



RESEARCH ARTICLE

REVISED Increased abundance of ADAM9 transcripts in the blood is associated with tissue damage [version 2; referees: 3 approved]

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Abstract

Background: Members of the ADAM (a disintegrin and metalloprotease domain) family have emerged as critical regulators of cell-cell signaling during development and homeostasis. ADAM9 is consistently overexpressed in various human cancers, and has been shown to play an important role in tumorigenesis. However, little is known about the involvement of ADAM9 during immune-mediated processes.

Results: Mining of an extensive compendium of transcriptomic datasets identified important gaps in knowledge regarding the possible role of ADAM9 in immunological homeostasis and inflammation: 1) The abundance of ADAM9 transcripts in the blood was increased in patients with acute infection but, 2) changed very little after *in vitro* exposure to a wide range of pathogen-associated molecular patterns (PAMPs). 3) Furthermore it was found to increase significantly in subjects as a result of tissue injury or tissue remodeling, in absence of infectious processes.

Conclusions: Our findings indicate that ADAM9 may constitute a valuable biomarker for the assessment of tissue damage, especially in clinical situations where other inflammatory markers are confounded by infectious processes.



This article is included in the Sidra Research channel.



This article is included in the Collective Data Discovery channel.

Open Peer Review

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REVISED Amendments from Version 1

In response to the reviewers we added background information at the beginning of the introduction section to present the rationale behind the data mining approach that was employed as well as the purpose of diagrams that were integrated to the figures. These in essence constitute “graphical legends” and allow presentation of the data in a semi-structured format, thus diagrams were moved accordingly below the plots in each figure. We also re-plotted the result of [Figure 1](#), retaining only neutrophil and monocyte data plot as per the reviewers’ suggestions. Additional data have been plotted as requested by the reviewers. We have also analyzed association of abundance of ADAM9 with degree of severity in trauma patients (GSE11375: [Figure 5](#)) and viral infections (GSE34205/GSE38900; Conclusions); and also added datasets generated in experimental models of injury *in vitro* and *in vivo* in human and mice ([Supplementary Figure 6](#)) to further document the involvement of ADAM9 in tissue inflammation/injury. Finally we have also updated the title of this article.

See referee reports

Introduction

Over the recent years “deep” molecular phenotyping technologies have become widely available to biomedical researchers. As a consequence collections of large-scale datasets held in public repositories are rapidly expanding. For instance, GEO, the NCBI Gene Expression Omnibus, is comprised of over 70,000 transcriptome data series, representing over 1.8 million individual profiles¹. Altogether publically available molecular and cellular phenotyping data of all types constitute the biomedical research community’s “collective data”. Collective data can and should be exploited not only by researchers who have acquired valuable background in quantitative sciences, but also by “mainstream” life scientists, whose research can also greatly benefit from this vast resource. A unique global perspective can for instance simply be gained from examining the abundance of a single analyte across tens or hundreds of “omics” studies. In this report potential gaps in knowledge pertaining to the role of the ADAM9 were investigated through interpretation of changes in abundance of ADAM9 RNA across public transcriptome datasets relevant to human immunology.

“ADAM metallopeptidase 9 (ADAM9) is a member of the ADAM (a disintegrin and metalloprotease domain) family. Members of this family are membrane-anchored proteins structurally related to snake venom disintegrins, and have been implicated in a variety of biological processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. The protein encoded by this gene interacts with SH3 domain-containing proteins, binds mitotic arrest deficient 2 beta protein, and is also involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. Several alternatively spliced transcript variants have been identified for this gene.” (Quoted from RefSeq²).

ADAM9 top functions include cellular adhesion, protein cleavage and shedding. ([Supplementary Figure 1](#)). Human ADAM9 protein cleaves and releases collagen XVII from the surface of skin keratinocytes³. This activity is enhanced in the presence of reactive oxygen species. Mouse ADAM9 protein cleaves and releases

epidermal growth factor (EGF) and fibroblast growth factor receptor 2IIIb (FGFR2IIIb) from the surface of prostate epithelial cells⁴. Following LPS treatment, ADAM9 protein catalytic domain cleaves Angiotensin-I converting enzyme (ACE) from the surface of endothelial cells⁵. Human ADAM9 protein disintegrin-cysteine-rich domain binds integrins and thus mediates cell adhesion⁶. Human ADAM9 protein enhances adhesion and invasion of non-small lung tumors which mediates tumor metastasis⁷. Mouse ADAM9 protein enhances tissue plasminogen activator (TPA)-mediated cleavage of CUB domain-containing protein 1 (CDCP1)⁸. This activity mediates lung tumor metastasis. Human ADAM9 protein mediates cell-cell contact interaction between stromal fibroblasts and melanoma cells at the tumor-stroma border, thus contributing to proteolytic activities required during invasion of melanoma cells⁹.

ADAM9 expression and regulation. ADAM9 has been reported as being expressed in various cell populations including monocytes¹⁰, activated macrophages¹¹, epithelial cells, activated vascular smooth muscle cells, fibroblasts⁹, keratinocytes and tumor cells. The abundance of ADAM9 RNA measured by RT-PCR is decreased *in vitro* in human melanoma cells after culture with collagen type I or with Interleukin 1 alpha (IL1 α) compared to mock stimulated conditions¹².

ADAM9 has been involved in disease processes including cancer, cone rod dystrophy and atherosclerosis. Homozygous mutation of the human ADAM9 gene results in severe cone rod dystrophy and cataract¹³. Mutation of the mouse ADAM9 gene results in no major abnormalities during development and adult life¹⁴. The abundance of ADAM9 RNA and protein measured by immunostaining and RT-PCR is increased *in vivo* in human prostate tumors compared to normal tissue¹⁵. The abundance of ADAM9 RNA measured by microarray and RT-PCR is increased *in vivo* in human advanced atherosclerotic plaque macrophages compared to normal tissue¹⁶. This increase is predictive of Prostate Specific Antigen (PSA) relapse.

It is known that ADAM9 is upregulated in some tumor cells during pathologic processes and also contributes to the formation of multinucleate giant cells from monocytes and macrophages¹¹. However, little is known about the activities of ADAM9 in regulating physiologic or pathologic processes, especially during acute infection or in response to tissue damage.

Methods

ADAM9 bibliography screening and literature profiling

Existing knowledge pertaining to ADAM9 was retrieved using NCBI’s National Library of Medicine’s Pubmed search engine with a query that included official gene symbol and name as well as aliases: “ADAM9 OR ADAM-9 OR “ADAM metallopeptidase domain 9” OR MCMP OR MDC9 OR CORD9”. As of January of 2015, 287 papers were returned when running this query. By reviewing this literature keywords were identified that were classified under six categories corresponding to cell types, diseases, functions, tissues, molecules or processes. Frequencies of these keywords were then determined for the ADAM9 bibliography as shown in [Supplementary Figure 1](#). This literature screen identified and prioritized existing knowledge about the gene ADAM9 and was used to prepare the background section of this manuscript and

provided the necessary perspective for the interpretation of ADAM9 profiles across other large-scale datasets.

Interactive data browsing application

We employed a resource that is described in details¹⁷ and is available publicly: <https://gxb.benaroyaresearch.org/dm3/landing.gsp>. Briefly: we have assembled and curated a collection of 172 datasets that are relevant to human immunology, representing a total of 12,886 unique transcriptome profiles. These sets were selected among studies currently available in NCBI's Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>).

The custom software interface provides the user with a means to easily navigate and filter the compendium of available datasets (<https://gxb.benaroyaresearch.org/dm3/geneBrowser/list>)^{17,18}. Datasets of interest can be quickly identified either by filtering on criteria from pre-defined lists on the left or by entering a query term in the search box at the top of the dataset navigation page, we also provided the GXB tutorial in YouTube video; <https://www.youtube.com/playlist?list=PLtx3tvflzJ9XkRKUz6ISEJpAhqKyuiCiD>.

Graphical legends

Diagrams have been incorporated within each figure. These have a dual purpose, first they provide readers with a graphical summary of the findings and second constitute an attempt at structuring information for future computational applications. Indeed, an important limitation of communicating biomedical knowledge in the form of research articles is that it consists in unstructured information (free text). This type of information is notoriously difficult to extract by computational means¹⁹. Standardized graphical summaries such as the ones provided in this manuscript constitute structured information that is both human readable and computationally tractable. The need for solutions will become more pressing as the biomedical literature continues to grow exponentially to such scales that it can only be very narrowly apprehended by research investigators. The graphical legends presented here merely serve as proof of concept.

Statistical analyses

All statistical analyses were performed using GraphPad Prism software version 6 (GraphPad Software, San Diego, CA).

Results and discussion

Dataset 1. Raw data of ADAM9 transcripts in blood in response to tissue damage

<http://dx.doi.org/10.5256/f1000research.6241.d138863>

All primary data presented in this manuscript are provided as data files. Detailed legends for each data file can be found in the text file 'Description of GSE datasets'.

Knowledge gap assessment

The seminal discovery was made while examining RNAseq transcriptional profiles. A knowledge gap was exposed when those results were interpreted in light of existing knowledge reported

in the literature. Next, the initial observation was validated and further extended by examining profiles of the gene of interest, ADAM9, across a large number of independent publically available transcriptome datasets. The completion of these tasks was aided by a custom data browsing application loaded with a curated compendium of 172 datasets relevant to human immunology sourced from the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) (<https://gxb.benaroyaresearch.org/dm3/landing.gsp>)¹⁷. Briefly, ADAM9 transcript was identified as a potential early stage discovery while browsing RNA-sequencing profiles of blood leukocyte populations (<https://gxb.benaroyaresearch.org/dm3/geneBrowser/show/396>), with the genes being ranked in alphabetical order. In this particular dataset whole blood sample of healthy donors, patients during acute infections (meningococcal sepsis, *E. coli* sepsis, *C. difficile* colitis), multiple sclerosis patients pre- and post- interferon treatment, patients with Type 1 diabetes and patients with amyotrophic lateral sclerosis (ALS) were obtained and monocyte, neutrophil, CD4 T cell, CD8 T cells, B cell, NK Cell isolated prior to profiling via RNA sequencing²⁰. The abundance of ADAM9 RNA measured by RNA-seq in human blood neutrophils and monocyte samples from subjects with sepsis was found to be markedly increased as compared to uninfected controls (Figure 1; [iFigure/GSE60424]²⁰). By comparison levels of abundance of ADAM9 RNA in lymphocytes and Natural Killer (NK) cells were low and no changes were observed in subjects with sepsis in these cell populations. Despite the small number of septic subjects included in the study (N=3) the robust increase in abundance that was observed prompted attempts to validate and further extend this initial observation in independent public datasets that were part of the compendium.

The abundance of ADAM9 increases during infection

Our data browsing tool allows the assessment of expression profiles across transcriptome datasets (<https://gxb.benaroyaresearch.org/dm3/geneBrowser/list>). In order to validate and extend our original observation we looked up ADAM9 transcriptome profiles for all available 172 datasets (<https://gxb.benaroyaresearch.org/dm3/geneBrowser/crossProject?probeID=ENSG00000168615&geneSymbol=ADAM9&geneID=8754studies>).

The abundance of ADAM9 RNA measured by microarrays in human blood samples was significantly increased as compared to uninfected controls in subjects with sepsis [iFigure/GSE28750]²¹ & [iFigure/GSE29536]²², in subjects with bacterial and influenza pneumonia [iFigure/GSE34205]²³, [iFigure/GSE40012]²⁴, in subjects with respiratory syncytial virus (RSV) infection [iFigure/GSE34205]²³ & [iFigure/GSE17156]²³ and in subjects with tuberculosis [iFigure/GSE19439]²⁵ & [iFigure/GSE34608]²⁶. Aggregated findings were reported in the form of flow charts that were generated using google docs presentations, with links to the source interactive graphs systematically provided as hyperlinks (Figure 2, Supplementary Figure 2 and Table 1). Altogether these data indicate that increase in abundance of ADAM9 can be detected in blood leukocytes, including monocytes and neutrophils fractions during bacterial and viral infection.

