NCOR1 and NCOR2 Exhibit Distinct Cellular and Transcriptomic Signatures in Human Abdominal Aortic Aneurysm

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Article

NCOR1 and NCOR2 Exhibit Distinct Cellular and Transcriptomic Signatures in Human Abdominal Aortic Aneurysm

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Abstract

Background/Objectives: Nuclear receptor corepressors NCOR1 and NCOR2 are key regulators of transcriptional repression, chromatin remodelling, and immunometabolic signalling. While NCOR1 has already been linked to vascular biology, its relevance in abdominal aortic aneurysm (AAA) remains unclear, particularly for NCOR2. This study aimed to investigate the expression, cellular localisation, and molecular interactions of NCOR1/2 in human AAA tissue. **Methods:** Human AAA samples (elective and ruptured) (n=45) and non-aneurysmal control aortas (n=18) were obtained from our Swiss Vascular Biobank. Transcriptomic profiling was performed using ribosomal RNA-depleted RNA sequencing. Differential expression and correlation analyses were performed using DESeq2/EdgeR and Spearman rank correlation with Benjamini–Hochberg correction. Cellular localisation was assessed through immunohistochemistry (IHC). **Results:** Bulk transcriptomic analyses showed no significant differences in NCOR1 or NCOR2 expression between AAA and controls. IHC revealed that NCOR1 was found in endothelial cells (ECs), smooth muscle cells (SMCs), and inflammatory infiltrates, while NCOR2 was primarily associated with macrophages. Correlation analyses suggest NCOR1 linking with various cellular markers, proteolytic enzymes, inflammatory mediators, and epigenetic regulators, including lncRNA MALAT1. NCOR2 showed distinct associations with remodelling enzymes, TGFB1 signalling, selective epigenetic modifiers, and lncRNA H19. **Conclusions:** The lack of transcriptional differences in NCOR1 and NCOR2 between AAA and controls does not exclude cell-type-specific regulation or functional relevance. The specific cellular distributions and molecular associations in human AAA imply that NCOR1 and NCOR2 play non-redundant roles in vascular remodelling, inflammation, and epigenetic regulation. Our findings highlight NCOR pathways as potential modulators of AAA pathophysiology and promising targets for future therapies.

Keywords: nuclear receptor corepressors NCOR1 and NCOR2; abdominal aortic aneurysm (AAA); vascular cells; epigenetics; lncRNAs

1. Introduction

Nuclear receptor corepressors NCOR1 and NCOR2, also known as N-CoR (nuclear receptor corepressor 1) and SMRT (silencing mediator of retinoid and thyroid receptors), are essential transcriptional coregulators of gene expression [1–6]. These proteins act as scaffolds for multi-protein corepressor complexes [1,5,7], which induce local chromatin modifications [1,3]. NCOR1/2 are also recognised as key factors in immunometabolic gene regulation, involving nuclear receptor signalling pathways that detect metabolic changes and regulate inflammation [1,6,8]. Furthermore, NCOR1 appears protective against atherosclerosis [9,10], whereas almost nothing is known about the role of

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NCOR2. Early mechanistic studies identified NCOR2 as a regulator of transcriptional repression of the inflammatory response [11]. Moreover, NCOR2 works alongside NCOR1 to control stimulus-dependent inflammatory signalling [12]. Current research describes NCORs as immunomodulators that coordinate inflammatory signalling, a process that plays an important role in many vascular pathologies.

Although NCOR1 and NCOR2 share similar functional domains, emerging evidence indicates that they play distinct roles and harbour distinct splice variants [13]. Furthermore, cell-type specificity has been observed [1,5]. Additionally, a study on endothelial cells found that NCOR1 plays a crucial role in angiogenesis, whereas NCOR2 had no effect [14]. Thus, NCOR1 and NCOR2 may have different target preferences depending on the cell type and signalling pathways.

As co-repressors, NCOR1 and NCOR2 are also essential epigenetic regulators [1,7]. They influence DNA methylation and the organisation of higher-order chromatin structures through interactions with other epigenetic modifiers [15]. Epigenetic regulation in vascular and immune cells is also controlled by a network of non-coding RNAs, especially long non-coding RNAs (lncRNAs) [16–19]. In the context of NCOR1/2, recent studies reveal an intriguing interaction between lncRNAs and their functions, which modulate the stability of repressor complexes and influence signal-dependent gene silencing [7,13,20]. Furthermore, epigenetic regulation and lncRNAs have been recognised as key contributors to vascular pathophysiologies [17,19,21]. Therefore, through epigenetic mechanisms or lncRNAs, NCOR complexes may regulate gene expression by altering chromatin structure and impacting transcription factor activity.

Given its broad roles in inflammation, metabolism, and epigenetics, NCOR1/2 have also been linked to cardiovascular diseases. In atherosclerosis, NCOR1 has been shown to be atheroprotective [9,10,15]. NCOR1 also contributes to vascular injury by maintaining the homeostasis of vascular smooth muscle cells (SMCs) [9]. Little is known about the roles of these metabolic immunomodulators in abdominal aortic aneurysm (AAA). AAA formation involves phenotypic modulation of SMCs and the infiltration of inflammatory cells [22–27]. These pathophysiologies, which have already been described in atherosclerosis and cardiovascular diseases, suggest that NCOR1/2 may also play important roles in AAA. Furthermore, epigenetic changes and lncRNAs, which are substantially affected by these immunometabolic regulators, have been recognised as important factors contributing to AAA [28–32]. To date, only two publications have mentioned the role of NCOR1 in AAA [9,22]. Du et al. used a mouse Ang II-induced AAA model and in vitro human SMCs [9]. Chen et al. identified key genes associated with AAA from the Gene Expression Omnibus database, including NCOR1, one of four transcription factors linked to AAA [22]. Regarding NCOR2, nothing is known about its role in AAA.

The aim of this study was therefore to analyse the expression and cellular localisation of NCOR1 and NCOR2 in human AAA tissue and to compare the results with those of the non-aneurysmal aorta from our Swiss Vascular Biobank (SVB) [33], conducting histological and transcriptomics analyses that focus on various markers of vascular and immune cells, as well as on epigenetic factors and lncRNAs.

2. Materials and Methods

2.1. Tissue Samples

Vascular tissue samples of human aortic tissue used in the study were obtained from patients scheduled for open aortic repair of the infrarenal abdominal aorta (n = 45) at the Department of Vascular Surgery (USZ/UZH) and collected in our Swiss Vascular Biobank (SVB). Non-aneurysmal healthy infrarenal aortic samples from the SVB used as reference (n = 18) were obtained during kidney transplantation and provided by the Department of Visceral Surgery (USZ/UZH). All patients gave appropriate written informed consent. The local ethics committee (Cantonal Ethics Committee Zurich, Switzerland) approved the tissue sample collection and analysis procedure (BASEC-No 2020-00378 and 2020-01844). The tissue samples were divided for consecutive histological and molecular-

 doi:10.20944/preprints202603.0777.v1

biological analyses. The part intended for histological studies was fixed in 4% formalin and embedded in paraffin (FFPE). Adjacent tissue pieces used for RNA analyses were collected in a specific cryoprotective solution and stored at -80°C [29]. The study was conducted in accordance with the World Medical Association Declaration of Helsinki.

