control group for star pattern analysis, which showed distorted heptagonal shapes with characteristic and readily distinguishable patterns for each group. Levels of putrescine ($p < 0.0034$), N^1 -acetylputrescine ($p < 0.0652$), and N^8 -acetylspermidine ($p < 0.0827$) were significantly increased in asthmatic lung tissue. The separation of the two groups was evaluated using multivariate analysis. In unsupervised learning, acetylated PAs including N^1 -acetylspermine were the main metabolites for discrimination. In supervised learning, putrescine and N^1 -acetylputrescine were evaluated as important metabolites.

Conclusions The present results provide basic data for understanding polyamine metabolism in asthma and may help to improve the therapy for severe asthma patients.

Keywords Metabolomics · Polyamine profiling analysis · Acetylated polyamines · Asthma · Lung tissue · Star pattern recognition analysis · Liquid chromatography–tandem mass spectrometry

1 Introduction

Endogenous di- and polyamines (PAs) are positively charged at physiological pH, which allows them to interact with negatively charged molecules such as DNA, RNA, and protein in

various biological processes (Wang and Casero 2006; Miller-Fleming et al. 2015; Tabor and Tabor 1984). PAs are related to playing important roles in cell survival and cell death pathway. Altered PA levels have been observed in proliferation, differentiation, and apoptosis. Increased PA levels were observed in various cancers, while their levels were reduced in aging processes related to cells (Minois et al. 2011; Pegg 2016; Seiler and Raul 2005; Nowotarski et al. 2013). In PAs metabolism, putrescine as precursor of spermidine and spermine is initially formed by the decarboxylation of ornithine and/or arginine in the PA metabolism pathway (Ohe et al. 2010), their acetylated derivatives are converted by putrescine acetyltransferase (PAT), spermidine/spermine acetyltransferase (SSAT), and acetylated polyamine oxidase (APAO) (Yoon and Lee 2014; Pegg 2008). Overproduced PAs are converted to acetyl forms that have reduced positivity and these are released to the

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outside of the cell or transferred to other needed sites (Seiler 2004; Wallace et al. 1988).

Asthma is a complex disease caused by both genetic and environmental factors; it is defined by chronic airway inflammation and hyperresponsiveness (Wenzel 2012). Corticosteroid treatment is currently used in asthma therapy and altered therapeutic method needs to be discovered because asthmatic severe patient are poor responders to corticosteroid treatment (Wood et al. 2012). Elevated levels of PAs were reported in the circulation of asthmatics with active symptoms (Kurosawa et al. 1992) and in lung tissue of ovalbumin (OVA)-induced asthmatic mice (North et al. 2013). In various studies, PAs have been shown to promote the pathogenic potential of inflammatory cells, such as mast cells and granulocytes (eosinophils and neutrophils) and differentiation and alternative activation of macrophages, which play an important role in asthma. Evident of these studies above were found to be associated with PA metabolism. Additionally, PAs were related to airway hyper-responsiveness and may induce airway smooth muscle contraction. These findings suggest that PAs could play an important role in respiratory diseases, and studies on the possibility of asthma treatment involving enzymatic regulation associated with polyamine metabolism are underway (Jain 2018). Indeed, systematic PA metabolism research is needed to improve asthma therapy. However, no systematic studies have been conducted on PA metabolism disorders, including acetylated PAs, in asthma.

Star pattern recognition analysis is a useful tool for visual discrimination of normal and disease states from a complex profile of metabolites. In our previous research, star pattern recognition analysis was effective for comparing normal and metabolic disease states (Kim et al. 2010, 2018; Paik et al. 2009; Shin et al. 2018). In the combination of metabolomics and multivariate statistical analysis, principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) are useful tools for determining the relative differences between different systems (Worley and Powers 2013). Therefore, in lung tissue of OVA-induced asthmatic mice, star pattern recognition analysis, PCA, PLS-DA, and OPLS-DA were performed for discrimination between control and asthma groups. In this study, PA profiling by LC-MS/MS was combined with pattern recognition analysis to understand the biochemical events and to monitor alterations in PA metabolism in lung tissue of mice with OVA-induced asthma.

2 Materials and methods

2.1 Chemicals and reagents

Putrescine, spermidine, spermine, N^1 -acetylputrescine, N^1 -acetylspermidine, N^8 -acetylspermidine,

N^1 -acetylspermine, 1,6-diaminohexane (internal standard; IS) and heptafluorobutyric acid (HFBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Diethyl ether, ethyl acetate, and sodium chloride (pesticide grade) were purchased from Kanto Chemical (Tokyo, Japan). Sodium hydroxide was supplied by Duksan (Ansan, Gyeonggi-do, Republic of Korea). HPLC grade water and methanol were purchased from Daejung Chemical (Siheung-si, Gyeonggi-do, Republic of Korea). Acetic acid was purchased from Wako Pure Chemical (Richmond, VA, USA). All other chemicals were analytical reagent grade.