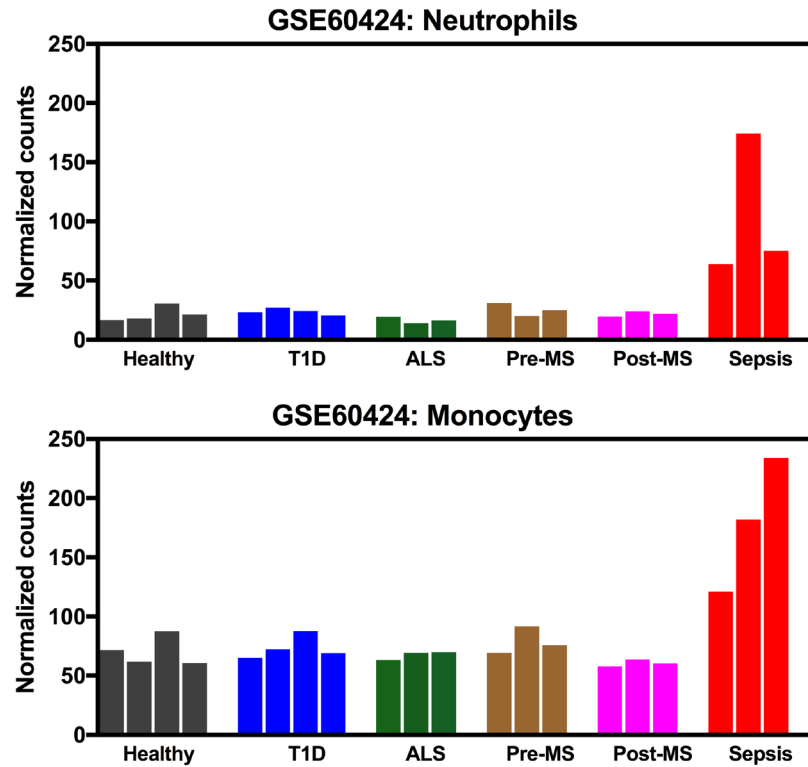


Figure 1. Elevated abundance of ADAM9 transcript in human monocytes and neutrophils during sepsis. The bar graphs show the abundance of ADAM9 transcripts measured by RNA-seq in neutrophils (top) and monocytes (bottom) isolated from blood collected in healthy donors (Black), patients with Type 1 diabetes (Blue), patients with ALS (Green), patients with multiple sclerosis pre- (Pre-MS; Brown) and post- (Post-MS; Pink) interferon treatment and patients with acute infections (sepsis; Red, caused by *Clostridium difficile*, *E. coli* and Meningococcal bacteria, respectively).

The abundance of ADAM9 increases only marginally following treatment with pathogen-associated molecules

Next, we investigated the regulation of ADAM9 transcription following leukocyte exposure to pathogens and pathogen-associated molecules. The abundance of ADAM9 RNA measured by microarrays in human blood cultures treated with Heat Killed *E.coli*, Heat Killed *Staphylococcus aureus* (HKSA) or Heat Killed *Legionella pneumophillum* (HKLP) for 6 hours was increased marginally as compared to unstimulated conditions [iFigure/GSE30101]²⁷. The abundance of ADAM9 RNA measured by microarrays in human blood cultures treated with Heat Killed *Acholeplasma laidlawii* (HKAS), *E. coli* LPS (E-LPS), Flagellin, PAM3, R837, Zymosan, Influenza virus, RSV, CpG, Poly:IC, for 6 hours was not changed as compared to unstimulated conditions (*Ex-vivo*) [iFigure/GSE30101]²⁷; IL8 [iFigure] and CXCL10 [iFigure] serve as positive controls. The abundance of ADAM9 RNA measured by microarrays in human blood samples from subjects treated with poly:IC for 1 day was marginally increased as compared to baseline samples [iFigure/GSE32862]²⁸; CXCL10 [iFigure] serves as a positive control (Figure 3 and

Supplementary Figure 3). Statistical analysis results are shown in Table 2. Taken together, these results showed that the abundance of ADAM9 was not changed or changed only marginally after stimulation with purified molecules bearing Pathogen Associated Molecular Patterns (PAMPs). These finding raised the question as to whether ADAM9 transcription might be activated instead by host-derived Damage-Associated Molecular Pattern molecule (DAMPs)^{29,30}.

The abundance of ADAM9 increases during tissue remodeling

Our dataset screen revealed in addition that changes in abundance of ADAM9 could be associated with tissue remodeling. The abundance of ADAM9 RNA measured by microarrays in human skin biopsy samples of subjects with lepromatous leprosy was significantly increased as compared to controls in subjects with tuberculoid leprosy [iFigure/GSE17763]³¹. The abundance of ADAM9 RNA measured by microarrays in human blood samples was significantly increased as compared to controls in pregnant subjects [iFigure/GSE17449]³². The abundance of ADAM9 RNA

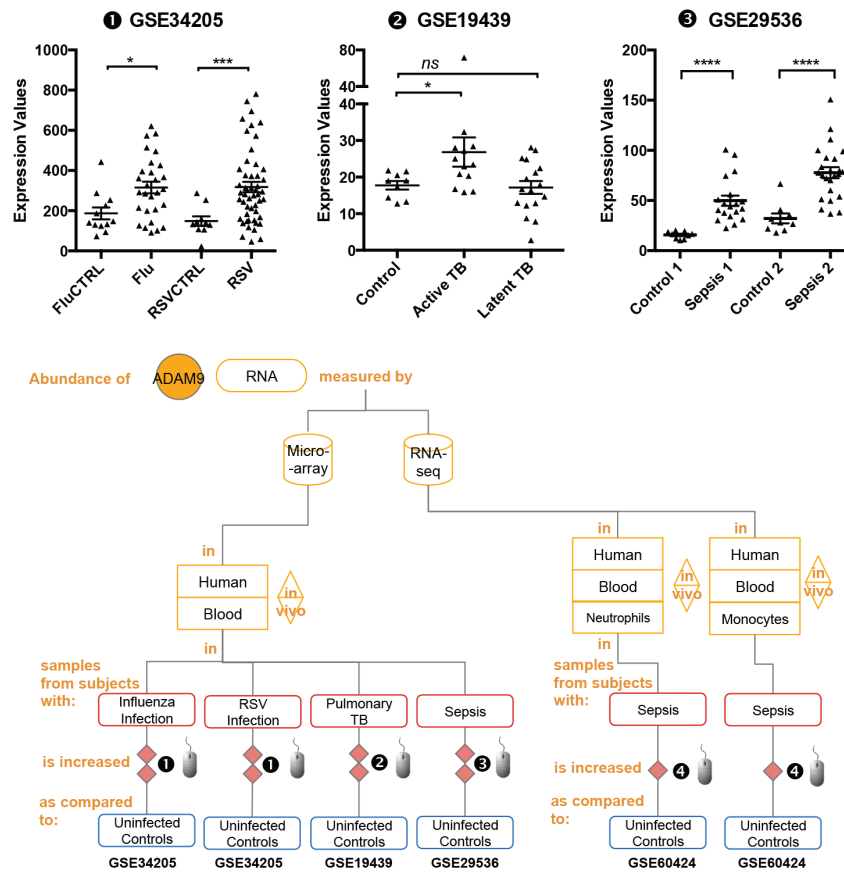


Figure 2. The abundance of ADAM9 increases during infection. mRNA expression levels for ADAM9 was measured by microarrays in whole blood obtained from children hospitalized with acute RSV and influenza virus infection (GSE34205), pulmonary tuberculosis patients (GSE19439) and patients with sepsis (GSE29536).

The graphical legend represents visually the information associated with the public datasets used for the meta-interpretation of ADAM9 transcriptional profiles. The flow chart indicates how data were generated. Diamonds indicate availability of supporting data and in the interactive version are hyperlinked to context-rich interactive plots. Links to these plots are also provided below:

1 GSE34205: In this study gene expression profiles were obtained from the whole blood of critically ill pediatric patients²³. Children hospitalized with acute RSV and influenza virus infection were offered study enrollment after microbiologic confirmation of the diagnosis. Blood samples were collected within 42–72 hours of hospitalization. Median age of subjects was 2.4 months (range 1.5–8.6). Uninfected subjects of similar demographics were recruited in the study and served as age-matched controls. Children with suspected or proven polymicrobial infections, with underlying chronic medical conditions (i.e congenital heart disease, renal insufficiency), with immunodeficiency, or those who received systemic steroids or other immunomodulatory therapies were excluded. As stated in the manuscript: “The Institutional Review Boards at the University of Texas Southwestern Medical Center and Baylor Institute for Immunology Research approved this study, and informed consent was obtained from legal guardians prior to any study-related procedure.” More details are available via the interactive data browsing application under the “study” tab.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Ka>

2 GSE19439: Whole blood was collected from patients with different spectra of tuberculosis (TB) disease and healthy controls²⁵. All patients were sampled prior to the initiation of any anti-mycobacterial therapy. Active Pulmonary TB: all patients confirmed by isolation of *Mycobacterium tuberculosis* on culture of sputum or bronchoalveolar lavage fluid. Latent TB: All patients were positive by tuberculin skin test (>14mm if BCG vaccinated, >5mm if not vaccinated) and were also positive by Interferon-Gamma Release assay (IGRA). As stated in the manuscript: “The local Research Ethics Committees (REC) at St Mary’s Hospital, London, UK approved the study”.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kb>

3 GSE29536: Whole blood was collected from culture positive patients meeting criteria for sepsis enrolled in two independent cohorts (Sepsis 1 and Sepsis 2)²². Uninfected controls recruited in this study were of similar demographics. As stated in the manuscript: “The study was performed by recruitment of patients who were suspected of having hospital or community acquired infection. Clinical specimens were collected for bacterial culture within 24 hours following the diagnosis of sepsis. All blood samples were obtained at the Khon Kaen Regional Hospital, Khon Kaen, Thailand as approved by Khon Kaen University Ethic Committee for Human Research (Project number HE470506)”.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jl>

4 GSE60424: Whole blood sample of healthy donors, patients during acute infections (meningococcal sepsis, *E. coli* sepsis, *C. difficile* colitis), multiple sclerosis patients pre- and 24 hours post- interferon treatment, patients with Type 1 diabetes and patients with ALS were obtained and monocyte, neutrophil, CD4 T cell, CD8 T cells, B cell, NK Cell isolated prior to profiling via RNA sequencing²¹.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kc>

Statistical significance was determined using Mann-Whitney U test. *ns*, not significant, * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$. The horizontal lines indicate mean \pm standard errors (SE).

Table 1. Increased abundance of ADAM9 during infection.

| GEO ID | A vs B | Avg A-Avg B | Avg A/Avg B | P value |
|----------|--------------------------------|-------------|-------------|----------|
| GSE34205 | Influenza vs Influenza CTRL | 129.0 | 1.7 | 0.0144 |
| | RSV vs RSV CTRL | 169.4 | 2.1 | 0.0009 |
| GSE19439 | Active TB vs Control | 9.1 | 1.5 | 0.0169 |
| | Latent TB vs Control | -0.6 | 1.0 | 0.8688 |
| GSE29536 | Sepsis 1 vs Control | 34.1 | 3.2 | < 0.0001 |
| | Sepsis 2 vs Control | 45.6 | 2.4 | < 0.0001 |
| GSE60424 | Sepsis vs Control (Neutrophil) | 82.7 | 4.6 | 0.0380 |
| | Sepsis vs Control (Monocyte) | 108.6 | 2.5 | 0.0121 |

Note : Avg = average abundance of ADAM9 within a given group. Statistical significance was determined using Mann-Whitney U test.

measured by microarrays in human blood monocytes samples from subjects with filariasis was significantly increased as compared to uninfected controls [iFigure/GSE2135]³³. These results are shown in Table 3, Figure 4 and Supplementary Figure 4. A common thread between these different states is that they involve extensive tissue remodeling, whether it involves the skin (leprosy), placental tissue (pregnancy) or lymphatic tissues (filariasis).

