The *NAMPT* Promoter Is Regulated by Mechanical Stress, Signal Transducer and Activator of Transcription 5, and Acute Respiratory Distress Syndrome–Associated Genetic Variants

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Abstract

Increased nicotinamide phosphoribosyltransferase (*NAMPT*) transcription is mechanistically linked to ventilator-induced inflammatory lung injury (VILI), with VILI severity attenuated by reduced *NAMPT* bioavailability. The molecular mechanisms of *NAMPT* promoter regulation in response to excessive mechanical stress remain poorly understood. The objective of this study was to define the contribution of specific transcription factors, acute respiratory distress syndrome (ARDS)-associated single nucleotide polymorphisms (SNPs), and promoter demethylation to *NAMPT* transcriptional regulation in response to mechanical stress. *In vivo* *NAMPT* protein expression levels were examined in mice exposed to high tidal volume mechanical ventilation. *In vitro* *NAMPT* expression levels were examined in human pulmonary artery endothelial cells exposed to 5 or 18% cyclic stretch (CS), with *NAMPT* promoter activity assessed using *NAMPT* promoter luciferase reporter constructs with a series of nested deletions. *In vitro* *NAMPT* transcriptional regulation was further characterized by measuring luciferase activity, DNA demethylation, and chromatin immunoprecipitation. VILI-challenged mice exhibited significantly increased *NAMPT* expression in bronchoalveolar lavage leukocytes and in lung endothelium. A mechanical stress-inducible region (MSIR) was identified in the *NAMPT* promoter from −2,428 to −2,128 bp. This MSIR

regulates *NAMPT* promoter activity, mRNA expression, and signal transducer and activator of transcription 5 (STAT5) binding, which is significantly increased by 18% CS. In addition, *NAMPT* promoter activity was increased by pharmacologic promoter demethylation and inhibited by STAT5 silencing. ARDS-associated *NAMPT* promoter SNPs rs59744560 (−948G/T) and rs7789066 (−2,422A/G) each significantly elevated *NAMPT* promoter activity in response to 18% CS in a STAT5-dependent manner. Our results show that *NAMPT* is a key novel ARDS therapeutic target and candidate gene with genetic/epigenetic transcriptional regulation in response to excessive mechanical stress.

Keywords: acute respiratory distress syndrome; cyclic stretch; nicotinamide phosphoribosyltransferase; B cell colony-enhancing factor; signal transducer and activator of transcription 5

Clinical Relevance

Nicotinamide phosphoribosyltransferase (*NAMPT*)/pre-B cell colony-enhancing factor is a key novel molecular marker and therapeutic target for acute lung injury. This study promotes the interpretation of the genetic and epigenetic regulation of *NAMPT* in response to excessive mechanical stress.

Acute respiratory distress syndrome (ARDS) is characterized by severe hypoxemia and a persistently high mortality

rate (~ 30%) (1, 2). Mechanical ventilation is a life-saving intervention in critically ill patients with respiratory failure due to

ARDS; however, excessive mechanical ventilation contributes directly to inflammatory lung injury, a process known

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as ventilator-induced lung injury (VILI) (3, 4). Like ARDS, VILI is also associated with augmented capillary leakage, acute inflammation, and increases in inflammatory cytokine expression (5).

We previously used high-throughput functional genomic approaches with extensive microarray-based lung gene expression profiling in canine and murine preclinical models of ARDS and in human patients with ARDS to search for novel ARDS/VILI biomarkers and therapeutic targets. These studies identified *NAMPT* (6), a gene encoding the cytozyme nicotinamide phosphoribosyltransferase (NAMPT), also known as pre-B cell colony-enhancing factor (PBEF) or visfatin (7), as a novel biomarker. NAMPT/PBEF,

originally named for its effects on the maturation of B cell precursors (8), is secreted from adipocytes (9). NAMPT/PBEF expression in human amniotic epithelial cell lines is up-regulated by mechanical stress and by inflammatory cytokines (10). Our prior studies demonstrated that NAMPT/PBEF is a direct neutrophil chemotactic factor that synergistically exacerbated VILI-mediated inflammatory injury *in vivo* by intratracheal delivery of recombinant NAMPT/PBEF (6, 11). Multiple indices of VILI-associated lung injury were reduced in heterozygous *NAMPT*^{+/-} mice, including peak inspiratory pressures and gene expression pathways (e.g., NFκB signaling, leukocyte extravasation, apoptosis, and Toll receptor