2.2 Preparation of standard solutions

Stock solutions of the seven PAs and IS were made up at 10.0 µg/mL in methanol/water (20:80, v/v) containing 0.05% HFBA. Working standard solutions were prepared by diluting each stock solution to 0.05 µg/mL and 1.0 µg/mL of IS in methanol/water (20:80, v/v) containing 0.05% HFBA, respectively. The calibration samples for PA profiling analysis in lung tissue matrix were prepared at four different amounts in the range of 0.05–5.0 ng (N^1 -acetylputrescine), 2.0–40.0 ng (putrescine, N^1 -acetylspermidine, N^8 -acetylspermidine and N^1 -acetylspermine), 10.0–250.0 ng (spermidine) and 500.0–10,000.0 ng (spermine) by mixing appropriate aliquots of each working solution in Table 2. All standard solutions were then stored at 4 °C.

2.3 Mouse model

Sixteen female mice (C57BL/6), 8–12 weeks old, weighing 20–22 g, were purchased from Orient Bio Inc. (Seongnam, Korea). Mice were housed in a controlled environment at 22 ± 2 °C and a relative humidity (% RH) of $50 \pm 5\%$ in polycarbonate cages and fed with a standard animal diet with free access to water. All mouse studies and experimental procedures were performed according to protocols approved by Suncheon National University Institutional Animal Care and Use Committee (SCNU IACUC, permit number: SCNU IACUC-2015-04) guidelines for the care and use of laboratory animals. All experiments were performed under sodium pentobarbital anesthesia, and every effort was made to minimize suffering. C57BL/6 mice were sensitized with an intraperitoneal injection of 10 mg of OVA and 1 mg of Imject Alum (Pierce) in 0.2 mL of saline on days 0 and 7. Control mice were injected with an equal volume of PBS. On days 14–16, mice received 10 µg of OVA dissolved in 50 µL of saline intratracheally under anesthesia with Zoletil/Rumpun. On day 17, lung tissue was collected after sacrifice.

2.4 Sample preparation for analysis of the seven PAs in mouse lung tissue

Mouse lung tissues were homogenized in distilled water using an ultrasonicator (IKA-Werke GmbH & Co.KG, Staufen, Germany) and were stored at -80°C until analysis. An aliquot of the homogenate equivalent to 5 mg of lung tissue including IS (10.0 ng) was added to acetonitrile (400 μL), vortex-mixed for 3 min, and centrifuged at 13,500 rpm (12,300 g) for 10 min. Supernatant was basified to $\text{pH} \geq 12$ using 1.0 M sodium hydroxide and then extracted using diethyl ether (3.0 mL) and ethyl acetate (2.0 mL), respectively. The combined extracts were evaporated to dryness under a gentle steam of nitrogen gas at 40°C , and dry residues were dissolved in 50 μL of methanol/water (20:80, v/v) containing 0.05% HFBA. Finally, a 5- μL aliquot was injected into the LC–MS/MS system for analysis.

2.5 Method validation for PA profiling analysis

Method validation was performed in pooled lung tissue samples ($n = 16$). The calibration range was selected by considering the minimum and maximum PA levels in lung tissue. Linearity of calibration curves was tested by least square regression analysis on the peak area ratios against increasing amount ratios. The limit of detection (LOD) and quantification (LOQ) for PAs were estimated based on the lowest concentration giving a signal greater than the sum of the mean blank signal plus three and ten times the standard deviation of the blank signal obtained through three blank measurements, respectively. The repeatability and accuracy were expressed as the percentage of relative standard deviation (%RSD) and percentage of relative error (%RE), respectively.

2.6 LC–MS/MS analysis

LC–MS/MS analysis in multiple reaction monitoring (MRM) mode was performed with a Shimadzu Nexera UPLC system (Shimadzu Corp., Kyoto, Japan) coupled with a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corp., Kyoto, Japan) with an electrospray ionization (ESI) interface in positive ionization mode. Chromatographic separation was performed using ACE C18-PFP column (150 mm $\times$ 2.1 mm, 3 μm) (Advanced Chromatography Technologies Ltd, United Kingdom). The column oven was maintained at 40°C and the auto-sampler temperature at 4°C . Mobile phase flow rate was 0.30 mL/min; the mobile phase was water containing 0.1% acetic acid with 0.05% HFBA (phase A) and methanol containing 0.1% acetic acid with 0.05% HFBA (phase B). Gradient elution of phase B was initiated from 20% (2 min), increased from 20 to 50% (6 min), and then held at 50% for 12 min. An aliquot (5 μL)

of each sample was injected into the LC–MS/MS system. The mass spectrometer interface temperature was maintained at 300°C . Nebulizing gas flow was 2.0 L/min. Desolvation line (DL) and heat block temperatures were 250°C and 400°C , respectively. Collision-induced dissociation (CID) gas pressure was 270 kPa.