The abundance of ADAM9 increases following tissue injury and sterile inflammation

Changes in ADAM9 transcript abundance were observed in additional datasets: The abundance of ADAM9 RNA measured by microarrays in human blood samples was significantly increased as compared to healthy controls in subjects with sarcoidosis [iFigure/GSE34608]²⁶, in subjects after severe blunt trauma [iFigure/GSE11375]³⁴, in subjects with chronic kidney disease [iFigure/GSE15072]³⁵, and in subjects who have undergone elective thoracic or abdominal surgery [iFigure/GSE28750]²¹. Furthermore, we found that the abundance of ADAM9 in trauma patients who did not survive (mean± 2SD; 121.3± 92.98) was significantly higher ($p < 0.05$) than those who survived (mean± 2SD; 90.86± 78.08) GSE11375. The abundance of ADAM9 RNA measured by microarrays in human blood samples from subjects treated with localized external beam radiation therapy for 42 days was significantly increased as compared to baseline samples [iFigure/GSE30174]³⁶. The abundance of ADAM9 RNA measured by microarrays in human blood monocytes samples from obese subjects was significantly increased as compared to lean controls [iFigure/GSE32575]³⁷. Finally, the abundance of ADAM9 RNA measured by microarrays

in human blood monocytes samples from subjects after severe trauma was significantly increased as compared to healthy controls [iFigure/GSE5580]³⁸. These results showed that increase in ADAM9 transcript abundance was associated with tissue injury and sterile inflammation (Table 4, Figure 5 and Supplementary Figure 5) and thus are consistent with the observations that are reported above associating increase in ADAM9 RNA with responses to Damage-Associated Molecular Pattern molecules (DAMPs) and tissue remodeling. Further evidence demonstrating the association of ADAM9 with tissue damage and injury was found in public transcriptome datasets generated by investigators employing mouse *in vivo* models and a human *in vitro* system (supplementary Figure 6): 1) abundance of ADAM9 transcript increased over time at 0, 2 hours, 3 days following thermal injury in a murine dermal burn wound model (GSE460); 2) An epidermal injury model (GSE30355) showed that abundance of ADAM9 was significantly higher in injured epidermis (sorted human keratinocyte (KC)) in comparison to uninjured cells (laser capture microscopy or *in vitro* cultured keratinocytes)³⁹. And 3) abundance of ADAM9 transcripts was increased lungs of C57BL/6 mice that developed acute lung injury after exposure to low-dose LPS and mechanical ventilation (GSE2411) in an *in vivo* model of lung inflammation and injury (GSE2411)⁴⁰.

Conclusions

This study is the first report describing the modulation of levels of ADAM9 transcripts in human whole blood and showing restriction of its expression to neutrophils and monocytes. In addition we observed that the abundance of ADAM9 was increased during acute infection but did not change after stimulation with pathogen-derived

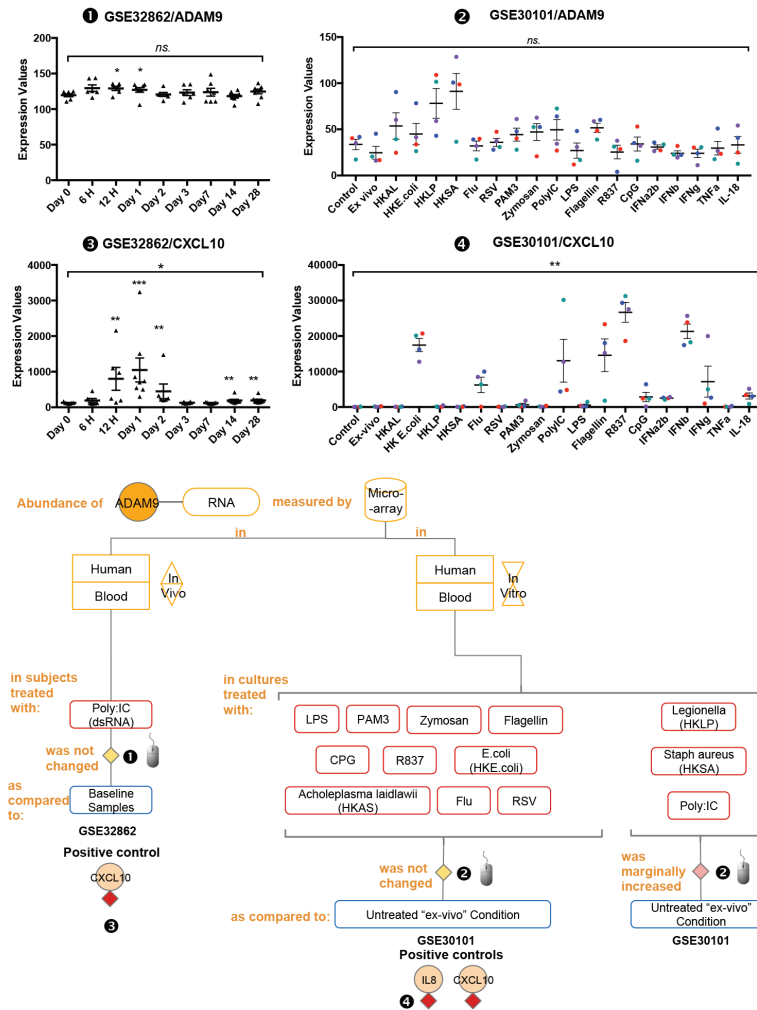


Figure 3. The abundance of ADAM9 increases only marginally following treatment with pathogen-associated molecules. mRNA expression levels for ADAM9 and CXCL10 were measured by microarrays in whole blood obtained from 8 healthy volunteers following sub-cutaneous administration of synthetic dsRNA (poly:IC) or placebo at baseline (day 0) and at 6 and 12 hours and 1, 2, 3, 7, 14, and 28 days (GSE32862), and from 4 healthy individuals (shown in different colors; Red, blue, green and purple) and stimulated *in vitro* for 6 hours with a wide range of immune stimuli including PAM3, Zymosan, Poly IC, E-LPS, Flagellin, R837, CpG Type A, heat-killed *Legionella pneumophila* (HKLP), heat-killed *Acholeplasma laidlawii* (HKAL), and heat-killed *Staphylococcus aureus* (HKSA); IL-18, TNF- α , IFN- α 2b, IFN- β , IFN- γ ; heat-killed *Escherichia coli* (HKEC), live influenza A virus and live (GSE30101).

The graphical legend represents visually the information associated with the public datasets used for the meta-interpretation of ADAM9 transcriptional profiles. The flow chart indicates how data were generated. Diamonds indicate availability of supporting data and in the interactive version are hyperlinked to context-rich interactive plots. Links to these plots are also provided below:

1 GSE32862 Whole blood was collected from 8 healthy volunteers following sub-cutaneous administration of synthetic dsRNA (poly:IC) or placebo at baseline (day 0) and at 6 and 12 hours and 1, 2, 3, 7, 14, and 28 days. As stated in the manuscript: "The study was approved by the Institutional Review Board of The Rockefeller University Hospital. Individual participants in this study provided written informed consent after appropriate review, discussion, and counseling by the clinical study team"²⁸.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kd>

2 GSE30101 Blood was collected from four healthy individuals and stimulated *in vitro* for 6 hours with a wide range of immune stimuli including PAM3, Zymosan, Poly IC, E-LPS, Flagellin, R837, CpG Type A, HKLP, HKAL, and HKSA; IL-18, TNF- α , IFN- α 2b, IFN- β , IFN- γ ; HKEC, live influenza A virus and live RSV. As stated in the manuscript: "The study was approved by the Baylor Research Institute Institutional Review Board at Baylor University Medical Center (Dallas, TX)"²⁷.

<http://www.interactivefigures.com:80/dm3/miniURL/view/KB>

3 See description above.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jr>

4 See description above.

<http://www.interactivefigures.com:80/dm3/miniURL/view/Jw>

Statistical significance was determined using one-way ANOVA and Dunnett's multiple comparisons test. *ns*, not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The horizontal lines indicate mean \pm standard errors (SE).

Table 2. Increased abundance of ADAM9 following treatment with PAMPs.

| GEO ID | A vs B | Avg A-Avg B | Avg A/Avg B | P value |
|----------|-----------------|-------------|-------------|----------|
| GSE32682 | Day 0 VS 6 H | 10.0 | 1.1 | 0.0734 |
| (ADAM9) | Day 0 VS 12 H | 9.5 | 1.1 | 0.0350 |
| | Day 0 VS Day 1 | 7.5 | 1.1 | 0.0140 |
| | Day 0 VS Day 2 | 1.1 | 1.0 | 0.9172 |
| | Day 0 VS Day 3 | 3.7 | 1.0 | 0.7133 |
| | Day 0 VS Day 7 | 4.3 | 1.0 | 0.6894 |
| | Day 0 VS Day 14 | -1.2 | 1.0 | 0.9305 |
| | Day 0 VS Day 28 | 5.3 | 1.0 | 0.1504 |
| GSE32682 | Day 0 VS 6 H | 66.9 | 1.5 | 0.4727 |
| (CXCL10) | Day 0 VS 12 H | 676.3 | 6.5 | > 0.9999 |
| | Day 0 VS Day 1 | 924.2 | 8.5 | 0.0023 |
| | Day 0 VS Day 2 | 324.0 | 3.6 | 0.0003 |
| | Day 0 VS Day 3 | 5.5 | 1.0 | 0.7133 |
| | Day 0 VS Day 7 | -3.8 | 1.0 | 0.8718 |
| | Day 0 VS Day 14 | 67.5 | 1.5 | 0.0093 |
| | Day 0 VS Day 28 | 71.0 | 1.6 | < 0.0059 |

Note : Avg = average abundance of ADAM9 within a given group. Statistical significance was determined using Mann-Whitney U test.

Table 3. Increased abundance of ADAM9 during tissue remodeling.

| GEO ID | A vs B | Avg A-Avg B | Avg A/Avg B | P value |
|----------|--|-------------|-------------|---------|
| GSE17763 | Lepromatous leprosy VS Tuberculoid leprosy | 13164.0 | 2.2 | 0.0012 |
| GSE17449 | Non pregnancy VS Pregnancy | 51.3 | 1.4 | 0.0366 |
| GSE2135 | Filariasis VS Post Treatment | 251.1 | 2.4 | 0.0313* |
| | Filariasis VS Healthy Control | 283.6 | 2.9 | 0.0197 |

Note : Avg = average abundance of ADAM9 within a given group. Statistical significance were determined using Mann-Whitney U test.
* (Pair samples) Statistical significance was determined using Wilcoxon test.