pathways) (6, 11). In addition, our study in *NAMPT* sequencing and subsequent genotyping identified single nucleotide polymorphisms (SNPs) within the *NAMPT* promoter to be significantly associated with enhanced susceptibility to sepsis and ARDS (6), suggesting that *NAMPT* is an ARDS susceptibility gene. The genetic association of *NAMPT* promoter SNPs with ARDS was replicated in a separate ARDS cohort and was found to be associated with the number of ventilator-free days and overall ARDS mortality (12). Finally, novel ARDS therapeutic strategies designed to reduce NAMPT enzymatic activity or bioavailability in preclinical models of ARDS and VILI resulted in significant VILI protection (11, 13).

Figure 1. Exposure to high tidal volume ventilation significantly increases murine nicotinamide phosphoribosyltransferase (NAMPT) protein expression in bronchoalveolar lavage (BAL) leukocytes and lung endothelium. Compared with spontaneously breathing (SB) mice (A), protein expression of NAMPT was increased in BAL leukocytes obtained from ventilator-induced inflammatory lung injury (VILI)-exposed mice (B). Protein expression of NAMPT was also increased in pulmonary endothelium from VILI-exposed mice (D) compared with SB mice (C).

These studies, highlighting the intimate involvement of *NAMPT* activity/expression in ARDS/VILI susceptibility and severity, led us to explore the molecular regulation of *NAMPT* promoter activity. Although the genetic and epigenetic factors involved in lung cell responses to mechanical stress are poorly understood, we now report significantly increased *NAMPT* expression in human pulmonary artery endothelial cells (ECs) exposed to 18% cyclic stretch (CS) and in murine lungs exposed to high tidal volume mechanical ventilation. Increased *NAMPT* expression evoked by 18% CS was mediated by the binding of *NAMPT* promoter to the transcription factor signal transducer and activator of transcription 5 (STAT5), resulting in increased promoter activity. STAT5 silencing attenuated *NAMPT* promoter activation in human ECs, and *NAMPT* expression was also significantly enhanced by DNA demethylation, an event significantly associated with increased STAT5 binding. Finally, two SNPs located in *NAMPT* promoter, rs59744560 (−948G/T) and rs7789066 (−2,422A/G), significantly linked to ARDS in two European descent cohorts (Chicago and Spain), increased basal and 18% CS-induced *NAMPT* promoter activation in a STAT5-dependent manner. Together, these findings suggest that *NAMPT* is an important ARDS candidate gene and novel therapeutic target with genetic/epigenetic transcriptional regulation highly influenced by excessive mechanical stress and ARDS-associated SNPs.

Materials and Methods

Cell Culture, Small Interfering RNA Transfection, and CS

Human pulmonary artery ECs were obtained from Lonza (Walkersville, MD) and were cultured as described previously (14) in the manufacturer's recommended endothelial growth medium-2. Cells were grown at 37°C in a 5% CO₂ incubator, and passages 6 to 9 were used for experiments. Media was changed 1 day before experimentation. For RNA interference, On-Target Plus small interfering RNAs (siRNAs) against STAT5A, STAT5B, and firefly luciferase (siCONTROL#2 used as negative control) were obtained from Dharmacon (Lafayette, CO) and transfected into ECs at a final concentration of 100 nM.

For CS studies, ECs were plated on Bioflex collagen I type cell culture plates (FlexCell International, Hillsborough, NC) and stimulated for 4 hours at 5 or 18% CS as previously described (15) on the FlexCell FX-5000 System (FlexCell International), mimicking low- and high tidal volume ventilation, respectively. For demethylation studies, ECs were treated with 5-aza-2'-deoxycytidine (5'-Aza) (Sigma-Aldrich, St. Louis, MO) for 72 hours at the indicated concentrations to inhibit DNA methyltransferase enzymes. Cells were harvested for RNA analysis by quantitative PCR (qPCR).

Models of VILI

Male C57BL/6J mice were induced VILI as previously described (11). Bronchoalveolar lavage (BAL) and lung tissue were collected from these mice and stained immunohistochemically with goat polyclonal anti-*NAMPT*/PBEF antibodies (Lampire Biological Laboratories, Pipersville, PA) as previously described (6).