2.2. Histological Evaluation

To evaluate tissue quality, aneurysm extent, and the composition of healthy aortic samples, FFPE blocks were sectioned into 5 µm slices and stained with Haematoxylin–eosin (HE) and Elastica van Gieson (EvG), focusing on cellular makeup, the extent of inflammation, and the distribution of collagen and elastin fibres. For immunohistochemistry (IHC), FFPE sections were mounted on pre-coated (0.1% poly-L-lysine; Merck/Sigma-Aldrich, Buchs, Switzerland) SuperFrost Plus slides (Thermo Fisher Scientific), and antigen retrieval was performed by heating in citrate buffer (pH 6.0). The primary antibodies used in this study were sourced from Lucerna-Chem (Luzern, Switzerland) and Agilent/Dako (Basel, Switzerland): anti-nuclear receptor corepressor NCoR1 (PA1-844A, Thermo Fisher/Invitrogen, Switzerland; abcam ab3482, 1:500), anti-nuclear receptor corepressor NCoR2 (PA1-843, 1:500; Thermo Fisher/Invitrogen), anti-SM-actin (SMCs, abcam ab5694, 1:1,000; Lucerna-Chem), anti-CD31 (ECs, ab134168, 1:40; Lucerna-Chem), anti-CD45 (leukocytes, M0701, 1:2,000; Agilent/Dako), and anti-CD68 for macrophages (M0814, 1:2,000; Agilent/Dako). All antibodies were diluted in DAKO REAL Antibody Diluent (Agilent/Dako). For primary antibody detection, mouse/rabbit-specific HRP/DAB (ABC) detection kits (ab64264, abcam; Lucerna-Chem) and Mayer's haematoxylin (Carl Roth, Switzerland) for nuclear counterstaining were used. All slides were scanned and digitised using the Precipoint-M8 Digital Microscope (Formafix, Langenfeld, Germany).

2.3. RNA Extraction and Quality Determination

For RNA sequencing and transcriptome analysis, RNA was isolated from fresh-frozen tissue samples using TRIzol (Thermo Fisher), which enables the extraction of total RNA, including non-coding RNAs. The RNA concentration was measured using the NanoDrop Lite Plus Spectrophotometer (Witec, Sursee, Switzerland). To assess the RNA quality of samples suitable for sequencing, RNA integrity numbers (RINs) and DV200 index (percentage of fragments greater than 200 nucleotides) were determined using TapeStation 4150 (Agilent). For transcriptome analysis, only samples with sufficient RNA quality—specifically, RIN values between 2 and 4 and a DV200 > 50%—were selected. Such quality standards are typical for atherosclerotic and aneurysmal tissue samples and provide reproducible, reliable data, as described previously [33].

2.4. Library Preparation and RNA Sequencing

The RNA library was prepared using the SMARTer Stranded Total RNASeq Kit (Clontech/Takara Bio, USA) following the manufacturer's protocol. The RNA sequencing was conducted in collaboration with the Functional Genomics Center Zurich (FGCZ/ETH, Switzerland). To include non-coding RNAs in the analysis, the ribosomal RNA depletion method was employed. The quality and quantity of the RNA library were again validated by determining the RIN. RNA sequencing was performed on the Illumina NovaSeq 6000 (Illumina, Berlin, Germany) with a sequencing depth of 200 M reads across 100 cycles.

2.5. Data Analysis

The data analysis of the sequenced RNA was performed using the SUSHI framework developed by FGCZ [34]. The quality control of individual reads was verified by FastQC. Read alignment was carried out with STAR [35], and read abundance was estimated using FeatureCounts from the R package subreads [36]. Differential expression analysis was conducted using the linear model approach from the Bioconductor packages DESeq2 and EdgeR280 [37]. The False Discovery Rate (FDR, adjusted P-value) was calculated with the Benjamini-Hochberg algorithm for multiple testing

 doi:10.20944/preprints202603.0777.v1

[38]. An FDR threshold of <0.1 was considered significant to balance sensitivity and discovery potential within the exploratory framework, recognising the increased likelihood of false positives. The datasets of RNA sequencing generated and analysed during the study are stored on FGCZ servers.

2.6. Statistical Analyses

The statistical analyses were conducted using IBM's SPSS software version 29.0 (SPSS Inc., Chicago, IL, USA). Data were tested for normality using the nonparametric Kolmogorov-Smirnov one-sample test. Due to the broad heterogeneity in individual sample values and the variation in the number of study samples, the Mann-Whitney U test was employed. Correlation analyses were performed using the Spearman correlation coefficient. In all cases, Benjamini-Hochberg correction for multiple testing and adjustments for sex and age were applied [38]. All tests were two-sided, and P < 05 (*), P < 0.01 (**), and P < 0.001 (***) were considered statistically significant.

3. Results

3.1. Patient Characteristics

The baseline characteristics of the patients included in the study are summarised in Table 1. A total of 45 patients with abdominal aortic aneurysm (AAA) were included: 30 undergoing elective AAA repair (eAAA), 15 with ruptured AAA (rAAA), and 18 healthy aortic controls.

Table 1. Patient characteristics.

	AAA	eAAA	rAAA	Ctrl	P-values
	(n = 45)	(n = 30)	(n = 15)	(n = 18)	
Sex (m/w %)	37 (82%)	26 (87%)	11 (73%)	12 (80%)	0.243
Age (years)	70.7 ± 10.1	68.6 ± 10.9	74.9 ± 6.6	53.9 ± 6.7	0.043 (<0.001)**
AAA diameter (mm)	69.7 ± 26.9	63.3 ± 25.2	82.2 ± 26.7	-	0.017*
Comorbidities					
Hypertension	40 (89%)	27 (90%)	13 (87%)	-	1.000
Hyperlipidaemia	16 (36%)	12 (40%)	4 (27%9	-	0.514
Nicotine abusus	17 (38%)	12 (40%)	5 (33%)	-	0.752
Diabetes mellitus	6 (13%)	3 (10%)	3 (20%)	-	0.384
Chronic kidney disease	29 (64%)	19 (63%)	10 (67%)	-	1.000
Coronary heart disease	16 (36%)	12 (40%)	4 (27%)	-	0.514
Peripheral artery disease	7 (16%)	5 (17%)	2 (13%)	-	1.000
Stroke	4 (9%)	3 (10%)	1 (7%)		1.000
Medication					
Antiplatelet therapy	34 (76%)	24 (80%)	10 (67%)	-	1.000
Alpha-blocker	4 (9%)	4 (13%)	-	-	0.285
Beta-blocker	21 (47%)	14 (47%)	7 (47%)	-	0.530
ACE inhibitors	12 (27%)	10 (33%)	2 (13%)	-	0.283
AT-blocker	5 (11%)	3 (10%)	2 (13%)	-	0.613
Statins	23 (51%)	16 (53%)	7 (47%)	-	1.000
Diuretics	7 (16%)	6 (20%)	1 (7%)	-	0.395

Age and AAA diameter were normally distributed; therefore, parametric t-test was used. For all other values, Fisher's exact test was applied. P-values comparing eAAA vs. rAAA; ** P-values in brackets show statistical comparison between AAA and Ctrl. AAA: abdominal aortic aneurysm, eAAA: elective AAA, rAAA: ruptured AAA, Ctrl: healthy aortic controls.