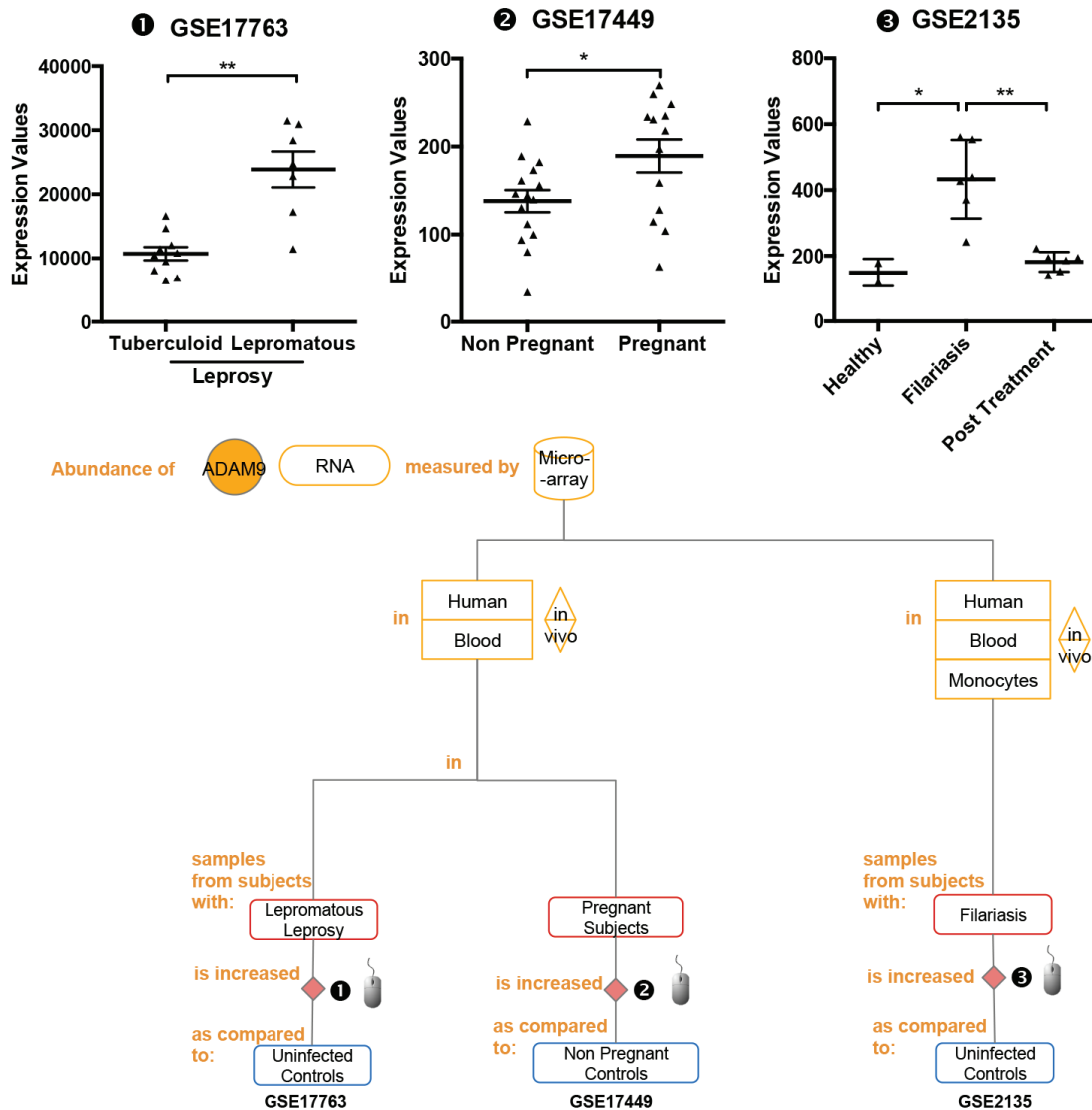


Figure 4. The abundance of ADAM9 increases during tissue remodeling. mRNA expression levels for ADAM9 was measured by microarrays in skin biopsies were obtained from patients with leprosy classified as tuberculoid leprosy or lepromatous leprosy (GSE17763), Peripheral Blood Mononuclear Cells obtained from 12 women (7 MS patients and 5 healthy controls) followed during their pregnancy (GSE17449) and monocyte from filariasis before and after treatment (GSE2135).

The graphical legend represents visually the information associated with the public datasets used for the meta-interpretation of ADAM9 transcriptional profiles. The flow chart indicates how data were generated. Diamonds indicate availability of supporting data and in the interactive version are hyperlinked to context-rich interactive plots. Links to these plots are also provided below:

❶ **GSE17763** Skin biopsies were obtained from patients with leprosy classified as tuberculoid leprosy (controlled disease, few skin lesions) or lepromatous leprosy (uncontrolled diseases, widespread lesions)³¹. All tuberculoid and lepromatous specimens were taken at the time of diagnosis before treatment, and reversal reaction biopsies (labeled as “reaction”) were taken upon follow from patients originally diagnosed with borderline lepromatous leprosy (UCLA Institutional Review Board # 92-10-591-31).

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Ke>

❷ **GSE17449** Peripheral Blood Mononuclear Cells were isolated from the blood of 12 women (7 MS patients and 5 healthy controls) followed during their pregnancy. As stated in the manuscript: This study was approved by the Ethical Committee of the San Luigi University Hospital (March 2006, approval n°87)³². Samples were obtained before pregnancy and at 9 months.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/KD>

❸ **GSE2135** Monocytes were isolated from the peripheral blood of patently infected filaria patients (either *Wuchereria bancrofti*, *Mansonella perstans*, or both), and from uninfected blood bank donors in Mali. As stated in the manuscript: “All study participants signed informed consent forms, and all protocols were approved by the institutional review boards at both the NIAID and the University of Mali (Bamako)”³³. Samples were collected from infected patients prior to and after antifilarial treatment.

<https://gxb.benaroyaresearch.org/dm3/miniURL/view/KB>

Statistical significance was determined using Mann-Whitney U test. *ns*, not significant, * $p < 0.05$ and *** $p < 0.001$. The horizontal lines indicate mean \pm standard errors (SE).

Table 4. Increased abundance of ADAM9 following tissue injury and sterile inflammation.

| GEO ID | A vs B | Avg A-Avg B | Avg A/Avg B | P value |
|------------|---------------------------------|-------------|-------------|----------|
| GSE34608 | Sarcoidosis VS Control | 56.4 | 1.9 | < 0.0001 |
| | Tuberculosis VS control | 56.9 | 1.9 | < 0.0001 |
| GSE11375 | Survived VS Control | 17.7 | 1.2 | 0.0367 |
| | Died VS Control | 45.2 | 1.6 | 0.0226 |
| | Survived VS Died | 27.5 | 1.3 | 0.0473 |
| GSE15072 | HD VS Healthy | 545.6 | 7.6 | < 0.0001 |
| | CKD VS Healthy | 94.3 | 2.1 | 0.0359 |
| GSE28750 | Post surgery VS Healthy | 153.2 | 5.1 | < 0.0001 |
| | Sepsis VS Healthy | 281.8 | 8.5 | < 0.0001 |
| GSE30174** | Healthy VS Baseline | 35.8 | 1.1 | 0.7243 |
| | Healthy VS 1h EBRT | -91.3 | 0.9 | 0.4727 |
| | Healthy VS D7 EBRT | 236.5 | 1.4 | 0.1419 |
| | Healthy VS D14 EBRT | 455.8 | 1.7 | 0.0068 |
| | Healthy VS D21 EBRT | 643.8 | 2.0 | 0.0021 |
| | Healthy VS D42 EBRT | 272.1 | 1.4 | 0.2150 |
| | Healthy VS 1 mo Post Tx | 85.8 | 1.1 | 0.5678 |
| GSE32575 | Obese before surgery VS control | 34.1 | 1.3 | < 0.0001 |
| | Obese post surgery VS control | 19.0 | 1.2 | 0.0001 |
| GSE5580 | TP mono VS HC mono | 247.1 | 1.7 | 0.0070 |
| | TP Leukocyte VS HC Leukocyte | 233.2 | 2.9 | 0.0006 |
| | TP T cell VS HC T cell | 57.9 | 3.0 | 0.0175 |

Note : Avg = average abundance of ADAM9 within a given group. Statistical significance was determined using Mann-Whitney U test. ** This dataset was tested by One-way ANOVA and Dunnett's multiple comparisons test, P value summary = 0.0042.

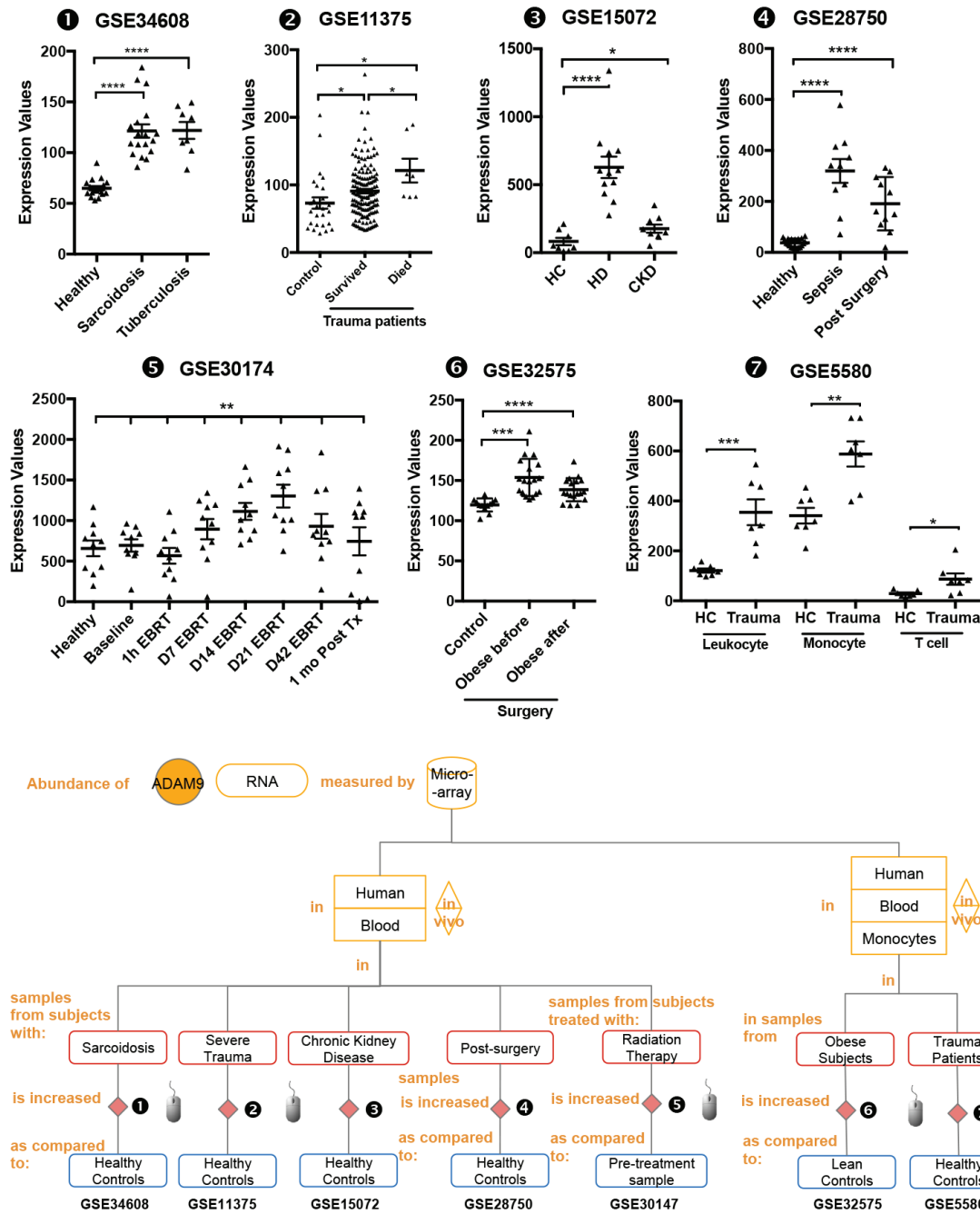


Figure 5. The abundance of ADAM9 increases following tissue injury and sterile inflammation. mRNA expression levels for ADAM9 was measured by microarrays in whole blood obtained from patients with active tuberculosis and sarcoidosis (GSE34608), from patients following severe blunt trauma within 12 hours of traumatic injury who survive and non-survive (GSE11375), from blood of patients with stage II-III Chronic kidney disease (CKD), patients undergoing hemodialysis treatment (HD) and healthy controls (GSE15072), from septicemic patients (GSE28750), from 10 men with non-metastatic prostate cancer; baseline (before External Beam Radiation Therapy - EBRT); days 1, 7, 14, 21, 42 of EBRT; and 30 days post-EBRT (GSE30174) and monocyte from blood of 18 morbidly obese subjects before and three months after bariatric surgery (GSE32575) and from seven subjects with defined multi organ dysfunction syndrome that developed after experiencing severe traumatic injury (GSE5580).