Chromatin Immunoprecipitation and qPCR

Chromatin immunoprecipitation (ChIP) of DNA bound to STAT protein complexes

was performed using the EZ-Magna ChIP assay (EMD Millipore, Billerica, MA) in accordance with the manufacturer's recommended procedure. STAT cross-linked protein/DNA complexes were immunoprecipitated using STAT2, STAT3, and STAT5 antibodies (sc-476, sc-482, and sc-835) (Santa Cruz Biotechnology, Santa Cruz, CA). qPCR was performed using SsoFast EvaGreen Supermix (BioRad, Hercules, CA) following the manufacturer's protocol.

Gene Cloning, Mutagenesis, and 5'-Deletion Mutations

Gene cloning, mutagenesis, and luciferase activity assays were performed as previously described (16).

Luciferase Reporter Gene Assays

All constructs were transfected into ECs, where a plasmid containing the Renilla luciferase gene was cotransfected as a control. Transfected cells were exposed to static conditions or 18% CS for 4 hours and lysed in passive lysis buffer. Luciferase activity was measured by Dual-Luciferase Assay Kits using the GloMax-Multi Detection System (Promega, Madison, WI). Relative activities were expressed as the ratio

Figure 2. Excessive mechanical stress increases *NAMPT* expression *in vitro*. (A) mRNA levels, as measured by RT-PCR, showed a significant increase in *NAMPT* transcription in endothelial cells (ECs) exposed to 18% cyclic stretch (CS), whereas significant changes were not observed in ECs exposed to 5% CS (* $P < 0.05$ versus 5% CS). (B) Exposure to 18% CS significantly increased full-length *NAMPT* promoter activity in ECs (* $P < 0.05$ versus 0 h). RLU, renilla luciferase in plasmid containing the Renilla luciferase gene.

of firefly luciferase in pGL3 to renilla luciferase in plasmid containing the Renilla luciferase gene. Five independent transfections and duplicate luciferase assays were performed for each condition.

Statistical Analysis

Metaanalysis of genotypic information from Chicago and Spanish cohort samples were conducted assuming a fixed effects model using a Mantel-Haenszel stratified analysis with Epidat 3.0. The ANOVA test was used for comparison of luciferase activities among different constructs. Statistical significance was defined at $P < 0.05$ in both tests.

Results

Excessive Mechanical Stress Increases NAMPT Expression *In Vivo* and *In Vitro*

Validating our prior studies in mice exposed to excessive mechanical stress (6), we identified increased NAMPT protein expression in BAL macrophages (cytoplasm and nucleus) (Figures 1A and 1B) and pulmonary endothelium (Figures 1C and 1D) in VILI-exposed mice. NAMPT expression was examined *in vitro* in lung ECs exposed to 5 and 18% CS, simulating low and high tidal volume ventilation, respectively (17). Analysis of EC NAMPT mRNA levels via RT-PCR after exposure to 5 or 18% CS demonstrated significant increases in NAMPT transcription in ECs exposed to 18% CS (4, 6, and 48 h) compared with ECs exposed to 5% CS (Figure 2A). These results were supported by studies using a 3-kb NAMPT promoter (−3,028 bp to +1 ATG) in luciferase reporter transfected into ECs and exposed to 18% CS with NAMPT promoter activity significantly increased 1 to 4 hours after exposure (Figure 2B).

Effects of 5'-Deletions on Mechanical Stress-Dependent NAMPT Promoter Activity

To determine the core promoter sequence and regulatory elements within the NAMPT promoter, we analyzed NAMPT luciferase reporter promoter activation in response to 18% CS using a nested deleted promoter with varying DNA length in ECs (Figure 3). These studies revealed that truncation of the NAMPT promoter from −2,428 to −1,228 (2,082–882 bp upstream of the transcription

start site [TSS]) decreased promoter activity by approximately 30% (Figure 3B), whereas truncation of the NAMPT promoter from −1,528 to −628 (1,182–282 bp upstream of TSS) increased promoter activity by approximately 50% (Figure 3B). These results suggest that the −2,428 to −1,228 region is potentially regulated by factors suppressing promoter activity, whereas the −1,228 to −328 region (882 bp upstream

and 18 bp downstream of TSS) appears essential for core NAMPT promoter activity.

ECs transfected with identical series of NAMPT promoter fragments were next exposed to 18% CS or static conditions (4 h), with exposure to 18% CS increasing NAMPT promoter activity in the presence of the −3,028 to −2,128 region (2,682–1,782 bp upstream of TSS) to 80 to approximately 100% (Figure 2B). These

Figure 3. Functional analysis of NAMPT promoter activity after EC exposure to 18% CS. A NAMPT promoter region sensitive to 18% CS was identified using a series of nested deletion constructs in conjunction with luciferase reporter activity assays. Each experiment was repeated four times, and the mean $\pm$ SD is shown in each bar graph (* $P < 0.05$ versus static).