2.7 Star pattern recognition and statistical analyses

The levels of three PAs and their four acetylated derivatives were determined from a calibration curve. Then, the quantitative level of each PA was normalized to the corresponding mean value of control mice group. These normalized values were then used to draw star graphs. Star graph was visually and readily distinguishable compared with numerical data from normal and abnormal. The star graphs were generated with Microsoft Excel (Microsoft, Redmond, WA). A nonparametric Mann–Whitney test was used to detect statistical differences in normalized metabolites between the OVA-induced asthma model and the control group, and p-values were adjusted with the false discovery rate (FDR). The Mann–Whitney test was performed with IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY). PCA (unsupervised learning) and PLS-DA and OPLS-DA (supervised learning) were applied for multivariate analysis. As powerful tools to determine differences between normal and abnormal, PCA have been used for trends and patterns analyses in data, PLS-DA and OPLS-DA have been used for discriminating between groups and for discovering the biomarker candidates. Multivariate statistical data analysis and classification analysis were performed on the log10-transformed, mean-centered and pareto-scaled data, and fivefold cross validation was applied for supervised learning. Multivariate statistical analyses were processed using the R statistical system (<http://www.R-project.org/>, version 3.5.0) with the *ropls* package (Babbar et al. 2006).

3 Results

3.1 Method validation for PA profiling analysis in lung tissue

Simultaneous profiling of three PAs and their four acetylated derivatives in lung tissue was validated under optimal conditions and mass spectra transitions are shown in Table 1. The range of calibration curves shown in Table 2, each of seven PAs showed good linearity ($r \geq 0.9960$) with LOD of 0.01–10.59 ng/mL and LOQ of 0.04–35.28 ng/mL. Repeatability and accuracy ranged from 0.6 to 15.3 (% RSD) and from -22.5 to 14.1 (% RE), respectively (Table 2). These were suitable for determination of PA levels in lung tissue samples (Table 3).

Table 1 Ion transitions and collision energy values for polyamines and the IS

No.	Polyamine	Precursor ion (m/z)	Product ions (m/z)					
			Transition 1 ^b (m/z)	CE1 ^d (V)	Transition 2 ^c (m/z)	CE 2 ^d (V)	Transition 3 ^c (m/z)	CE 3 ^d (V)
1	<i>N</i> ¹ -Acetylputrescine	131	72 (100) ^a	– 16	114 (87)	– 14		
2	Putrescine	89	72 (100)	– 11				
3	1,6-Diaminohexane (IS)	117	100 (100)	– 12	55 (55)	– 18	83 (12)	– 15
4	<i>N</i> ¹ -Acetylspermidine	188	100 (89)	– 17	72 (100)	– 22		
5	<i>N</i> ⁸ -Acetylspermidine	188	114 (76)	– 18	171 (100)	– 16		
6	Spermidine	146	72 (100)	– 16	112 (24)	– 15	84 (19)	– 23
7	<i>N</i> ¹ -Acetylspermine	245	112 (100)	– 22	100 (78)	– 20	129 (58)	– 13
8	Spermine	203	112 (100)	– 20	84 (55)	– 32	129 (40)	– 13

^aRelative intensities of product ions (%) in parentheses^bQuantitative ion^cQualitative ions^dCollision energy**Table 2** Linearity, LOD, LOQ, repeatability and accuracy of seven polyamines in mice lung tissue metrics by LC–MS/MS

No.	Polyamine	Calibration range (ng)	Linearity (r)	LOD ^a	LOQ ^b	Repeatability (%) ^c		Accuracy (%) ^d	
						Low ^e	High ^f	Low ^e	High ^f
1	<i>N</i> ¹ -Acetylputrescine	0.05–5.0	0.9985	0.01	0.04	15.3	7.2	14.1	0.3
2	Putrescine	2.0–40.0	0.9971	0.04	0.14	12.7	7.4	– 14.4	– 4.0
3	<i>N</i> ¹ -Acetylspermidine	2.0–40.0	0.9960	0.08	0.27	11.2	12.2	– 6.4	– 4.5
4	<i>N</i> ⁸ -Acetylspermidine	2.0–40.0	0.9985	0.13	0.44	11.5	12.4	– 0.3	– 3.7
5	Spermidine	10.0–250.0	0.9989	0.45	1.49	1.8	2.2	– 16.9	– 5.6
6	<i>N</i> ¹ -Acetylspermine	2.0–40.0	0.9964	0.02	0.08	3.7	8.0	– 22.5	– 0.2
7	Spermine	500.0–10,000.0	0.9999	10.59	35.28	0.6	4.7	– 3.5	– 1.3