The proportion of male patients was high across all AAA groups (82%), with no significant differences between eAAA, rAAA, and controls. Patients with AAA were significantly older than

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controls (70.7 ± 10.1 vs 53.9 ± 6.7 years, P < 0.001). Comparing eAAA and rAAA, no statistically significant difference in age was observed. The mean maximal aneurysm diameter was significantly larger in rAAA compared to eAAA (82.2 ± 26.7 mm vs 63.3 ± 25.2 mm, P = 0.017). The prevalence of cardiovascular risk factors and comorbidities in AAA patients was high, with hypertension present in nearly all patients (89%), followed by chronic kidney disease (64%) and coronary heart disease (36%). No significant differences were found between eAAA and rAAA regarding any cardiovascular comorbidities or medication (Table 1). Antiplatelet therapy was the most used medication (76%), followed by statins (51%) and beta-blockers (47%). Overall, the eAAA and rAAA groups were well matched in sex, cardiovascular risk profile, comorbidities, and medications, with the only differences in age and diameter. Concerning healthy aortic controls obtained from organ donors undergoing kidney transplantation, clinical information was limited to age and sex only due to data protection regulations.

3.2. Overall Differential Gene Expression Analysis in AAA and Controls

To assess overall transcriptional expression patterns, differential gene expression analysis was performed comparing RNA-sequencing results from AAA samples, non-aneurysmal control aortas, and elective versus ruptured AAAs. Volcano plot analysis demonstrated significant transcriptional changes in many genes in AAA compared to controls, exceeding the predefined threshold of log2 fold change ≥ 0.5 and FDR < 0.1 (Figure 1A). In AAA, 5,552 genes were upregulated, while 3,063 were downregulated.

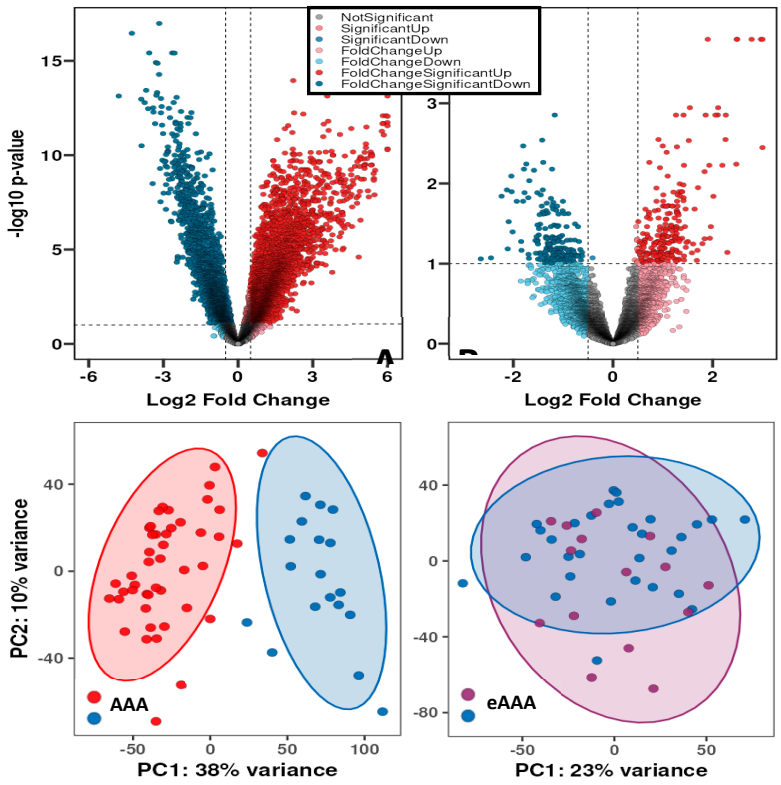

Figure 1. Volcano plot showing differentially expressed genes in the abdominal aortic aneurysm (AAA) samples compared to control healthy aorta (Ctrl) (A) and between elective (eAAA) and ruptured (rAAA) aneurysm samples (B). Principal component analysis (PCA) of the study groups, (C) AAA against Ctrl, (D) eAAA vs rAAA. The volcano plot shows −log10-transformed FDR as a function of the difference between the study groups. The broken lines indicate a 0.5 log2-fold change with FDR < 0.1.

Of these genes, 2,264 were lncRNAs (1,435 with unknown functions or not yet characterised). The principal component analysis (PCA) (Figure 1C) shows a clear separation between the groups (AAA, control).

 doi:10.20944/preprints202603.0777.v1

In contrast, comparison of eAAA and rAAA revealed a significantly smaller set of differentially expressed genes (Figure 1B), with 238 upregulated and 183 downregulated (including 78 lncRNAs). This suggests that, while aneurysm formation involves broad transcriptomic changes, rupture is characterised by more subtle differences in gene expression. The sets of differentially expressed genes were linked to extracellular matrix degradation, remodelling, and SMC phenotypic switching (various proteolytic enzymes, collagen fibres, markers of synthetic smooth muscle cells), as well as inflammation (including various cytokines, including T-cells, macrophages and others). Furthermore, the PCA showed considerable overlap and no clear separation of individual samples between eAAA and rAAA (Figure 1D).

3.3. Differential Expression Analysis of NCOR1 and NCOR2 in AAA and Controls

Given the scope of the study, the differential expression analysis concentrated on NCOR1 and NCOR2. Surprisingly, in contrast to previous research on human carotid plaques showing significantly reduced NCOR1 expression in carotid atherosclerotic lesions [10], violin plot analysis revealed no significant difference in NCOR1 expression between AAA and control aorta (P = 0.419) (Figure 2A). Similarly, NCOR2 expression did not differ significantly between the two study groups (P = 0.617) (Figure 2B). Furthermore, when comparing elective and ruptured aneurysms, the expression patterns for NCOR1 and NCOR2 were almost identical (P = 0.950 and P = 0.907) (Figure 2C–D).