Figure 4. Exposure to 18% CS significantly increases signal transducer and activator of transcription 5 (STAT5) binding to the NAMPT promoter. Quantitative PCR (qPCR) of chromatin immunoprecipitation was performed for STAT2, STAT3, and STAT5. Primers for the 5'- (A) and 3'- (B) STAT binding sites on the NAMPT promoter were designed to capture *in silico* transcription factor-binding motifs that were specific to STAT family transcription factors. The sites of these motifs were located in the mechanical stress-inducible region. STAT occupancy was normalized to the change in qPCR product of static input fractions and 18% CS input fractions (* $P < 0.05$ versus input).

18% CS-mediated increases were abolished by truncation of the NAMPT promoter from -2,428 to -2,128 (2,082–1,782 bp upstream of TSS) (Figure 3B). These data are highly suggestive of a mechanical stress-inducible region (MSIR) at -2,428 to -2,128 containing critical NAMPT promoter responsive elements.

NAMPT Promoter Responses to Mechanical Stretch Are Regulated by STAT5

We analyzed the -2,428 to -2,128 sequence using Genomatix (www.genomatix.de) and TESS software (www.cbil.upenn.edu/cgi-bin/teess) and identified STAT family transcription factors as potentially capable of binding to this genomic region (Figure 3A). To address whether specific STAT transcription factors interact with the putative STAT binding sites within the NAMPT promoter, we evaluated STAT2, STAT3, and STAT5 and performed ChIP assays in ECs exposed to 18% CS versus static condition using two sets of primers targeting the 5' and 3' STAT binding sites. Compared with static cells, 18% CS significantly increased the occupancy of STAT5 at the NAMPT promoter in ECs relative to an input control at the 5' STAT binding site (Figure 4A) and at the 3' STAT binding site (Figure 4B). These data suggest that STAT5 is a critical transcription factor that mediates 18% CS-induced NAMPT promoter activation.

DNA Demethylation Increases NAMPT Transcription and STAT5 Binding to the NAMPT Promoter

To address epigenetic regulation of the NAMPT promoter in response to 18% CS, we assessed the status of NAMPT promoter

methylation. Exposure to 18% CS induced demethylation of the promoter region (-2,000 to -1,800 bp) relative to ATG, corresponding to a region containing *in silico* PAX5 binding sites (see Figure E1 in the online supplement). To verify that STAT5 binding to the NAMPT promoter is influenced by DNA demethylation, we performed ChIP assays in 5'-Aza-exposed or control carrier (PBS)-exposed ECs and determined that 5'-Aza significantly increases STAT5 NAMPT promoter occupancy as well as STAT3 (Figure 5A). Consistent with these results, 5'-Aza incubation dramatically increased NAMPT mRNA levels compared with controls (~2.5-fold) (Figure 5B), similar to the effect of 18% CS. In ECs pretreated with 5'-Aza, exposure to 18% CS did not further increase NAMPT mRNA levels (data not shown).

Two NAMPT Promoter Variants are Associated with ARDS Susceptibility and Increased Promoter Activity via STAT5

Our prior sequencing studies identified two NAMPT promoter SNPs (rs9770242 and rs61330082) that were found in subsequent

genotyping studies to be highly associated with ARDS susceptibility and severity (6). Additional sequencing studies in Chicago and Spanish ARDS cohorts identified NAMPT SNPs rs59744560 (-948G/T) and rs7789066 (-2,422A/G, cohort) as significant ARDS-associated SNPs (Tables 1 and 2). The consistency of allele effects was evidenced by a stratified metaanalysis yielding per-allele odds ratios of 3.29 and 2.73 for -948T and -2,422G, respectively. *In silico* studies identified SNPs -948T and -2,422G with significant potential for altering binding of two transcription factors, glucocorticoid receptor (GR) and nuclear factor of activated T cells, to the NAMPT promoter compared with -948G and -2,422A, respectively. Using site-directed mutagenesis, minor alleles of the two variants were inserted into the NAMPT promoter reporter and transfected into ECs. Compared with -948G and -2,422A, ARDS-associated alleles -948T and -2,422G significantly increased NAMPT promoter activities under static conditions. These ARDS-associated allele promoter activities were further enhanced by 18% CS. Reductions in EC STAT5 protein levels (using siRNA against STAT5a/b), however, attenuated the enhanced promoter response produced by the ARDS-associated SNPs (Figure 6).