The graphical legend represents visually the information associated with the public datasets used for the meta-interpretation of ADAM9 transcriptional profiles. (https://docs.google.com/presentation/d/12yvt11_LmMOAsozjilAe8MwwKORGgHsO60hpdK2hHsQ/edit#slide=id.g496fd210c_046). The flow chart indicates how data were generated. Diamonds indicate availability of supporting data and in the interactive version are hyperlinked to context-rich interactive plots. Links to these plots are also provided below:

- ❶ **GSE34608** blood was collected from patients with active tuberculosis and sarcoidosis as well as uninfected controls. As stated in the manuscript: "The study was approved by the Ethical Committee 1 of the Charité University Medicine, Campus Mitte in Berlin, the University of Luebeck, and the University of Freiburg in Germany"²⁶.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jt>
- ❷ **GSE11375** blood was collected from patients following severe blunt trauma within 12 hours of traumatic injury. As stated in the manuscript: "The study was approved by review board the Harborview Medical Center and the University of Texas Medical Branch-Galveston"³⁴.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/K8>
- ❸ **GSE15072** Peripheral Blood Mononuclear Cells were isolated from the blood of patients with stage II-III Chronic kidney disease (CKD), patients undergoing hemodialysis treatment (HD) and healthy controls. As stated in the manuscript, "The study was carried out according to the Declaration of Helsinki and approved by the institutional ethical board of the University Hospital 'Policlinico di Bari', Bari, Italy"³⁵.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/KE>
- ❹ **GSE28750** Blood was collected from sepsis patients with clinical evidence of systemic infection based on microbiology diagnoses (n=10). Participants in the post-surgical (PS) group were recruited pre-operatively and blood samples collected within 24 hours following surgery (n=11). Healthy controls (HC) included hospital staff with no known concurrent illnesses (n=20). As stated in the manuscript: "The study protocol was approved by institutional review boards (IRBs)/Human Research Ethics Committees (HRECs) from Mater Health Services (MHS), Uniting Care, the Royal Brisbane & Women's Hospital and the Nepean Hospital Human Research Ethics Committee, prior to the recruitment of study volunteers"²¹.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/K6>
- ❺ **GSE30174** Blood samples were collected from ten subjects at 7 timepoints for microarray analysis: baseline (before External Beam Radiation Therapy - EBRT); days 1, 7, 14, 21, 42 of EBRT; and 30 days post-EBRT. Baseline data obtained from subjects were compared to data obtained from age-, race-, and gender-matched healthy controls. As stated in the manuscript: "The protocols were approved by the Institutional Review Board of the National Institute of Health (NIH), Bethesda, Maryland, USA"³⁶.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/K4>
- ❻ **GSE32575** CD14+ monocytes were isolated from the blood of 18 morbidly obese subjects (BMI: 45.1±1.4 kg/m²) before and three months after bariatric surgery. Six lean age-matched female (BMI: 20.3±0.5 kg/m², mean±SEM) were used as controls. As stated in the manuscript: "This study complies with the Declaration of Helsinki and the Medical Ethics Committee of the Katholieke Universiteit Leuven approved the study protocol. All human participants gave written informed consent"³⁷.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/K5>
- ❼ **GSE5580** Monocytes were isolated from the peripheral venous blood of seven subjects with defined multi organ dysfunction syndrome that developed after experiencing severe traumatic injury. Blood was also obtained from seven age-, sex-, and ethnicity-matched healthy subjects. As stated in the manuscript: " Informed consent was obtained from 18 severely injured patients and 22 healthy subjects under a protocol approved by the University of Rochester School of Medicine Institutional Review Board"³⁸.
<https://gxb.benaroyaresearch.org/dm3/miniURL/view/KC>

Statistical significance was determined using Mann-Whitney U test or one-way ANOVA and Dunnett's multiple comparisons test (GSE30174). ns, not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The horizontal lines indicate mean ± standard errors (SE).

molecules. It was not changed *in vivo* following administration of synthetic double stranded RNA (polyIC), a treatment that mimics viral exposure. Notably, it was not increased either in patients during the early acute phase of HIV infection when an intense immunological response is detected in absence of clinical symptoms [iFigure/GSE29536](#)²². However, ADAM9 transcript abundance was increased in the blood of patients as a result of tissue damage, sterile inflammation and tissue remodeling. Therefore, in addition to its widely reported role in the pathogenesis of cancer the constellation of findings that we are reporting point towards the involvement of ADAM9 in immune-mediated processes and suggest that ADAM9 may constitute a valuable marker for assessing tissue damage, whether it occurs as result of acute infection, traumatic injury or medical procedures such as surgery or radiation therapy. Furthermore, our observations may also be of high significance in the context of acute infections since unlike "generic" markers of inflammation, that could also be used to assess tissue injury in other settings, ADAM9 would not be confounded by the host responses

to the pathogen and may therefore accurately reflect damage to the patient tissues or organs ([Figure 6](#)). Thus ADAM9 blood transcript levels, or possibly levels of circulating proteins, could potentially be employed for triage of patients presenting with symptoms of infection in the emergency room or for monitoring of patients in intensive care units. The functional significance of elevated levels of this proteinase in blood of patients is unclear. While it has been associated with tissue repair increase in protein or transcript levels in the circulation may be an indication of catastrophic tissue damage that will lead to poor outcomes. This is suggested for instance by the fact that abundance of ADAM9 in patients who did not survive was significantly higher than those who survive (GSE11375 - profiling of responses in the blood of trauma patient). In another dataset GSE34205/GSE38900 (Viral infections) we also show that abundance of ADAM9 is correlated with degree of severity in pediatric viral infection (RSV, influenza and HRV infection), moreover level of ADAM9 transcript in patients who were ventilated were significantly higher than that who were non-ventilated.

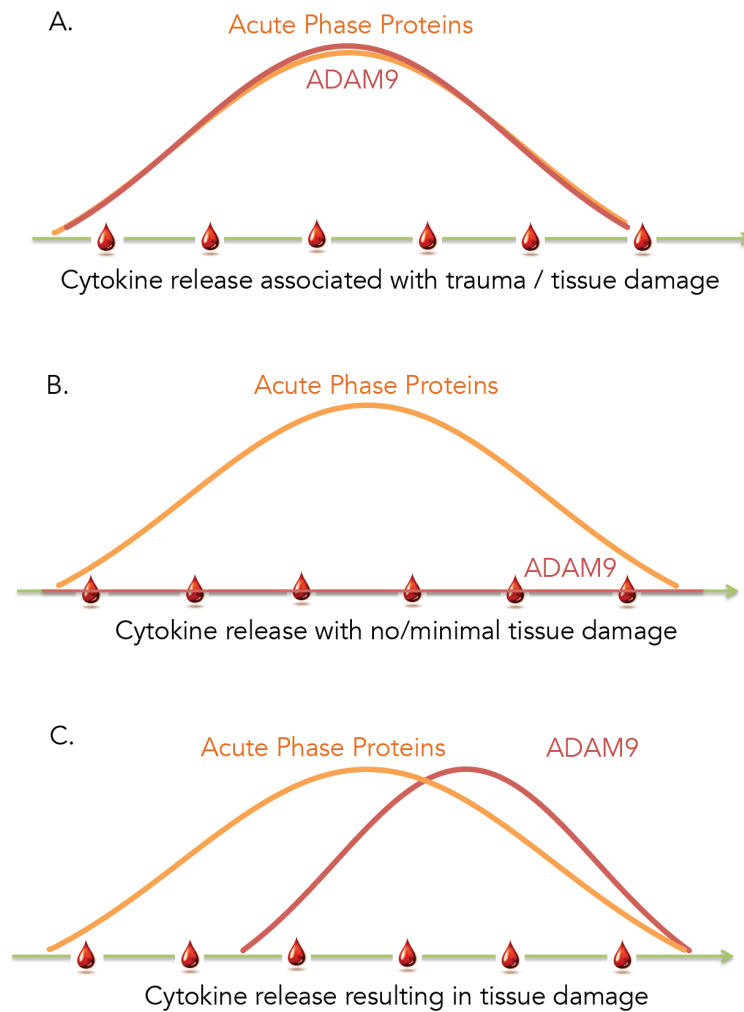


Figure 6. Proposed Model. **A.** Sterile inflammation resulting from tissue injury caused for instance by severe trauma, surgery or radiation therapy can be monitored via the use of prototypical markers of inflammation (acute phase proteins) with ADAM9 levels increasing in concert. **B.** Acute infection also causes a measurable inflammatory response that is the direct result of the antimicrobial response mounted by the immune system. This response can develop in absence of substantial tissue injury and thus does not cause an increase in abundance of ADAM9. **C.** When substantial tissue injury occurs as a result of the infection the abundance of ADAM9 rises, which detection enables the identification and triage of critically ill subjects.

Our analytic approach consisted in the interpretation of transcriptional profiles of a single gene across multiple systems-scale profiling studies. The data from the different studies were not merged in a single unified meta-analysis. Thus it would be more appropriate to qualify this work as a “meta-interpretation”. It proved successful at identifying among a constellation of findings a common thread, the concomitant elevation of ADAM9 with conditions associated with extensive tissue damage. Concerns with regards to the quality of the public data used as input for meta-interpretation, for instance the introduction of uncontrolled confounding factors that may be technical (batch effects) or biological (demographics, treatment), should be mitigated by the fact that

conclusions are based on data drawn from not one but multiple studies, and that these were vetted by institutional review boards and peer review. These mechanisms should ensure that only a minority of those studies would be affected by critical design or technical flaws. However, we also recognize that *in silico* cross-validation of seminal observations does not obviate the need for follow on studies or experimentation. Finally, the fact that the approach presented relies on interpretation of transcriptional profiles derived from a relatively large number of transcriptional studies presents another challenge given that the amount of background information that can be provided for each study cannot be exhaustive. The data browsing web application that we have used attempts

to address this limitation by providing readers access to interactive figures that they can drill into to access detailed sample and study information. Taken together this study provides an original framework for the design of strategies aiming at leveraging vast amounts of high resolution molecular phenotyping data available in public repositories.

Data availability

All primary data presented in this manuscript can be accessed along with contextual information via the data browsing application described above and is also available in NCBI's GEO public repository. GEO accession numbers (starting with GSE) are provided where appropriate throughout this manuscript along with the primary reference associated with the GEO record.

F1000Research: Dataset 1. Raw data of ADAM9 transcripts in blood in response to tissue damage, [10.5256/f1000research.6241.d138863](https://doi.org/10.5256/f1000research.6241.d138863)⁴¹

Author contributions

DR and DC designed the analysis strategy, interpreted the data, prepared figures and drafted the manuscript. CK, BK, GL participated in the mining of the dataset compendium. All authors read and approved manuscript.

Competing interests

No competing interests were disclosed.

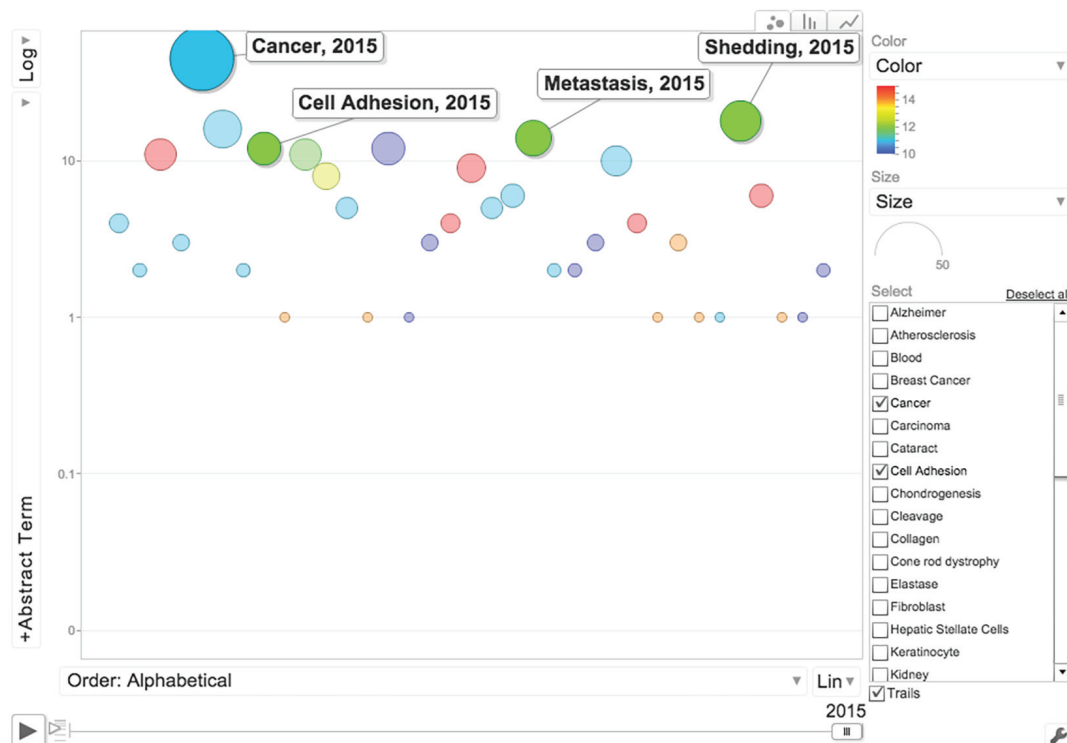
Grant information

The author(s) declared that no grants were involved in supporting this work.