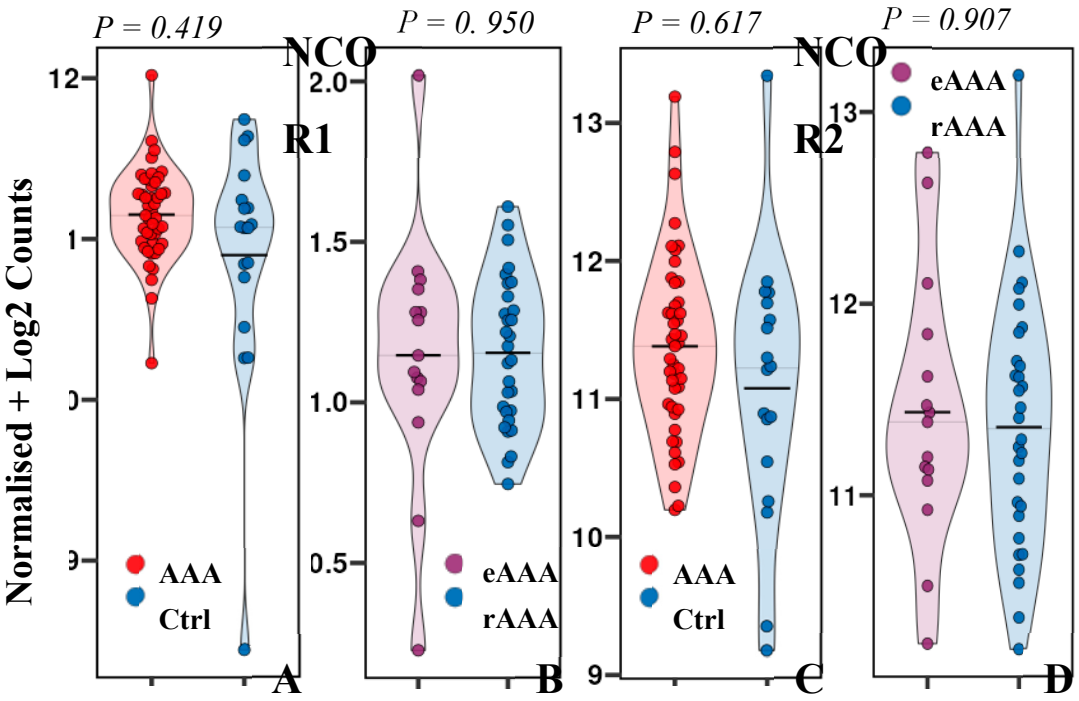

Figure 2. Violin plots comparing the expression of NCOR1 (A and C) and NCOR2 (B and D) in abdominal aortic aneurysm (AAA) against healthy control aorta (Ctrl) (A and B) and between elective (eAAA) and ruptured AAA (rAAA).

Based on the results shown above, which indicate no differences between eAAA and rAAA in the expression of NCOR1 and NCOR2 (as discussed in the Discussion, paragraph Study limitations), we focused on only two study groups in the further analyses: AAA and healthy aortic controls.

3.4. Histological Analysis of NCOR1 and NCOR2 in AAA and Controls

To assess the cellular localisation of NCOR1 and NCOR2 expression, corresponding immunohistochemistry (IHC) was performed (Figure 3). Again, also in histology, no significant

 doi:10.20944/preprints202603.0777.v1

differences were observed between eAAA and rAAA. Therefore, Figure 3 displays only representative images of a healthy control aorta and AAA in general. In the controls, NCOR1 expression was detected solely in smooth muscle cells (SMCs) within the media and in pericytes surrounding larger vessels in the adventitia (Figure 3A). Regarding NCOR2, no expression was observed in healthy aortas (Figure 3B). In contrast, multiple cell types were found to be positive for both immunometabolic factors in aneurysmal tissue (Figure 3C–F). NCOR1 was positive in ECs, SMCs, CD45, and CD68 cells (Figure 3E). In contrast, NCOR2 was only detected in macrophages (CD68) (Figure 3F).

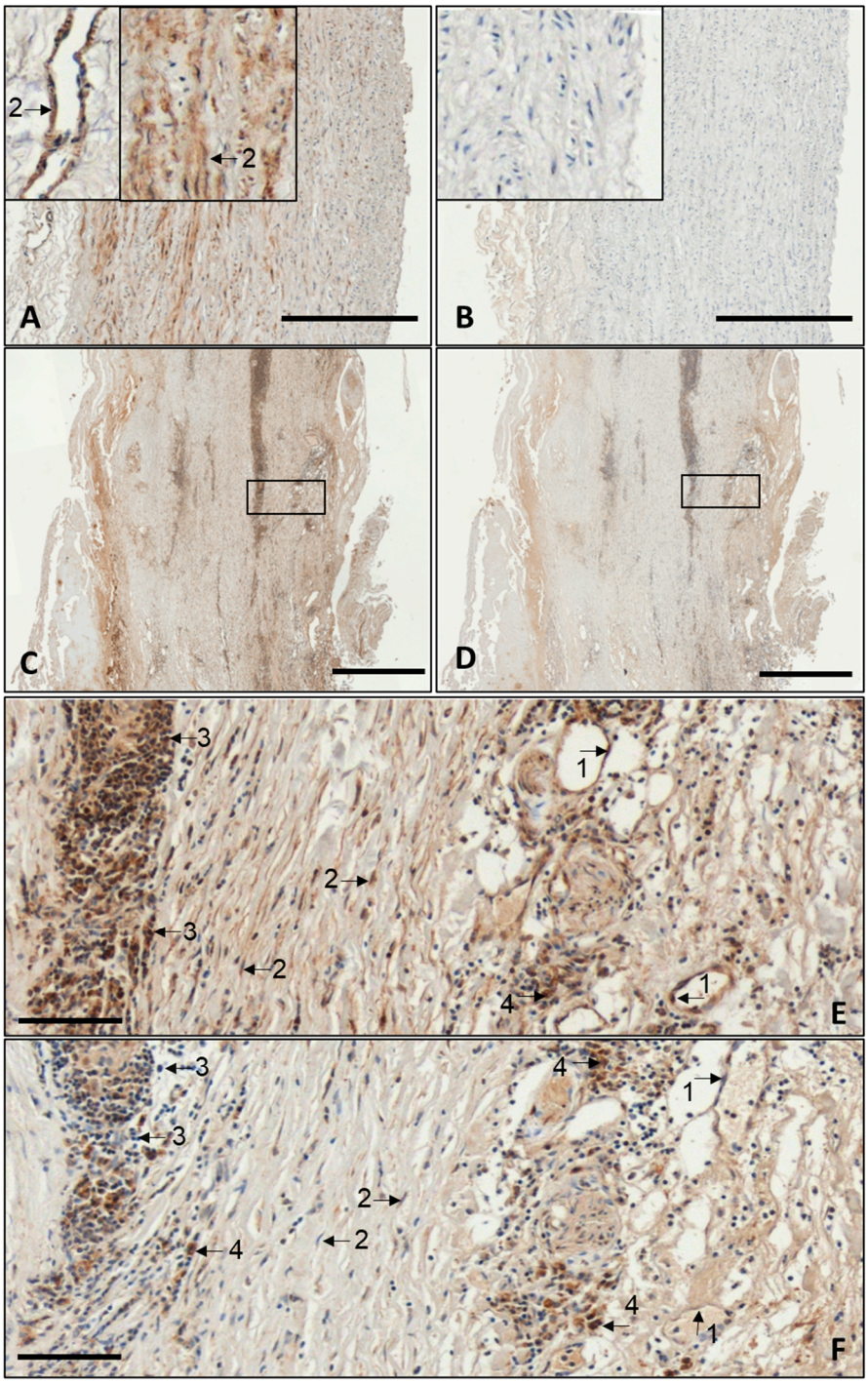

Figure 3. Representative Images of histological analysis of NOCR1 and 2 in human AAA tissue samples and aortic controls (Ctrl). The images A, B, C, and D show overview stains of control aorta with NCOR1 (A) and NCOR2 (B), as well as AAA tissue stained with NCOR1 (C) and NCOR2 (D). Images E and F provide more detailed insight into the expression of NCOR1 and NCOR2 in individual cells within the AAA wall: (E) NCOR1; (F) NCOR2. Arrows:

 doi:10.20944/preprints202603.0777.v1

endothelial cells (ECs) (1), smooth muscle cells (SMCs) (2), leukocytes CD45 (3), and macrophages (CD68) (4). Scale bars represent 500 μm on figures A-D, 100 μm on figures E and F. AAA: abdominal aortic aneurysm.