Discussion

Despite improved understanding of the pathophysiology of ARDS, the underlying mechanisms for the injurious effects of mechanical ventilation in the setting of ARDS remain unclear, and effective pharmacotherapy has not emerged. We previously used genomic-intensive approaches to identify potential ARDS and VILI susceptibility candidate genes (18, 19)

Figure 5. Demethylation significantly increases NAMPT transcription and recruitment of STAT5. (A) 5-Aza-2'-deoxycytidine (5'-Aza) significantly increased mRNA levels of NAMPT (* $P < 0.05$ versus 0 μ M). (B) NAMPT promoter demethylation by 5'-Aza significantly increased STAT5 and STAT3 binding to NAMPT promoter (* $P < 0.05$ versus control).

Table 1. Association of Acute Respiratory Distress Syndrome Risk in Relation to Nicotinamide Phosphoribosyltransferase –2,422 and –948 Polymorphisms

Polymorphism	Genotype	Odds Ratio	Confidence Interval	P Value
–2,422 T/C	TT	Referent	–	–
	CC	2.73	1.18–6.30	0.015
–948 G/T	GG	Referent	–	–
	TT	3.29	1.07–10.12	0.03

and determined that *NAMPT*, the gene encoding the proinflammatory cytokine NAMPT/PBEF, is a potential VILI candidate gene and novel biomarker in sepsis and ARDS (6, 11). *NAMPT* SNPs in the promoter region were determined to confer susceptibility to sepsis-induced ARDS (6), a finding that was validated in an independent cohort with increased ARDS mortality (12). However, the underlying molecular mechanism involved in *NAMPT* gene regulation in the settings of ARDS and VILI remains poorly understood.

In this study, we demonstrate that excessive levels of mechanical stress (18% CS), but not homeostatic levels (5% CS), rapidly increase *NAMPT* promoter activity, transcription, and protein expression that is significantly associated with specific inducible promoter segments. Through serial progressive 5' to 3' unidirectional deletions and promoter activity assays, we detected a distal promoter or enhancer region (–3,028 to –2,428 bp), a proximal promoter region (–1,228 to –628 bp), and a negative regulatory region (–2,428 to –1,228 bp). *In silico* analysis also revealed that the proximal promoter of the *NAMPT* gene is conserved across vertebrate species,

suggesting a common regulation mechanism of the *NAMPT* gene across species (see Table E1). Our study demonstrates that the mechanical stress-inducible region critical for *NAMPT* responses to 18% CS resides within the promoter region between –2,400 and –2,100 bp. Although additional genetic and epigenetic mechanisms of *NAMPT* regulation are also in play (20), the current studies indicate that mechanical stress-mediated increases in *NAMPT* expression involve a group of transcription factors known as the STATs (signal transducer and activator of transcription proteins). STATs are recognized as activated by mechanical stretch (21), and we recently reported mechanical stress-mediated increased promoter activity within the HMGB1 gene as STAT3 dependent (22). CS significantly increases JAK2/STAT5 phosphorylation (23) and STAT5 translocation to the nucleus (24). Leveraging *in silico* information generated by predictions from Genomatix, UCSC Genome Browser (GRCh37/hg19) (25), and previous reports (8), we identified transcription factor-binding sites for

STAT5 and determined that STAT5 exerts critical positive regulatory control in *NAMPT* responses to mechanical stretch. STAT5 activation is characterized by translocation into the nucleus and subsequent binding to the corresponding DNA sequence to regulate target gene transcription and to promote cell differentiation, proliferation, and survival (26). STAT5a and STAT5b are fraternal twins of signal transduction and transcriptional activation. STAT5 is frequently activated by cytokines and induces cytokine functions (IL-2, IL-3, IL-5, and GM-CSF) (27) and promotes B cell development by controlling cell survival (28). In prior and current studies, we have demonstrated involvement of the STAT pathway in response to excessive pathological mechanical stress and VILI development (11) via enhanced STAT5 (and STAT3) binding to the *NAMPT* promoter and up-regulated *NAMPT* promoter activity, confirming *NAMPT*'s critical role in mechanical ventilation-induced ARDS and exacerbation of ARDS (6, 8, 10, 11).