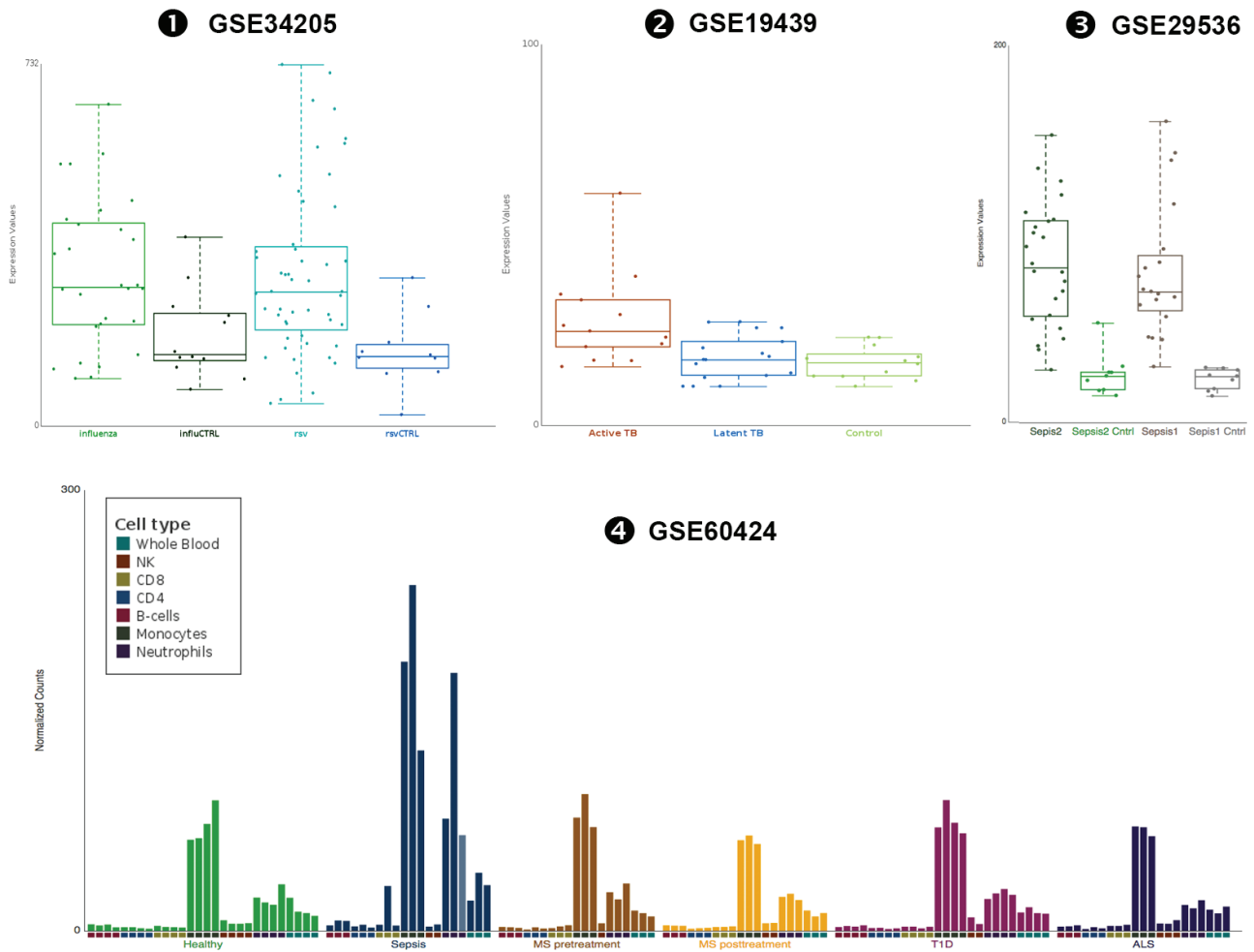
Acknowledgements

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Supplementary Figures

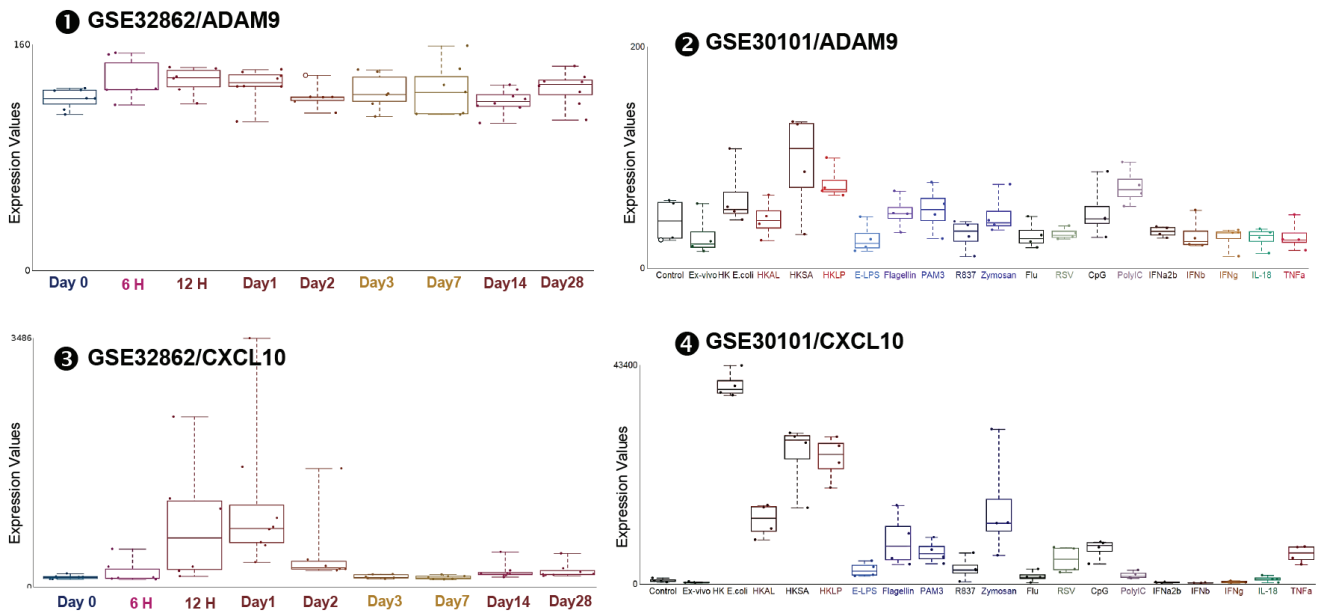


Supplementary Figure 1. ADAM9 Literature Profile. This bubble graph indicates the frequency of abstracts of papers published on ADAM9 containing curated terms (Y-axis: Abstract Term) as of January 2015. Terms are ordered alphabetically along the X-axis. The colors indicate different categories (Blue: Cell type, Green: Disease, Red: Tissue, Light blue: Cellular component, Yellow: Function). The size of the bubbles is proportional to the number of abstract terms. **(Interactive version: Additional file iFIGURE 1 - X-axis drop down menu, select "Order: Alphabetical"; Y-axis drop down menu, select Log Scale; Click "Play" (bottom left corner)).**



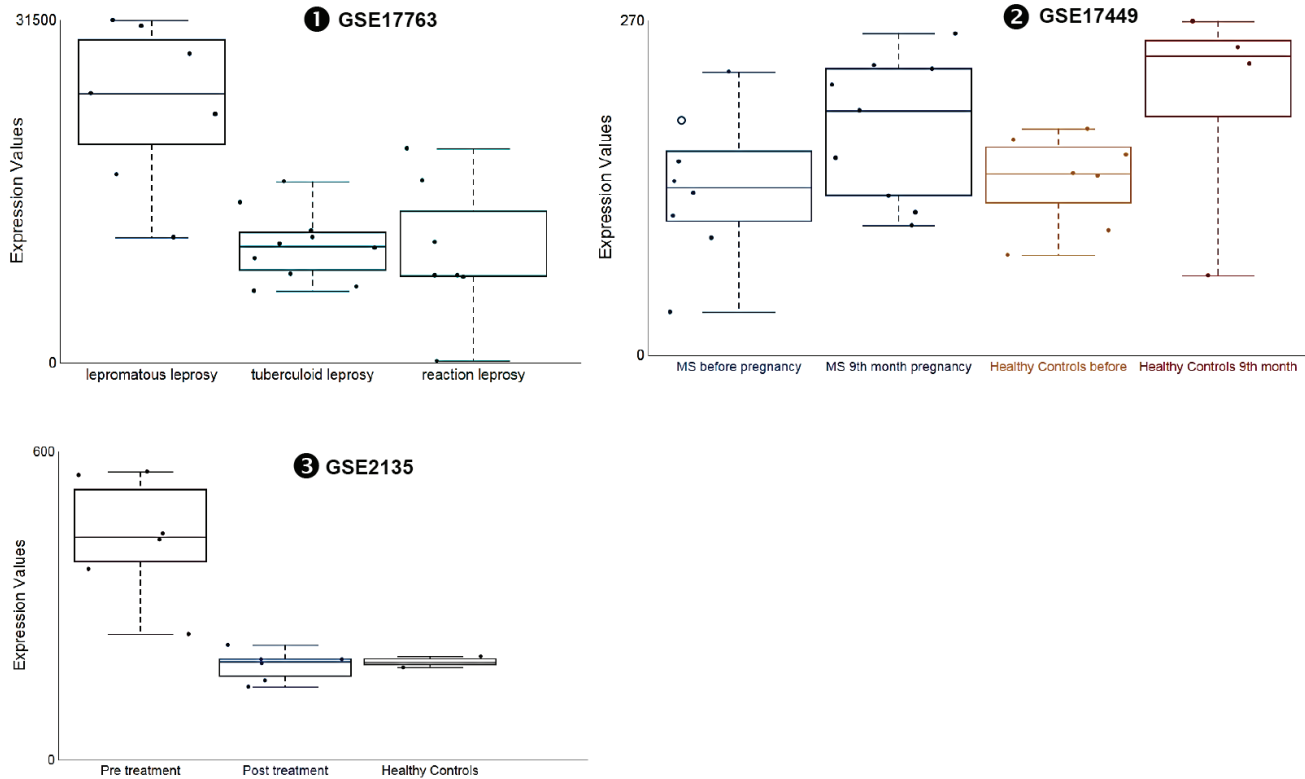
Supplementary Figure 2. These plots are exported directly from the GXB tool and show data supporting the notion that the abundance of ADAM9 increases during infection as presented in **Figure 2** of the manuscript. The interactive version of this plot can be accessed via the links provided below:

- ① <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Ka>
- ② <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kb>
- ③ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jl>
- ④ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kc>