3.5. Correlation Analysis of NCOR1 and NCOR2 with Factors Associated with AAA

Since IHC cannot capture all cellular features within AAA, correlation analyses of NCOR1 and NCOR2 expression with known markers of ECs, SMCs, immune cells, and neovascularisation were conducted (Tables 2 and 3). Additionally, correlation analyses were performed for other aneurysm-associated factors, including proteolytic enzymes, epigenetic factors, and lncRNAs (Tables 3 and 4). As already mentioned in the Materials and Methods section, to minimise potential bias and false-positive or false-negative correlations, Benjamini-Hochberg correction for multiple tests and adjustment for age and sex were performed.

Association with smooth muscle cell markers. The NCOR1 expression in healthy aortas was positively correlated with SMTN, a contractile marker of SMCs (r = 0.618, P < 0.05), as well as with the expression of some extracellular matrix components such as FN1, FBN1, and the factor PDGFRB (r = 0.519, 0.581, 0.616, P < 0.05) (Table 2), along with collagen COL1A1 and COL3A1 (r = 0.496 and 0.542, P < 0.05). Conversely, in AAA tissue samples, NCOR1 exhibited significant inverse correlations with multiple SMC genes, including both contractile and synthetic phenotypes (ACTA2, SMTN, FN1, COL1A1, COL3A1, FBN1, and PDGFRB; r = -0.373 to -0.572, P < 0.05, 0.01, and < 0.001) (Table 2). Interestingly, NCOR2 did not show any significant associations with SMC markers in either healthy aortas or AAA tissue, suggesting a potential lack of its effect in SMCs compared to NCOR1.

Table 2. Correlations between NCOR1 and 2 and various markers of SMCs and ECs.

	contractile SMCs		synthetic SMCs				
	ACTA2	**SMTN**	**FN1**	**COL1A1**	**COL3A1**	**FBN1**	**PDGFRB**
	non-aneurysmal healthy aorta (Ctrl)						
NCOR1	n.s.	0.618*	0.581*	0.542*	0.496*	0.519*	0.616*
NCOR2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	abdominal aortic aneurysm (AAA)						
NCOR1	-0.418*	-0.373*	-0.447**	-0.572***	-0.547***	-0.433*	-0.504***
NCOR2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

	ECs		Neovascularisation		
	CDH5	**VCAM1**	**VEGFB**	**TIE1**	**ANG**
	non-aneurysmal healthy aorta (Ctrl)				
NCOR1	n.s.	n.s.	n.s.	n.s.	n.s.
NCOR2	n.s.	n.s.	n.s.	n.s.	n.s.
	abdominal aortic aneurysm (AAA)				
NCOR1	-0.340*	n.s.	n.s.	-0.349*	-0.367*
NCOR2	n.s.	-0.464***	0.660***	n.s.	n.s.

Spearman correlation coefficient. Benjamini-Hochberg (B-H) correction for multiple variables and adjustment for sex and age were applied to determine statistical significance: * P < 0.05, ** P < 0.01, *** P < 0.001; n.s. not significant. ECs: endothelial cells, SMCs: smooth muscle cells; NCOR1 and 2: nuclear receptor co-repressor 1 and 2; ACTA2: alpha-smooth muscle actin, SMTN: smoothelin, FN1: fibronectin, COL1A1: collagen 1, COL3A1: collagen 3, FBN1: fibrillin-1, PDGFRB: platelet-derived growth factor receptor beta, CDH5: VE-cadherin, VCAM1: vascular cell adhesion molecule 1, TIE1: tyrosine kinase with immunoglobulin-like domain 1, VEGF: vascular endothelial growth factor, ANG: angiopoietin.

Association with endothelial markers. Neither NCOR1 nor NCOR2 expression correlated with endothelial cell (EC) markers in intact healthy aorta (Table 2). In contrast, in AAA tissue samples, NCOR1 showed inverse correlation with several EC- and angiogenesis-related genes. Notably, higher NCOR1 expression was associated with lower CDH5 (VE-cadherin) (r = -0.34, P < 0.05), as well as reduced expression of the angiogenic factor TIE1 (r = -0.349, P < 0.05) and ANG (r = -0.367, P < 0.05). This suggests that NCOR1 may be downregulated in endothelial- and neovessel-rich areas of AAA.

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NCOR2 showed a different pattern, being strongly negatively associated with the expression of the EC-marker VCAM1 (r = -0.464, P < 0.001). Furthermore, NCOR2 correlated positively with VEGFB (r = 0.660, P < 0.001), indicating that the effects of NCOR1 and NCOR2 differ in neovascularisation.

Association with immune cell markers. In AAA tissue samples, NCOR1 expression showed significant positive correlations with certain T-lymphocyte markers (Table 3). Specifically, NCOR1 correlated with CD3D, CD3E, CD3G, and CD247 (r = 0.325 to 0.412, P < 0.05). NCOR1 also weakly correlated with the T-cell chemokine CXCL9 (r = 0.384, P < 0.05), indicating a link between NCOR1 and T-cells. Additionally, NCOR1 exhibited a strong positive correlation with the apoptotic caspase CASP8 (r = 0.555, P < 0.001). In contrast, NCOR2 showed a more limited immune profile, with no significant associations with T-cell markers. Instead, NCOR2 was positively correlated with NFκB2 (r = 0.519, P < 0.01) and TGFB1 (r = 0.632, P < 0.001). Notably, NCOR2 was significantly inversely associated with CXCL10 (r = -0.505, P < 0.01).

Table 3. Correlations between NCOR1/2 and various markers of inflammation and ECM degradation in AAA.

	Inflammatory cells								Apoptosis
	CD3D	**CD3E**	**CD3G**	**CD247**	**NFKB2**	**TGFB1**	**CXCL9**	**CXCL10**	**CASP8**
NCOR1	0.325*	0.371*	0.412*	0.375*	n.s.	n.s.	0.384*	n.s.	0.555***
NCOR2	n.s.	n.s.	n.s.	n.s.	0.519**	0.632***	n.s.	-0.505**	n.s.

	Proteolytic enzymes / ECM degradation				
	MMP2	**MMP14**	**ADAM15**	**ADAMTS2**	**ADAMTS7**
NCOR1	-0.445**	-0.649***	-0.417*	-0.473**	-0.499**
NCOR2	n.s.	n.s.	n.s.	0.441**	0.476**

Spearman correlation coefficient. Benjamini-Hochberg (B-H) correction for multiple variables and adjustment for sex and age were applied to determine statistical significance: * P < 0.05, ** P < 0.01, *** P < 0.001; n.s. not significant. NCOR1 and 2: nuclear receptor co-repressor 1 and 2, CD3D, CD3E, CD3G, CD247: T-cell surface glycoproteins, NFKB2 : Nuclear factor NF-kappa-B p100 subunit, TGFB1: Transforming growth factor beta 1, CXCL9 and 10: Chemokine (C-X-C motif) ligand 9 and 10, CASP8: Apoptosis marker caspase 8, MMP: matrix metalloproteinase, ADAM: A disintegrin and metalloproteinase, ADAMTS: A disintegrin and metalloproteinase with thrombospondin motifs.