^aLOD, limit of detection (ng/mL)^bLOQ, limit of quantification (ng/mL)^cRelative standard deviation (% RSD)^dRelative error (% RE)^eIndicate low value for, *N*¹-acetylputrescine 0.2 ng, putrescine 25.0 ng, *N*¹-acetylspermidine 15.0 ng, *N*⁸-acetylspermidine 10.0 ng, spermidine 40.0 ng, *N*¹-acetylspermine 15.0 ng, spermine 1500.0 ng^fIndicate high value for, *N*¹-acetylputrescine 5.0 ng, putrescine 40.0 ng, *N*¹-acetylspermidine 25.0 ng, *N*⁸-acetylspermidine 40.0 ng, spermidine 250.0 ng, *N*¹-acetylspermine 25.0 ng, spermine 10000.0 ng

3.2 Profiling analysis of PAs and univariate statistical analysis

Seven PAs, putrescine, spermidine, spermine, *N*¹-acetylputrescine, *N*¹-acetylspermidine, *N*⁸-acetylspermidine and *N*¹-acetylspermine, were identified and quantified in lung tissues of control and asthmatic mice by LC–MS/MS. Their quantitative levels are shown in Table 3. Spermine was the most abundant, followed by spermidine and putrescine. Putrescine ($p < 0.0034$), *N*¹-acetylputrescine ($p < 0.0652$), and *N*⁸-acetylspermidine ($p < 0.0827$) were significantly increased in the asthma group compared with the control group according to the Mann–Whitney test. The FDR calculated by the Benjamini–Hochberg procedure indicated

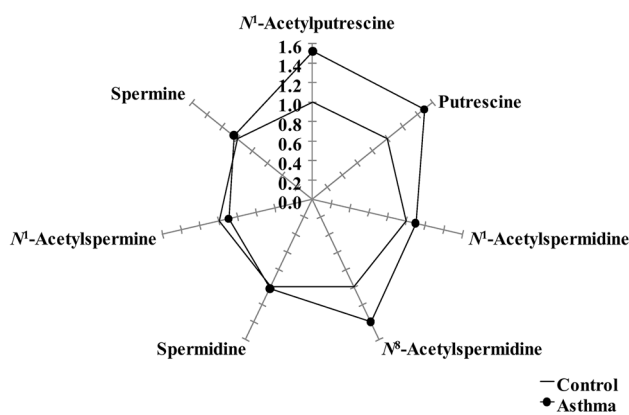
putrescine, *N*¹-acetylputrescine, and *N*⁸-acetylspermidine Benjamini–Hochberg p -values of $p < 0.0238$, $p < 0.1929$, and $p < 0.1929$, respectively (Table 3). The elevation of putrescine ($p < 0.0034$) showed a similar pattern to that of the previously reported acute allergic lung inflammation mouse model (North et al. 2013).

3.3 Star pattern recognition analysis

Values of seven PAs in asthmatic mice were normalized to the corresponding mean values of control mice varied from 0.89 to 1.52 in lung tissue. In comparison to the control mean values, the levels of *N*¹-acetylputrescine, putrescine, and *N*⁸-acetylspermidine in asthmatic lung tissues were

Table 3 The level of seven polyamines, Mann–Whitney test and FDR in lung tissue

No.	Polyamine	Amount (ng/mg of lung tissue) (Mean $\pm$ SD)		Normalized values ^a	Mann–Whitney test (p-values)	FDR ^b (p-values)
		Control (<i>n</i> = 10)	Asthma (<i>n</i> = 6)			
1	<i>N</i> ¹ -Acetylputrescine	0.097 $\pm$ 0.071	0.148 $\pm$ 0.066	1.52	0.0652	0.1929
2	Putrescine	3.944 $\pm$ 0.744	5.860 $\pm$ 1.198	1.49	0.0034	0.0238
3	<i>N</i> ¹ -Acetylspermidine	2.382 $\pm$ 0.851	2.625 $\pm$ 0.696	1.10	0.5152	0.7213
4	<i>N</i> ⁸ -Acetylspermidine	1.987 $\pm$ 1.142	2.768 $\pm$ 1.339	1.39	0.0827	0.1929
5	Spermidine	30.295 $\pm$ 3.578	30.716 $\pm$ 4.070	1.01	0.7449	0.7449
6	<i>N</i> ¹ -Acetylspermine	1.773 $\pm$ 0.939	1.579 $\pm$ 0.684	0.89	0.6644	0.7449
7	Spermine	834.470 $\pm$ 136.137	874.267 $\pm$ 243.100	1.05	0.3855	0.6747

^aValues normalized to the corresponding control composition values^bFalse discovery rate by Benjamin–Hochberg method**Fig. 1** Star symbol plots of mean data from the lung tissues of asthma and control groups drawn on the basis of the level of the polyamines after normalization to the corresponding control mean values

increased by 52%, 49%, and 39%, while *N*¹-acetylspermine was reduced by 11% (Table 3). The star graphic pattern for lung tissue from the asthma group was badly distorted and was readily distinguishable and characteristic from the heptagonal shape of the control group (Fig. 1).