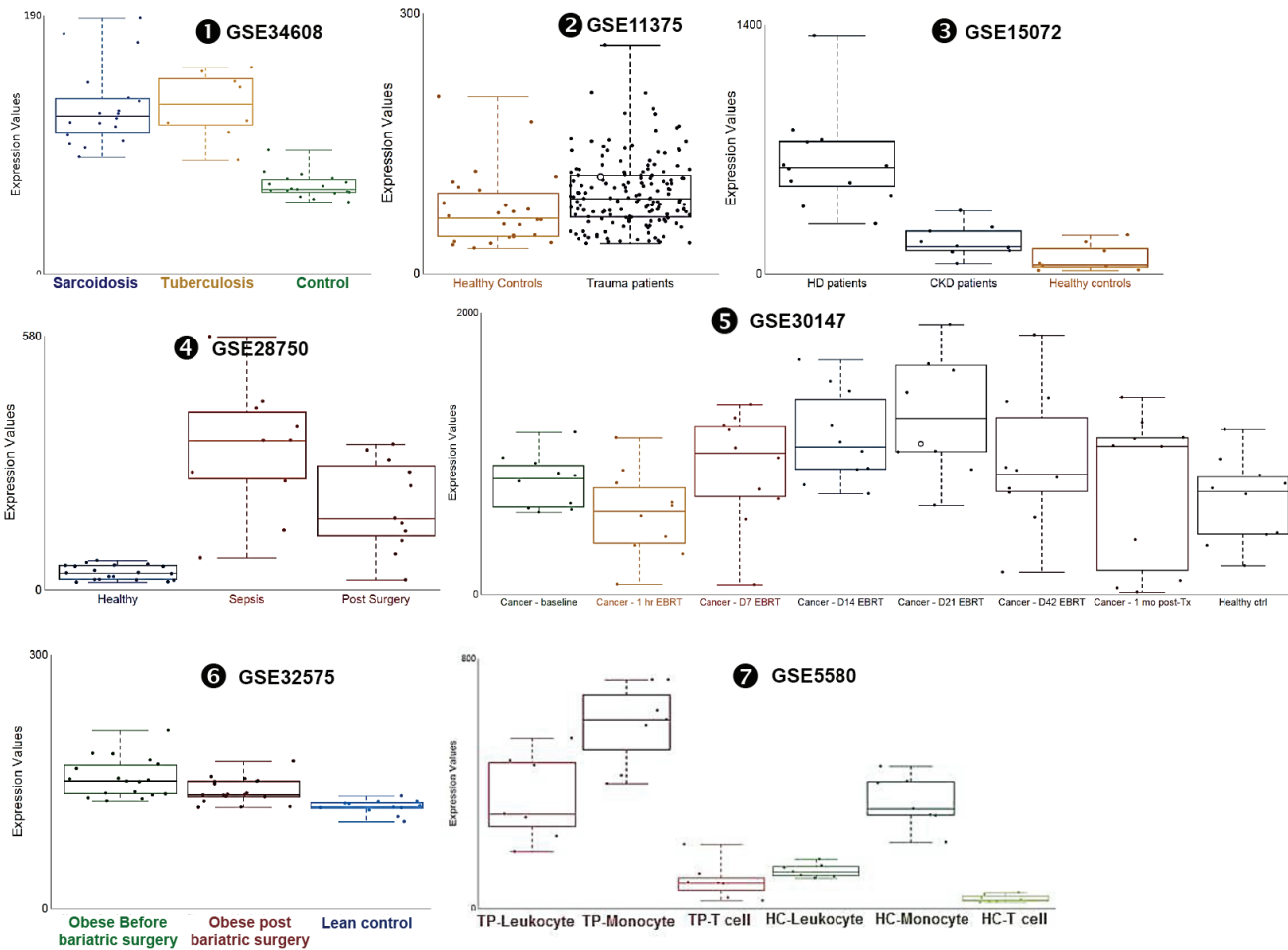
Supplementary Figure 3. These plots are exported directly from the GXB tool and show data supporting the notion that the abundance of ADAM9 increases only marginally following treatment with pathogen-associated molecules as presented in Figure 3 of the manuscript. The interactive version of this plot can be accessed via the links provided below:

- ❶ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Kd>
- ❷ <http://www.interactivefigures.com:80/dm3/miniURL/view/KB>
- ❸ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jr>
- ❹ <http://www.interactivefigures.com:80/dm3/miniURL/view/Jw>



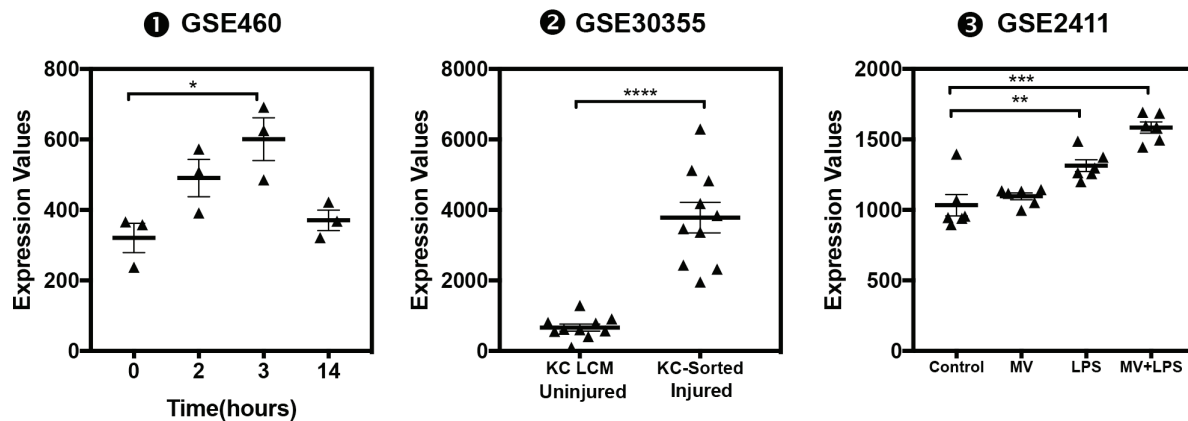
Supplementary Figure 4. These plots are exported directly from the GXB tool and show data supporting the notion that the abundance of ADAM9 increases during tissue remodeling as presented in **Figure 4** of the manuscript. The interactive version of this plot can be accessed via the links provided below:

- ① <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Ke>
- ② <https://gxb.benaroyaresearch.org/dm3/miniURL/view/KD>
- ③ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/KB>



Supplementary Figure 5. These plots are exported directly from the GXB tool and show data supporting the notion that the abundance of ADAM9 increases following tissue injury and sterile inflammation. The interactive version of this plot can be accessed via the links provided below:

- ❶ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/Jt>
- ❷ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/K8>
- ❸ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/KE>
- ❹ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/K6>
- ❺ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/K4>
- ❻ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/K5>
- ❼ <https://gxb.benaroyaresearch.org/dm3/miniURL/view/KC>



Supplementary Figure 6. The abundance of ADAM9 increases in different models of tissue injury. mRNA expression levels for ADAM9 was measured by microarrays in a murine inflammatory lung injury and mechanical ventilation model (GSE2411), an *in vitro* human epidermal injury model (GSE30355) and mouse thermal injury model (GSE460). These raw data were exported from GEO and were plotted by GraphPad Prism.

❶ **GSE460** Thermal injury was induced in mice, and skin excised at 0 hours, 2 hours, 3 days and 14 days post-injury.

https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS353:96738_at

❷ **GSE30355** Keratinocyte response to injury was evaluated in (1) epidermal samples isolated by laser capture microscopy and (2) cultured KCs

https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4608:1570042_a_at

❸ **GSE2411** Twenty four C57/B6 male mice were randomized to four groups: 1) Control, 2) mechanical ventilation (MV), 3) LPS, and 4) MV+LPS. Expression profiling of whole lungs revealed a significant augmentation of ADAM9 transcript levels in the combined MV+LPS group relative to the other 3 conditions.

https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS1276:1416094_at

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[Data Source](#)

Open Peer Review

Current Referee Status:



Version 2

Referee Report 09 December 2016

doi:[10.5256/f1000research.10567.r17151](https://doi.org/10.5256/f1000research.10567.r17151)



Adaikalavan Ramasamy

The Jenner Institute, University of Oxford, Oxford, UK

The authors have done a thorough job of addressing the reviewers comment. The article is very easy to read now and presents a very useful application of their tool. Thank you.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 28 November 2016

doi:[10.5256/f1000research.10567.r18034](https://doi.org/10.5256/f1000research.10567.r18034)



Andreas Ludwig

Institute of Pharmacology and Toxicology, RWTH Aachen University Hospital, Aachen, Germany

Rinchai and co-workers nicely addressed all raised concerns. However, we still have two minor comments.

1. Figure 1 could be further improved. The presentation as scatter plot similar to the other figures as well as statistical analysis would strengthen the message and support the introduction to the readership.
2. Method section – statistical analyses: Please include “... and are indicated within the figure legends.”

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 28 November 2016

doi:[10.5256/f1000research.10567.r17152](https://doi.org/10.5256/f1000research.10567.r17152)



**Caroline A. Owen**

Pulmonary Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

The authors have adequately responded to the concerns that were raised in the initial review. There is now a more comprehensive explanation of the study design and methods. The conclusions are balanced and justified.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 24 August 2015

doi:[10.5256/f1000research.6696.r10054](https://doi.org/10.5256/f1000research.6696.r10054)

**Caroline A. Owen**

Pulmonary Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

The title and abstract of the manuscript:

Both are appropriate.

The design, methods and analysis of the results from the study:

The methods and design have been explained, and the analyses are appropriate for the topic being studied. The results show impressive increases in ADAM9 gene expression in blood leukocytes in some disease states. However there are issues regarding the design/content of the study:

1. It is not clear from reading only the manuscript whether the controls and disease groups are matched with respect to age, sex, and/or race/ethnicity, and/or whether the disease groups studied have co-morbidities that might contributed to the differences in ADAM9 gene expression observed between the groups. It would be necessary to read many of the cited papers in order to obtain this information.
2. The microarray results do not appear to have been validated by performing real-time qPCR studies (for example) on any of the samples.
3. In the *in vitro* studies, details on the concentrations and incubation times for the agonists have not been provided in the methods, text or legends. It is possible that the concentrations and time points studied were not optimal for detecting increases in ADAM9 gene expression.

Data presentation:

All of the results have been presented in the manuscript. However, in general more details about the experimental conditions in the figure legends would have been helpful to the reader.

Discussion and conclusions:

The discussion section could be expanded to include a discussion of the limitations of the study. The discussion could have included a section on how the changes in ADAM9 gene expression detected in

blood leukocytes might influence the pathogenesis of progression of the diseases that were studied based upon the known activities of this proteinase.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 11 Oct 2016

Damien Chaussabel, Sidra Medical and Research Center, Qatar

We thank the reviewer for the valuable feedback and suggestions to improve our manuscript.

The title and abstract of the manuscript:

Both are appropriate.

The design, methods and analysis of the results from the study:

The methods and design have been explained, and the analyses are appropriate for the topic being studied. The results show impressive increases in ADAM9 gene expression in blood leukocytes in some disease states. However there are issues regarding the design/content of the study:

1. It is not clear from reading only the manuscript whether the controls and disease groups are matched with respect to age, sex, and/or race/ethnicity, and/or whether the disease groups studied have co-morbidities that might contributed to the differences in ADAM9 gene expression observed between the groups. It would be necessary to read many of the cited papers in order to obtain this information.

Authors: *This is a good point as such factors may indeed potentially confound analysis and undermine the conclusions advanced by the authors of those studies (since it would presumably affect not only ADAM9 but all the transcripts being measured).*

Mechanisms exist that should help ensure that the study design and choice of selection of control subjects is appropriate at least in most studies:

- 1. One is IRB review that to some extent will evaluate the study design elements such as inclusion and exclusion criteria for case and control groups and will help ensure that results of the study will be meaningful and justify risk to the study population.*
- 2. The second mechanism is peer review. Having conducted such studies ourselves and reviewed submissions of others concerns often come up regarding factors that might potentially confound analyses and that would need be addressed before publication.*

In addition, the process of loading dataset and sample as well as study information in GXB as well as QC checks provide an additional opportunity to identify "faulty" designs.

These steps can of course only mitigate risk since even study investigators may not be aware of all the factors that could potentially confound the analysis. One of the potential advantages of the analytic strategy that we have employed is that it factors in results not from one but several studies

carried out by different investigators in different geographic locations, often using different technology platforms. Thus the conclusions we derive from such “meta-interpretation” is likely to be rather robust with only a minority of the studies potentially being affected by study design.

It should also be noted that details concerning study design have been incorporated in GXB and therefore the reader does not have to access the original manuscript in order to locate the relevant information. Sample information is also available via GXB and can be accessed by 1) hovering of the mouse cursor over individual data points; 2) overlaying the information on the interactive bar graph; 3) accessing the table listing all available sample information.

We have acknowledged the point raised in the review and include some of the considerations outlined above in the conclusion of our manuscript:

“Concerns with regards to the quality of the public data used as input for meta-interpretation, for instance the introduction of uncontrolled confounding factors that may be technical (batch effects) or biological (demographics, treatment), should be mitigated by the fact that conclusions are based on findings from not one but multiple studies, and that all of them were vetted by institutional review boards and peer review. These mechanism should ensure that only a small minority of those studies would be affected by critical design or technical flaws.”

2. The microarray results do not appear to have been validated by performing real-time qPCR studies (for example) on any of the samples.

Authors: Confirmation by real-time PCR was not available for all studies, and when they were had not been performed for ADAM9 since it was not a focus of the systems-scale analyses. We did not have direct access to study samples and could not check levels of ADAM9 transcript ourselves. It should be noted that doing so would in any case only serve to validate the accuracy of the technology platform that the authors employed rather than the intrinsic value of ADAM9 as a biomarker. The approach that we employ provides a means for *in silico* validation of findings from an initial study across additional independent patient cohorts. However, we recognize that it does not ultimately obviate the need for follow on studies/experimentations.

We added the following sentence in the conclusion:

“We also recognize that such *in silico* cross-validation of our seminal observation does not obviate the need for follow on studies or experimentation.”