Association with ECM degradation enzymes. Striking differences were also observed in how NCOR1 and NCOR2 relate to proteolytic enzymes in AAA. NCOR1 was significantly inversely correlated with many matrix-degrading proteases (Table 3). Higher NCOR1 expression was significantly associated with lower levels of MMP2 (r = -0.445, P < 0.01) and MMP14 (MT1-MMP; r = -0.649, P < 0.001), key metallo-proteinases that drive elastic and collagen fibre degradation. Similarly, NCOR1 showed significant negative correlations with ADAM15 (r = -0.417, P < 0.05), and ADAMTS family proteases involved in collagen and elastin cleavage, including ADAMTS2 (r = -0.473, P < 0.01), and ADAMTS7 (r = -0.499, P < 0.01). In contrast, NCOR2 showed positive correlations with ADAMTS2 (r = 0.441, P < 0.01) and ADAMTS7 (r = 0.476, P < 0.01), suggesting a link to reparative vascular remodelling.

Associations with epigenetic factors. AAA tissues showed numerous intriguing correlations between NCOR1/2 and many epigenome-regulating genes (Table 4). For example, NCOR1 levels were significantly associated with several histone methyltransferases (HMTs), including KMT2A, KMT2C, EHMT1, EZH1, EZH2, and KMT3, as well as numerous histone demethylases (HDMs), including KDM2A/B, KDM3B, KDM4A/C, KDM5A/D, and KDM8A. Similarly, NCOR1 was significantly positively associated with several histone acetyltransferases, including KAT6A/B and KAT8, as well as histone deacetylases HDAC1 and HDAC6. Moreover, NCOR1 also showed a positive correlation with DNA methyltransferase DNMT1. These widespread positive correlations indicate a strong link between NCOR1 and epigenetic enzymes in AAA.

In contrast to NCOR1, NCOR2 exhibited an almost reciprocal pattern for many epigenetic factors (Table 4). NCOR2 expression was positively correlated with histone methyltransferases (HMTs)

 doi:10.20944/preprints202603.0777.v1

similar to NCOR1, but interestingly, with different ones (KMT2D, SETD1A/B, and KMT5C). Surprisingly, histone demethylases (HDMs) showed negative correlations with NCOR2, KDM1B, KDM3A, KDM4C, KDM5A, and KDM6A. Regarding histone deacetylases (HDACs), the correlations were positive for HDAC4, 5, and 7, and negative for HDAC2 (Table 4). Interestingly, unlike NCOR1, NCOR2 showed a significant correlation with the DNA methyltransferase DNMT3A. Additionally, mixed results were observed for the sirtuin deacetylases, another important class of epigenetic regulators. While NCOR1 showed no correlations with sirtuins, NCOR2 exhibited a negative association with SIRT1 and a significant positive association with SIRT2 and SIRT6.

These data suggest that in AAA, NCOR1 and NCOR2 are important epigenetic modulators of disease progression, operating, however, within different epigenetic contexts.

Table 4. Correlations between NCOR1 and 2 and various markers of epigenetics and lncRNAs in AAA.

	HMTs								
	KMT2A	**KMT2C**	**KMT2D**	**SETD1A**	**SETD1B**	**EHMT1**	**EZH2**	**KMT3A**	**KMT5C**
NCOR1	0.486**	0.525**	n.s.	n.s.	n.s.	0.468**	0.465**	0.537**	n.s.
NCOR2	n.s.	n.s.	0.642***	0.628***	0.653***	n.s.	n.s.	n.s.	0.476**

	HDMs											
	KDM1B	**KDM2A**	**KDM2B**	**KDM3A**	**KDM3B**	**KDM4A**	**KDM4C**	**KDM5A**	**KDM5D**	**KDM6A**	**KDM7A**	**KDM8**
NCOR1	n.s.	0.452**	0.477**	n.s.	0.446**	0.392*	0.555***	0.487**	0.441**	n.s.	n.s.	.532**
NCOR2	-0.447**	n.s.	n.s.	-0.453**	n.s.	n.s.	-0.464**	-0.556***	n.s.	-0.487**	-0.403*	n.s.

	HATs			HDACs					
	KAT6A	**KAT6B**	**KAT8**	**HDAC1**	**HDAC2**	**HDAC4**	**HDAC5**	**HDAC6**	**HDAC7**
NCOR1	0.469**	0.419*	0.401*	0.451**	n.s.	n.s.	n.s.	0.447**	n.s.
NCOR2	n.s.	n.s.	n.s.	n.s.	-0.441**	0.467**	0.709***	n.s.	0.490**

	DNMTs		Sirtuins			lncRNAs		
	DNMT1	**DNMT3A**	**SIRT1**	**SIRT2**	**SIRT6**	**H19**	**MALAT1**	**GAS5**
NCOR1	0.449**	n.s.	n.s.	n.s.	n.s.	n.s.	0.485**	n.s.
NCOR2	n.s.	0.453**	-0.511**	0.452**	0.589***	0.620***	n.s.	-0.437**

Spearman correlation coefficient. Benjamini-Hochberg (B-H) correction for multiple variables and adjustment for sex and age were applied to determine statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; n.s. not significant. NCOR1 and 2: nuclear receptor co-repressor 1 and 2, HMTs: Histone methyltransferases KMT: Lysine (K) histone methyltransferase, HDMs: Histone demethylases, KDM: Lysine (K) histone demethylase, HATs: Histone acetyl transferases, KAT: Lysine (K) histone acetyl transferase; SIRTs: sirtuins, lncRNAs: long non-coding RNAs.

Associations with lncRNAs. The correlation analysis of NCOR1 and NCOR2 with lncRNAs also reflected the differences between these two immunometabolic modulators. NCOR1 expression was strongly positively correlated with the pro-proliferative lncRNA MALAT1 ($r = 0.485$, $P < 0.01$). NCOR2, on the other hand, showed strong positive correlations with H19 ($r = 0.620$, $P < 0.001$) and was negatively associated with the senescence-associated lncRNA GAS5 ($r = -0.437$, $P < 0.01$).

4. Discussion

Interestingly, in contrast to the previous data from human atherosclerotic carotid lesions [10,15], our transcriptome analyses revealed no significant bulk differences in the expression of NCOR1 or NCOR2 between AAA and control aortas. However, significant differences were observed in the expression of NCOR1 and NCOR2 at the cellular level. While NCOR1 was broadly detectable in AAA across endothelial, smooth muscle, and inflammatory cells, NCOR2 showed a more restricted pattern, being mainly associated with macrophage-rich areas. Correlation profiling linked NCOR1 and NCOR2 to distinct signatures regarding their cellular association, extracellular matrix–remodelling, epigenetics, and lncRNAs, supporting non-redundant roles of both corepressors in AAA.