Epigenetic mechanisms of *NAMPT* regulation are also involved in *NAMPT* promoter regulation as 18% CS significantly increased DNA demethylation of PAX5 *in silico* binding sites and DNA demethylation by 5'-Aza increased *NAMPT* transcription and enhanced STAT5 as well as STAT3 recruitment to the *NAMPT* promoter. Because STAT3:STAT5 heterodimers are elicited by M-CSF binding to *cis*-inducible element sites with high affinity to promote STAT5-dependent genes (29), our data suggest the possibility that

Table 2. Summary of *NAMPT* Single Nucleotide Polymorphisms Significantly Associated with Phenotypes of Various Diseases

Position*	Location†	SNP_ID	Alleles	African American-Associated Phenotypes‡	European American-Associated Phenotypes‡	Spanish-Associated Phenotype‡
100287367	–2,422T > C	rs7789066	T/C	—	—	ARDS
100286480	–1,535C>T (–1,543C>T)	rs61330082	C/T	ARDS	ARDS, CAD	—
100285946	–1001G>T	rs9770242	G/T	—	ARDS	—
100285893	–948G > T	rs59744560	G/T(C/A)	—	ARDS, DM2	—
100285368	–423A > G	rs1319501	A/G	—	Risk of MI	—

Definition of abbreviations: ARDS, acute respiratory distress syndrome; CAD, coronary artery disease; DM2, type 2 diabetes mellitus; MI, myocardial infarction.

*Position in chromosome 1 according to NCBI build 36.

†Reference sequence is NM_005746.2.

‡References for associated phenotypes (6, 31, 34, 35).

Figure 6. Influence of mechanical stress, STAT5, and acute respiratory distress syndrome (ARDS)-associated variants on NAMPT promoter activities. Activities of the NAMPT promoter containing the recently identified risk alleles for ARDS in Chicago or Spanish ARDS cohorts ($-948T$ or $-2,422G$, respectively) were assessed. NAMPT promoter activity was significantly increased in NAMPT constructs containing either $-948T$ or $-2,422G$ upon response to 18% CS compared with the wild-type (WT) NAMPT promoter constructs. Reductions in EC STAT5 protein levels (using small interfering RNA [siRNA] against STAT5a/b compared with negative control siRNA) (inset) partially or fully abolished the effects of $-948T$ and $-2,422G$ on promoter activities in response to 18% CS (* $P < 0.01$ versus static; # $P < 0.05$ versus wild type; + $P < 0.05$ versus 18% CS).

STAT5 may form heterodimers with STAT3 to regulate the NAMPT promoter. During maturation of pro-B cells, STAT5 also cooperates with PAX5 to promote distal immunoglobulin heavy-chain gene transcription (30). Under 18% CS, increased recruitment of STAT5 could synergize with demethylation of PAX5-binding elements to promote NAMPT transcription.

Our studies identified two ARDS-associated NAMPT promoter SNPs

($-2,422A/G$ and $-948G/T$), and, despite study limitations, including relatively small groups of patients with ARDS and controls and lack of cross validation in the two ARDS cohorts, our *in vitro* experiments confirmed robust and significant influence of these SNPs on NAMPT promoter activity in response to 18% CS challenge. Functionality of the $-948G/T$ (C/A) variant is supported by recent studies associating this NAMPT

SNP with low-grade inflammation in patients with diabetes (31). Under static conditions, silencing of STAT5 failed to influence the activity of promoters with $-2,422A/G$ or $-948G/T$. However, in response to 18% CS, reductions in STAT5 expression significantly attenuated SNP-dependent enhanced NAMPT promoter activity with either NAMPT variant. Because GR acts as a transcriptional coactivator for STAT5 and enhances STAT5-dependent transcription (32) and because STAT5 combines with nuclear factor of activated T cells to promote *Foxp3* transcription (33), the effects of the $-948G/T$ and $-2,422A/G$ SNPs may synergize with STAT5 to significantly enhance NAMPT transcription with the influence of these cotranscription factors during pathological stress. Together, these studies demonstrated that STAT5 significantly regulates NAMPT transcription under pathological mechanical stress.

Interaction of the NAMPT promoter with inflammation-related transcription factor STAT5, NAMPT promoter demethylation, and ARDS-associated SNPs regulate NAMPT promoter activity, thereby contributing to VILI and ARDS development. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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