3.4 Multivariate statistical analysis

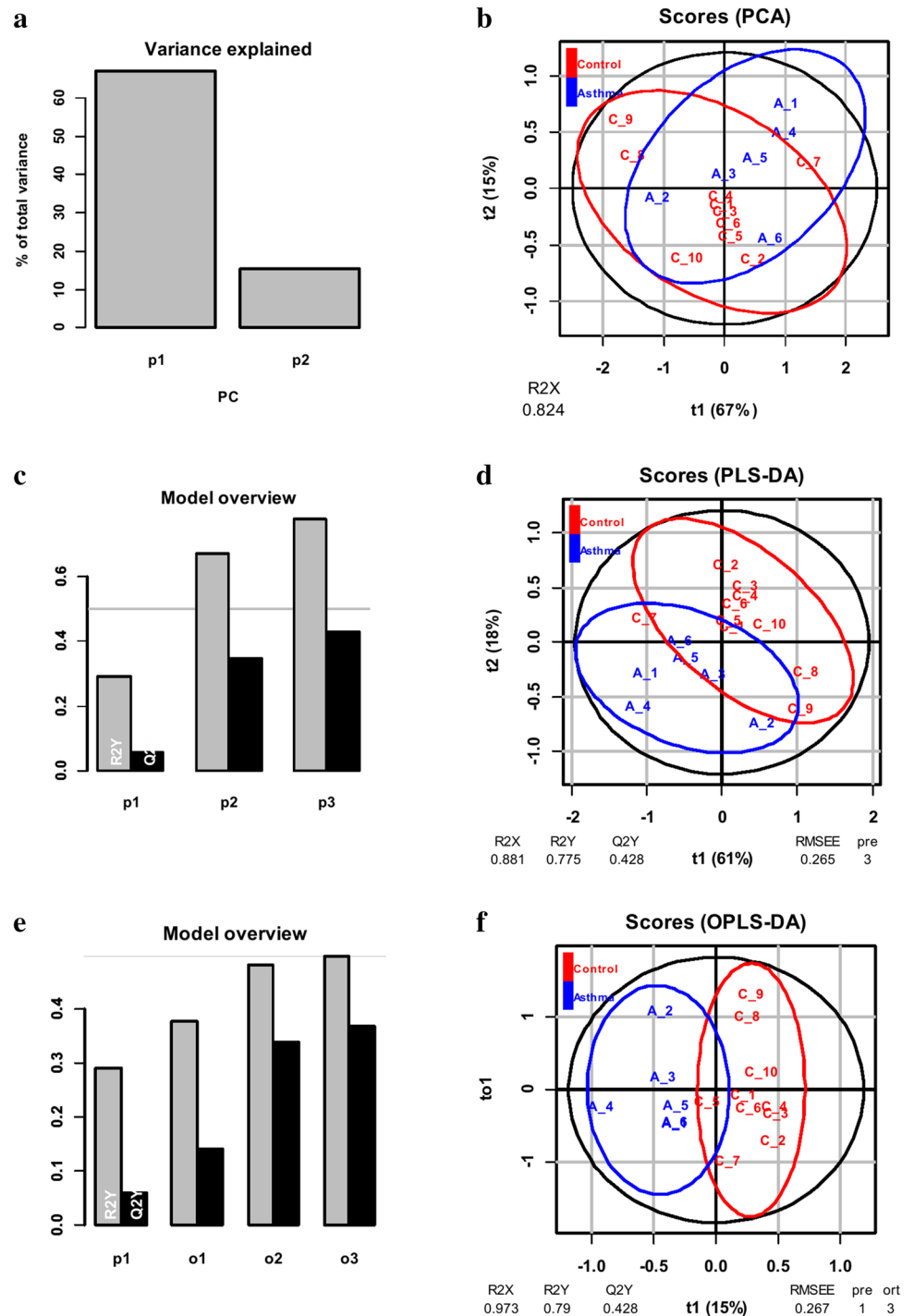
PCA analysis, a powerful type of unsupervised learning, was performed with PC1 and PC2. The PCA score plot showed unclear separation between the asthma and control groups; the first component can explain 67% of the total variances (82.4%) with two components (Fig. 2a). For supervised learning, PLS-DA and OPLS-DA were performed to obtain better differentiation and were conducted with five-fold internal cross-validation. The PLS-DA and OPLS-DA were calculated with three PLS components (p1, p2 and p3 in Fig. 2c) and one predictive component (p1 in Fig. 2e) with three orthogonal components (o1, o2 and o3 in Fig. 2e), respectively. And, overfitting confirmed that overfitting did