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Unlike previous observations in human carotid atherosclerotic plaques, where NCOR1 expression was significantly reduced compared to controls [10,15], our transcriptomic data showed no significant difference in NCOR1 or NCOR2 expression between AAA and healthy aorta. In this context, we suggest that disease-specific factors differentiate AAA from atherosclerosis. In atherosclerotic lesions, NCOR1 was linked to macrophages and foam cell-rich plaques, thereby reducing lipid uptake pathways [10]. However, whereas carotid atherosclerosis is an intimal disease, in AAA, the pathology extends through all aortic layers with extensive matrix remodelling rather than forming an intimal lipid-rich core [23,27,39]. Moreover, although macrophages are present in AAA tissue, our previous study [26] demonstrated that lymphocytes, including B- and T-cells, predominate among the inflammatory infiltrates, whereas advanced atherosclerotic plaques are usually characterised by macrophage-rich lesions [15,39,40]. Our findings suggest that in AAA, downregulation of NCOR1 in one cell type (e.g., dedifferentiating SMCs) might be countered by upregulation in other cell types (e.g., inflammatory cells), resulting in cell-specific but no overall changes when analysing the whole tissue. This assumption was confirmed by a recent study using a mouse AAA model, finding that NCOR1 expression can be increased in aneurysmal aortas as a compensatory response [9]. Therefore, the absence of significant bulk mRNA changes between AAA and healthy aorta does not imply that NCOR is not involved in AAA. Instead, it highlights that their regulation in AAA is highly context- and cell-type-dependent.

Despite minimal transcriptional differences, distinct localisation patterns of NCOR1 and NCOR2 were observed at the protein level. NCOR1 shows broad cell-type expression in AAA, whereas NCOR2 appears to be mainly expressed in immune cells, such as macrophages. These findings align with previous mechanistic studies emphasising cell-type specificity in NCOR utilisation [5]. A prior study demonstrated that SMC-specific NCOR1 is vital for maintaining the contractile phenotype and vessel wall integrity [9]. Conversely, NCOR2 was not detected in SMCs of AAA lesions. Instead, the prominent presence of NCOR2 in macrophages supports its established role as an immunomodulatory corepressor in myeloid cells [10–12].

The extended correlation analyses discussed further below offer further insight into the possible role of NCOR1 and NCOR2 in the pathogenesis of AAA. However, it is important to mention that even after correcting for multiple testing and adjusting for age and sex, our interpretations remain speculative, as correlation does not prove causation.

NCOR1 expression showed positive correlations with multiple SMC markers, including SMTN, as well as with various extracellular matrix (ECM) components produced by these cells. These data indicate that in the healthy aortic wall, higher NCOR1 levels are associated with a contractile phenotype and increased synthesis of vessel wall stabilising structural proteins [9]. In contrast, NCOR1 expression in AAA was inversely correlated with many SMC-related genes, spanning both contractile markers (ACTA2, SMTN) and synthetic/ECM genes (FN1, COL1A1, COL3A1, FBN1), implying that SMCs within the aneurysm wall have lost much of their NCOR1 expression. Du et al. reported that loss of NCOR1 drives SMCs towards a “de-differentiated” state with heightened MMP production and AAA formation [9]. Thus, our human data suggest that SMCs in aneurysms exhibit reduced NCOR1 expression, consistent with NCOR1 downregulation in these cells as a feature of pathological remodelling. The opposing trends between healthy and aneurysmal tissue underscore a redistribution of NCOR1 away from SMCs during AAA development, potentially contributing to SMC dysfunction. These results are supported by the downregulation of PDGFRB along with NCOR1 in SMCs. PDGFRB reflects active phenotypic switching and loss of contractile stability [9,24]. In contrast to NCOR1, NCOR2 showed no association with SMC signatures in either healthy or diseased aorta.

AAA tissues are characterised by extensive neovascularisation. In the intact healthy aorta, neither NCOR1 nor NCOR2 showed any notable correlation with EC or angiogenic markers. However, in AAA samples, NCOR1 expression became inversely correlated with VE-cadherin (CDH5) and with reduced levels of *TIE1* receptor and angiopoietin. These findings are in line with recent mechanistic work demonstrating that NCOR1 can impact angiogenesis [14]. Our data suggest

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that in AAA, NCOR1 expression is locally suppressed to allow neovascularisation. In contrast, NCOR2 levels correlated positively with VEGFB, suggesting its role in maintaining the existing vasculature. Again, these data confirm the distinct roles of NCOR1 and NCOR2 in vascular tissue.

Chronic inflammation is a key factor in AAA development [23,27], and our analysis shows that NCOR1 and NCOR2 are associated with distinct immune cell signatures. In aneurysmal tissues, NCOR1 expression exhibited significant positive correlations with various T-lymphocyte markers and cytokine signalling. These findings are unexpected, given that NCOR1 was reported to suppress inflammatory genes in macrophages [10,12,41]. However, in atherosclerosis, where macrophages predominate, AAA contains more T-cells [26]. Furthermore, it has been reported that in resting macrophages, NCOR1 represses certain pro-inflammatory genes, and its loss leads to hyperactivation of those pathways [42]. We also observed a strong positive correlation between NCOR1 and CASP8, an apoptotic caspase, suggesting that NCOR1 may also be involved in active cell death. These findings imply that NCOR1, while generally anti-inflammatory at the cellular level, may be mobilised within areas of intense immune activation to limit tissue damage [10]. In contrast to NCOR1, NCOR2 showed a more selective and inverse relationship with immune markers. NCOR2 exhibited a strong positive correlation with TGF-β1 and NF-κB. TGF-β1 has already been described to be upregulated in AAA during tissue remodelling [43]. The strong link between NCOR2 and TGFβ1 suggests that NCOR2 expression may be associated with immunomodulation and tissue repair. The positive correlation between NCOR2 and NF-κB2 might indicate activation of the non-canonical NF-κB pathway. Interestingly, NCOR2 was inversely correlated with the chemokine CXCL10, which promotes Th1-type T cell trafficking [44]. This aligns with the known role of NCOR2 (SMRT) in restraining full transcriptional activation of pro-inflammatory pathways [1,12,20]. Collectively, these immune-correlation patterns suggest that NCOR1 and NCOR2 operate in distinct inflammatory contexts within AAA. Whereas NCOR1 seems to be linked to an active inflammatory state, such as T-cell infiltration and cell apoptosis [4,10,15], NCOR2 appears to be associated with tissue remodelling [1].

Further correlation analyses indicated that NCOR1 and NCOR2 are also linked to ECM degradation, again in opposite ways. NCOR1 expression significantly negatively correlated with various proteolytic enzymes (MMP2 and 14, ADAM15, ADAMTS2, and 7). These correlations imply that higher levels of NCOR1 expression protect the ECM from proteolysis and help maintain aortic wall stability [9]. In contrast, NCOR2 showed positive correlations with ADAMTS2 and ADAMTS7. ADAMTS2 is a procollagen peptidase that assists in collagen maturation [45], whereas ADAMTS7 has been linked to SMC migration and matrix remodelling in atherosclerosis [46]. The results suggest that NCOR2 might promote specific proteolytic activity to enhance targeted vascular remodelling.

As transcriptional corepressors, NCOR1 and NCOR2 play a crucial role in the epigenetic regulation of gene expression [3,4,7,15]. This was also confirmed by our correlation analysis. Interestingly, in the AAA tissue, both NCOR1 and NCOR2 were linked to different epigenetic modifiers. NCOR1 expression showed positive correlation with a wide range of epigenetic regulators, including HMTs, HDMs, HATs, and HDACs. Additionally, NCOR1 levels were associated with increased expression of the DNA methyltransferase DNMT1. These findings imply a significant role for NCOR1 in chromatin reprogramming at multiple levels. It is notable that both methyltransferases and demethylases, as well as histone acetyltransferases and deacetylases, were positively associated with NCOR1 expression. This suggests that NCOR1 is active in various transcriptional reprogramming processes, such as e.g. SMC phenotype switching or activation/repression of specific inflammatory cytokines or cells [9]. The positive correlations may reflect a stress response programme triggered by injury or inflammation within the diseased aortic wall. Conversely, NCOR2 showed a distinct, more selective set of epigenetic associations. While NCOR2 also correlated positively with HMTs, these involved different enzymes from those linked to NCOR1. Moreover, NCOR2 was negatively associated with HDMs. These results suggest that NCOR2 promotes histone methylation while suppressing demethylation, thus maintaining gene silencing [1]. Regarding HATs, no correlation with NCOR2 was observed. In contrast, the correlation with HDACs was largely

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positive, similar to NCOR1, but involved different deacetylases. This data indicates that the epigenetic influence of NCOR2 differs from that of NCOR1. Furthermore, as previously described, NCOR2 appears to cooperate with NCOR1 to support and specify its epigenetic effects [12,20]. Additionally, like NCOR1, NCOR2 was also associated with certain sirtuins (SIRT1, 2, and 6). Sirtuins are a specific class of histone deacetylases. Unlike classical HDACs and histone acetyltransferases, which mainly regulate acetylation in an ATP-dependent manner, sirtuins require NAD+ as a cofactor, linking their activity tightly to redox balance, energy availability, and stress signalling [47,48]. This metabolic dependence positions sirtuins critically within ageing pathways and immunometabolic regulation. In summary, NCOR1/2 expression in AAA seems to have a broad epigenetic reprogramming signature, reflecting its engagement with a wide network of chromatin modifiers during disease progression.

Long non-coding RNAs (lncRNAs) have become essential regulators in cardiovascular diseases, including AAA [16,19,28,30]. They influence chromatin organisation, transcription factor activity, and gene expression profiles. Our study identified distinct correlations between NCOR1/2 and specific lncRNAs, suggesting that these corepressors may also interact with different lncRNA-regulated networks in AAA. NCOR1 expression was strongly positively correlated with MALAT1, while NCOR2 was positively correlated with H19 but negatively with GAS5. These varying lncRNA associations provide further evidence that NCOR1 and NCOR2 operate in separate regulatory niches. MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1) has been linked to promoting EC proliferation, angiogenesis, and SMC migration [18,49]. H19 is highly upregulated in both human and experimental AAAs and has been shown to drive aneurysm progression [29,49]. Our data indicate that high NCOR2 expression is associated with elevated H19 levels. One possible link could involve the SMC phenotype, as H19 has been associated with SMC apoptosis and loss in AAA [29]. It is also plausible that H19 influence NCOR2 expression. GAS5 (Growth Arrest Specific 5) inversely correlates with NCOR2 and has been reported to promote AAA and SMC apoptosis [32]. Lower GAS5 levels in areas with high NCOR2 expression suggest that cells in those areas may be active or proliferating. This aligns with the observation that NCOR2 is present in macrophages engaged in remodelling. In summary, the correlations involving lncRNAs reinforce the assumption that NCOR1/2 interact with distinct regulatory networks in AAA, involving SMC physiology, immune response, and epigenetics. However, the findings suggest that these lncRNAs may also modulate NCOR complexes and consequently influence their expression.

Our findings have several potential implications for a therapeutic approach in AAA and related vascular diseases. First, the data highlight NCOR1 as a potential protective factor in the vessel wall, particularly by supporting SMC stability and suppressing destructive processes. Increasing NCOR1 activity in SMCs might offer a new strategy to reinforce the aortic wall against aneurysmal dilatation. Agonists or modulators that promote NCOR1 recruitment could indirectly enhance its positive effect in AAA pathophysiology [10]. Conversely, NCOR2 could be targeted to reduce immune-driven damage in AAA. Moreover, modulating NCOR1/2 or their key upstream/downstream partners, which are involved in essential metabolic processes, could provide multiple benefits by decreasing inflammation, improving SMC function, and restoring the epigenetic state of the diseased aorta [5,15]. Consequently, NCOR1 and NCOR2 are promising new therapeutic targets in AAA, aiming to slow aneurysm progression and strengthen the aortic wall.

Study limitations. This study has several notable limitations. First, the sample size and heterogeneity. While we analysed a reasonably large cohort (45 AAA and 18 controls), human AAA tissues are highly variable in composition, which can confound transcriptomic analyses. Furthermore, we did not see any differences between eAAA and rAAA. To properly compare these two disease conditions, the tissue samples of patients with ruptured AAA had to be removed at the site of rupture. This, however, is often not possible, and excision farther away from the ruptured site might not truly reflect the aortic wall most prone to rupture. Regarding the correlation analyses, some observed associations might be influenced by outlier samples or specific sub-regions of the aneurysm wall. Furthermore, the study is observational by design. Therefore, we cannot establish causality

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from the ascertained correlations. The associations between NCORs and other factors contributing to AAA do not demonstrate that they directly regulate these factors, or vice versa. As a result, our interpretations are speculative, as correlation does not imply causation. Nonetheless, we have provided potential explanations and relevant literature supporting our assumptions. Therefore, the associations between NCOR1, NCOR2, and various cellular, inflammatory, matrix remodelling, epigenetic, and lncRNA factors are plausible, even if not proven. In addition, control aorta samples were obtained from organ donors of a younger age. The significant age difference between controls and AAA patients could be a potential confounder. It is here worth mentioning that obtaining a healthy aorta of a matched AAA cohort of 70 years on average is almost impossible. Therefore, the findings of our current work should be regarded as hypothesis-generating.

Conclusions. Despite the above-described limitations, our study provides, for the first time, a comprehensive view of NCOR1 and NCOR2 in human AAA, revealing distinct expression patterns, cellular localisations, and molecular associations that suggest non-redundant roles in the underlying disease. Our data align with findings from related vascular pathologies but also raise new questions, particularly about NCOR2's function in AAA. Therefore, more nuanced approaches, such as spatial transcriptomics or single-cell RNA sequencing, are needed to assess NCOR1/2 activity in specific cell subpopulations within AAA. Clarifying these roles could lead to new diagnostic markers and innovative therapeutic targets for AAA and possibly other chronic vascular diseases.

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Abbreviations

NCOR1: Nuclear receptor corepressor 1; NCOR2: Nuclear receptor corepressor 2; SMRT: silencing mediator of retinoid and thyroid receptor; AAA: abdominal aortic aneurysm; eAAA: elective AAA; rAAA: ruptured AAA; lncRNA: long non-coding RNA; EC: endothelial cell; SMC: smooth muscle cell; ECM: extracellular matrix; SVB: Swiss Vascular Biobank; FFPE: formalin-fixed-in-paraffin-embedded; HE: Haematoxylin-Eosin staining; EvG: Elastica van Gieson staining; IHC: immunohistochemistry; RIN: RNA integration number (stage of DNA degradation; FGCZ: Functional Genomic Center Zurich; FDR: false discovery rate; CD45: Cellular marker of T-cells; CD68: Cellular marker of macrophages.

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