not occur because R2Y and Q2Y increase with the number of components (Fig. 2c, e). The PLS-DA score plot showed a separation of the two groups, R2X, R2Y, and Q2 were 0.881, 0.775, and 0.428, respectively (Fig. 2b). The OPLS-DA score plot showed the best separation of two groups better than PCA and PLS-DA, and R2X, R2Y, and Q2 were 0.973, 0.790, and 0.428, respectively (Fig. 2c). For discrimination between the two groups in the multivariate analysis, the loading score and VIP score were used, highly score obtained polyamines were selected for evaluation. Top-4 ranked *N*¹-acetylspermine (0.541), *N*⁸-acetylspermidine (0.497), *N*¹-acetylputrescine (0.451), and *N*¹-acetylspermidine (0.410) were selected in PCA loading score of PC1. In PLS-DA, *N*¹-acetylspermine (1.487), putrescine (1.384), and *N*¹-acetylputrescine (1.068) were selected with VIP scores above 1.0. Likewise, in OPLS-DA, putrescine (1.798), *N*¹-acetylputrescine (1.536), and *N*⁸-acetylspermidine (1.066) had VIP scores above 1.0. All polyamines scores are shown in Table 4, while the loading score ranking, VIP scores, and Mann–Whitney test are shown in Fig. 3.

4 Discussion

In asthma, the apoptosis of inflammatory cells in lung tissue is inhibited and the growth and differentiation of inflammation-related cells is promoted through an increase of inflammation-related cytokines (Jain 2018). Also, damage to epithelial cells due to airway inflammation will be repaired in the injury-repair cycle (Bousquet et al. 2000). For these reasons, there is a potential for supra-optimal levels of polyamines in asthmatics. The results of the current study are similar to previous reports of increased ornithine-derived polyamines in asthmatic mouse model (North et al. 2013). The cell may undergo proliferation, differentiation, apoptosis or quiescence depending on PA levels and cell type, and as the concentration of PA in the cell increases (Seiler and Raul 2005), PA is converted to a reduced acetylated

Fig. 2 Multivariate statistical analysis of lung tissue of OVA-induced asthma and control mice. C_ and A_ indicate control and OVA-induced asthma mice, respectively: **a** PCA variance explained; **b** PCA score plot; **c** PLS-DA model overview; **d** PLS-DA score plot; **e** OPLS-DA model overview; **f** OPLS-DA score plot



form and released out of the cell to maintain a constant level (Seiler 2004; Wallace et al. 1988). In the previous report, increased SSAT by up-regulation of *Sat1* expression was related to various cellular stresses including inflammation (Porter et al. 1991; Casero and Pegg 1993; Maxwell et al. 2003; Hector et al. 2004; Casero et al. 1989; Babbar et al. 2007, 2006; Pirnes-Karhu et al. 2012; Wang and Casero 2006), thus, there is a potential for increased acetylated

PAs in the asthma group. And, in the current results, it was assumed that the increased *N*¹-acetylputrescine was converted to release putrescine synthesized from ornithine. In the unsupervised learning PCA and the supervised learning PLS-DA, investigated all acetylated PAs including *N*¹-acetylspermine obtained a high score in loading score of PCA, although not separated into two groups. In PLS-DA, *N*¹-acetylspermine, putrescine, and *N*¹-acetylputrescine were

Table 4 The PCA loading score, VIP score of PLS-DA and OPLS-DA as multivariate analysis results

No	Polyamine	Unsupervised learning		Supervised learning	
		PCA loading score		PLS-DA	OPLS-DA
		PC1	PC2	VIP score	VIP score
1	<i>N</i> ¹ -Acetylputrescine	0.451	0.415	1.068	1.536
2	Putrescine	0.280	0.118	1.384	1.798
3	<i>N</i> ¹ -Acetylspermidine	0.410	− 0.311	0.793	0.477
4	<i>N</i> ⁸ -Acetylspermidine	0.497	0.286	0.919	1.066
5	Spermidine	0.011	0.389	0.239	0.055
6	<i>N</i> ¹ -Acetylspermine	0.541	− 0.527	1.487	0.015
7	Spermine	0.102	0.453	0.449	0.192

evaluated using the VIP score (> 1.0). Oddly, only *N*¹-acetylspermine was not statistically significant, but it was reduced in the asthma group. This finding requires further investigation because of the large weighting in separating groups in PCA and PLS-DA. On the other hand, in the supervised learning, putrescine had higher significance and a similar ranking order to the *p*-values of the Mann–Whitney test. The

high VIP scores (> 1.0) of putrescine, *N*¹-acetylputrescine, and *N*⁸-acetylspermidine were evaluated in OPLS-DA. A variety of statistical approaches were used to compare the control and asthma groups to select PAs that discriminated between the two groups. Our results may be helpful in interpreting polyamine metabolic pathways of asthma that have not yet been systematically studied (Fig. 4). However, since statistical processing of a small number of samples may lead to incorrect analysis, further studies of the metabolic pathways should be conducted.

5 Conclusion

Simultaneous profiling of three PAs and their four acetylated derivatives was validated by LC–MS/MS in MRM mode. Under optimal conditions of the tissue matrix, this method showed good linearity ($r \geq 0.9960$) with LODs of 0.01–10.59 ng/mL, LOQs of 0.04–35.28 ng/mL, repeatability (% RSD = 0.6–15.3) and accuracy (% RE = − 22.5 to 14.1). When this method was applied to the analysis of PAs in lung tissue samples of control and asthmatic mice, all seven PAs were identified and their levels determined. The seven PAs showed a trend toward overall increased

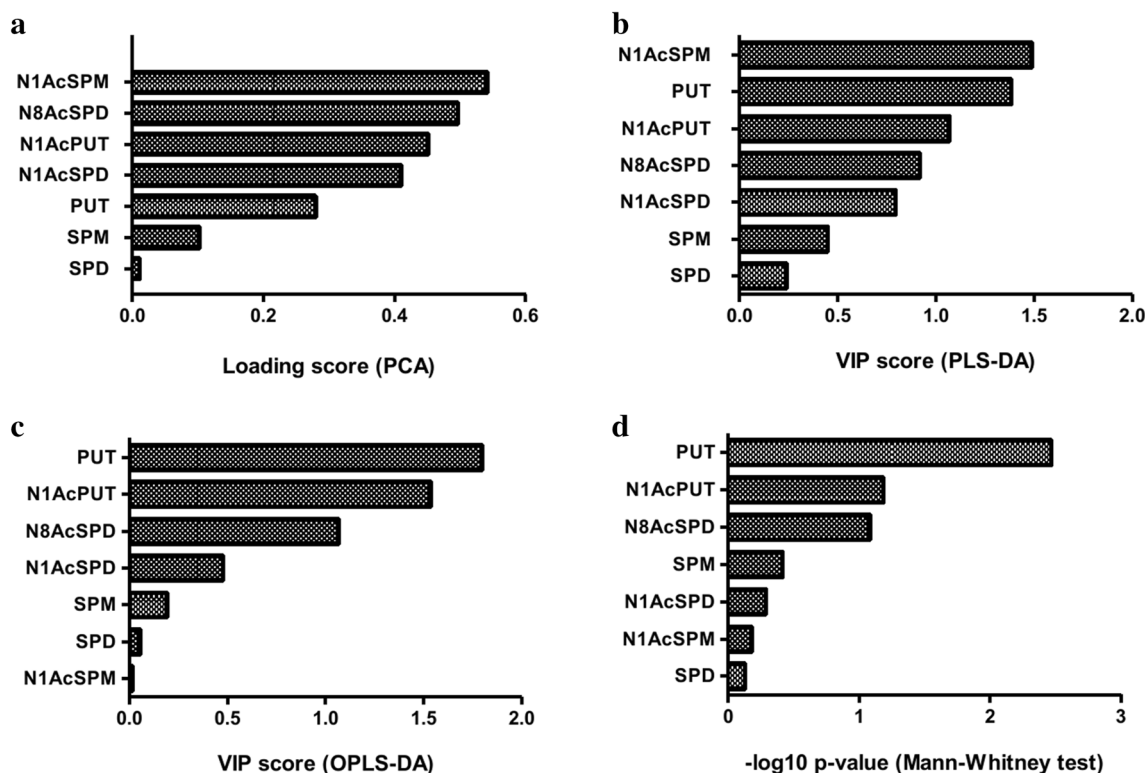


Fig. 3 Bar plot of univariate and multivariate statistical analysis: **a** loading score of polyamines ranked by result of PCA; **b** VIP score of polyamines ranked by result of PLS-DA; **c** VIP score of polyamines ranked by result of OPLS-DA; **d** $-\log_{10}$ p-values of polyamines

ranked by the statistical significance of the Mann–Whitney test. *PUT* Putrescine, *SPD* Spermidine, *SPM* Spermine, *N1AcPUT* *N*¹-Acetylputrescine, *N1AcSPD* *N*¹-Acetylspermidine, *N8AcSPD* *N*⁸-Acetylspermidine, *N1AcSPM* *N*¹-Acetylspermine

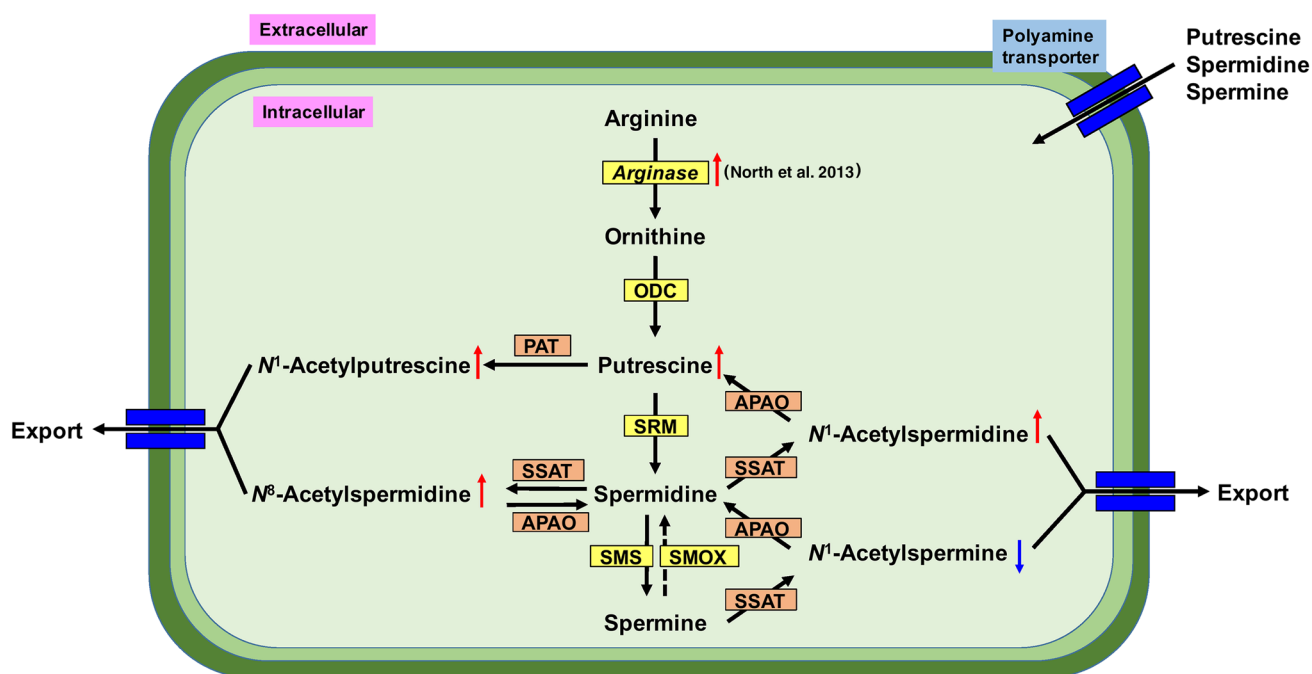


Fig. 4 Polyamine metabolic pathway including acetylated derivatives with lung tissue of OVA-induced asthmatic mice model compared to control (Jain 2018)

levels, and putrescine ($p < 0.0034$), N^1 -acetylputrescine ($p < 0.0652$), and N^8 -acetylspermidine ($p < 0.0827$) were significantly increased in the asthma group compared with the control group. In the star pattern recognition analysis results, the levels of N^1 -acetylputrescine, putrescine, and N^8 -acetylspermidine in asthmatic lung tissue were increased by more than 39% compared to the control mean values. Acetylated PAs played important roles in unsupervised learning PCA, and the importance of putrescine and including acetyl PAs was increased in supervised learning PLS-DA and OPLS-DA, it was similar to ranking of p-values by Mann–Whitney test. Putrescine, N^1 -acetylputrescine, and N^8 -acetylspermidine were significantly increased, while N^1 -acetylspermine was reduced. All of these were evaluated as important metabolites in PCA and PLS-DA, but more research is needed. The present study results could be the basis for understanding polyamine metabolism and asthma and the altered polyamine metabolic patterns with acetylated PAs may useful in designing and improving therapy for severe asthma patients.

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Author contributions H-SL performed method development, optimization and validation including application to asthma mice. Y-HH

performed sampling of asthma model. CS, MJ and JM performed optimization of method. HJP, YK, THS and SC performed pre-analytical experiments and sample preparation. AKP performed interpretation of statistical analysis. HK, S-TY and GL performed interpretation of the results. M-JP designed the experiments and supervised this work. All of authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee in the Suncheon National University.

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