3. In the *in vitro* studies, details on the concentrations and incubation times for the agonists have not been provided in the methods, text or legends. It is possible that the concentrations and time points studied were not optimal for detecting increases in ADAM9 gene expression.

Data presentation:

All of the results have been presented in the manuscript. However, in general more details about the experimental conditions in the figure legends would have been helpful to the reader.

Authors: As requested by the reviewer we have added details in each dataset as shown in Figure legends through the manuscript. As mentioned above we have employed GXB as an interface between the readers and the papers that originally described the study and its findings. Information

regarding study design or samples has been structured within GXB and can be accessed directly from the manuscript and in only a few clicks. It can also be represented graphically. We have also added a few sentences to highlight this point in the manuscript:

“Finally, the fact that the approach presented relies on interpretation of transcriptional profiles derived from a relatively large number of transcriptional studies presents another challenge given that the amount of background information that can be provided for each study cannot be exhaustive. The data browsing web application that we have used attempts to address this limitation by providing readers access to interactive Figures that they can drill into to access detailed sample and study information.”

Furthermore, we selected CXCL10 as a positive control to show that large levels of induction could be obtained for genes known to respond to those stimuli. Expression values for this gene range from nearly 10 up to nearly 40,000 units. And although this dataset is indeed publicly available we happen to have been the contributors (as is the case of a number of datasets being reanalyzed here) and had performed dose ranging and time course experiments prior to selection of the stimulation conditions.

Discussion and conclusions:

The discussion section could be expanded to include a discussion of the limitations of the study. The discussion could have included a section on how the changes in ADAM9 gene expression detected in blood leukocytes might influence the pathogenesis of progression of the diseases that were studied based upon the known activities of this proteinase.

Authors: *A new section has been added in the conclusion specifically to discuss limitations of the study (see additions mentioned above). We are yet unsure of the functional significance of elevation in levels of ADAM9, which on one hand may be beneficial to mediate tissue repair; on the other hand the fact that ADAM9 proteins or transcripts levels are found elevated in blood may be an indication of extensive tissue damage and be associated with poor outcome. Indeed we now for instance report in the context of GSE11375 (profiling of responses in the blood of trauma patient) that abundance of ADAM9 in patients who did not survive was significantly higher than those who survive. In another dataset GSE34205/GSE38900 (Viral infections) we now show that abundance of ADAM9 is correlated with degree of severity in pediatric viral infection (RSV, influenza and HRV infection), moreover level of ADAM9 transcript in patients who were ventilated were significantly higher than that who were non-ventilated. We have added these statements in the discussion.*

Competing Interests: No competing interests were disclosed.

Referee Report 13 August 2015

doi:10.5256/f1000research.6696.r9961



Andreas Ludwig, Daniela Drey Müller

Institute of Pharmacology and Toxicology, RWTH Aachen University Hospital, Aachen, Germany

Rinchai and co-workers nicely present a re-analysis of existing genomic datasets, demonstrating an useful tool for quick establishment of functional hypotheses. By this, they suggest a novel function of

ADAM9 as biomarker for tissue damage. The article is well written, but several concerns should be addressed before indexing.

1. Introduction: The authors give a nice review of the current literature. However, a link to their one study is missing. The introduction should include the motivation (“knowledge gap assessment”). Otherwise, readers could expect a detailed physiologic analysis of ADAM9 in tissue damage.
2. Methods: The description is very nice but could be adapted to journal style and shortened. The possibilities offered by the software could be summarized in a table.
3. Figure 1: The colour scheme in the figure and the legend are sorted differentially. In general, the figure is overloaded and the colour scheme not helpful. Differences were only observed for monocytes and neutrophils. These results should be included in figure 1, whereas the other results should be included as supplementary figure.
4. Supplementary Figure 2 to 5: I don’t see any additional information by this second plot type.
5. What is the difference between Suppl. Figure 2 4 and Figure 2?
6. Why are not all datasets mentioned in the text also shown and listed within the figures? This is very obvious for Figure 2.
7. The diagrams within the figures are very redundant especially due to the detailed description within the text. This space should be used to present more original data sets.
8. The tables should be summarized within one table. Further, the table should include all datasets analysed and mentioned in the text.
9. Figure 3: It would be helpful to mark the values for the different individuals, maybe by different colours to avoid the impression of a general outlier. Otherwise, changes after PAMP treatment could be possible.
10. Conclusion: To address the point of infection the authors include a stimulation of blood samples. However, this is not sufficient to draw the conclusion of a biomarker for tissue damage (also as a result of infection). Experiments with tissue cells, including scratch assays, stimulations with cytokines, and conditioned media from blood samples would provide further information and address the tissue damage effect in comparison to the infection effect.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 11 Oct 2016

Damien Chaussabel, Sidra Medical and Research Center, Qatar

We thank the reviewers for their valuable feedback and suggestions to improve our manuscript.

Rinchai and co-workers nicely present a re-analysis of existing genomic datasets, demonstrating an useful tool for quick establishment of functional hypotheses. By this, they suggest a novel function of ADAM9 as biomarker for tissue damage. The article is well written, but several concerns should be addressed before indexing.

1. Introduction: The authors give a nice review of the current literature. However, a link to their one study is missing. The introduction should include the motivation (“knowledge gap assessment”). Otherwise, readers could expect a detailed physiologic analysis of ADAM9 in tissue damage.

Authors: *As suggested we added a paragraph at the beginning of the introduction section to present the rationale behind the data mining approach that was employed. We experienced issues with one of our servers at some time. We checked the links and references provided in the introduction and it seems to be working fine now.*

2. Methods: The description is very nice but could be adapted to journal style and shortened. The possibilities offered by the software could be summarized in a table.

Authors: *We also took care of this. Since the description of the GXB tool has now been published and the code made openly available in Github we now point readers to these resources and shortened the paragraph describing the features of the software accordingly (Speake C, et al., J Transl Med 2015, Rinchai D, et al., F1000R 2016). A link to a tutorial video has also been added to the methods (<https://www.youtube.com/playlist?list=PLtx3tvflzJ9XkRKUz6ISEJpAhqKyuiCiD>).*

3. Figure 1: The colour scheme in the figure and the legend are sorted differentially. In general, the figure is overloaded and the colour scheme not helpful. Differences were only observed for monocytes and neutrophils. These results should be included in figure 1, whereas the other results should be included as supplementary figure.

Authors: *Thank you for pointing this out. In the original version of the Figure we used the graphic exported directly with GXB. However, we agree that it is difficult to read, especially without interactive features that allow overlay of sample information, sorting and mouse overs. Another reviewer also suggested retaining only the neutrophil and monocyte data the plot for Figure 1 and we have made these changes accordingly.*

4. Supplementary Figure 2 to 5: I don't see any additional information by this second plot type.

Authors: *Supplementary Figures 2-5 represent the data exactly how they can be visualized interactively in the GXB. We felt this might be helpful given the fact that we provide links throughout the manuscript that lead to interactive version of these plots. We are now providing this rationale in the legend of the supplementary figures.*

5. What is the difference between Suppl. Figure 2 4 and Figure 2?

Authors: *Same rationale as stated above, we used Supplementary Figures representing the original data exactly how they can be visualized interactively in the GXB. The links to the interface of each graph are provided in legends of each Supplementary Figures.*

6. Why are not all datasets mentioned in the text also shown and listed within the figures? This is very obvious for Figure 2.

Authors: *We in fact initially tried to show the results from all of the studies. But since some of the Figures make reference to a rather large number of datasets and that we are able to provide links to interactive graphs we decided to only select a subset of the key studies that best support the points that we were making. In response to the reviewers' comments we are now listing all the studies in the Figure legend and accompanying Table. Readers can access the data for each study by clicking the associated hyperlinks. All the studies mentioned in the text are also represented on the graphical abstract.*

7. The diagrams within the figures are very redundant especially due to the detailed description within the text. This space should be used to present more original data sets.

Authors: *We did not properly communicate the purpose of these diagrams that constitute graphical legend and allow presentation of the data in a semi-structured format that is both human and machine readable. We are now providing a rationale (see below) and have re-positioned them at the bottom of the Figure, which will hopefully work better.*

Rationale: "Diagrams have been incorporated within each Figure. These have a dual purpose, first they provide readers with a graphical summary of the findings and second constitute an attempt a structuring information for future computational applications. Indeed, an important limitation of communicating biomedical knowledge in the form of research articles is that it consists in unstructured information (free text). This type of information is notoriously difficult to extract by computational means [e.g. Chaussabel D. Am J Pharmacogenomics 2004; 4: 383-93]. Standardized graphical summaries such as the ones provided in this manuscript constitutes structured information that is both human readable and computationally tractable. The need for such solutions will become more pressing as the biomedical literature continues to grow exponentially to such scales that it can only be very narrowly apprehended by research investigators."

8. The tables should be summarized within one table. Further, the table should include all datasets analysed and mentioned in the text.

Authors: *We thanks to reviewer for raising this point. As mentioned earlier we have added studies that have been previously omitted. But as far as merging all datasets in one table we were concerned after making an attempt that it would be difficult for reader to track down information about a given dataset if the list is too extensive. Also we reverted to the original format where separate tables are linked to each individual figure.*

9. Figure 3: It would be helpful to mark the values for the different individuals, maybe by different colours to avoid the impression of a general outlier. Otherwise, changes after PAMP treatment could be possible.

Authors: As suggested by reviewers, we labeled the value of different individual by using different colors in the PAMPs treatment dataset (GSE30101). We found that ADAM9 levels didn't show significant outlier response, with the exception of the green subject that shows low response to HKSA in comparison to the other subjects. This could be explained by donor-specific variation in the subject's ability to respond. Overall the magnitude of response to such stimuli remains especially low, especially when compared to CXCL10 which served as a positive control and did not reach significance. Donor information was not available for GSE32862

10. Conclusion: To address the point of infection the authors include a stimulation of blood samples. However, this is not sufficient to draw the conclusion of a biomarker for tissue damage (also as a result of infection). Experiments with tissue cells, including scratch assays, stimulations with cytokines, and conditioned media from blood samples would provide further information and address the tissue damage effect in comparison to the infection effect.

Authors: Indeed we are reporting an association and direct evidence will have to be obtained experimentally. We have changed the title to better reflect this fact, and it now reads: "Increased abundance of ADAM9 transcripts in the blood is associated with tissue damage".

Confirmation experiments will require the analysis of large number of sample for validation and will require further investigation, we are working to secure necessary funding and ethics approval for follow on studies, which is taking more time than anticipated. Some datasets available in GEO include experiments that are relevant, this is for instance the case GSE30101 where whole blood that was stimulated with microbe-derived as well as host-derived factors such as pathogen-associated molecular patterns (PAMPs), inflammatory cytokines (IL18, TNF), as well as interferon type I and Type II). Prompted by your comment we also have checked expression of ADAM9 transcript in additional datasets. Transcript abundance were for instance measured in the lungs of C57BL/6 in a model of lung inflammation and injury (GSE2411) [Kennedy-Crispin M. et al., J Invest Dermatol 2012]. The result showed that the abundance of ADAM9 was significantly higher in mice that developed acute lung injury after exposure to low-dose LPS and mechanical ventilation (GSE2411). In an experimental model of epidermal injury (GSE30355) the abundance of ADAM9 was significantly higher in injured epidermis (sorted keratinocyte (KC)) as comparison to uninjured condition (laser capture microscopy or in vitro cultured keratinocytes) [Altemeier WA. et al., J Immunol 2005]. Additionally, in a murine dermal burn wound model ADAM9 transcript of mouse thermal injury induced increased over the time at 0, 2 hours, 3 days respectively (GSE460).

We have added a description of these findings in the result section under the paragraph entitled "The abundance of ADAM9 increases following tissue injury and sterile inflammation" and are also including a new Supplementary Figure 6.

Competing Interests: No competing interests were disclosed.

Referee Report 16 July 2015

doi:10.5256/f1000research.6696.r8789



Adaikalavan Ramasamy

The Jenner Institute, University of Oxford, Oxford, UK

Rinchai *et al.* suggest a novel role for ADAM9 by mining existing dataset. This clever re-use of existing dataset is a demonstration on how scientists can test new hypothesis quickly, inexpensively and with more robustness. They also provide a web tool based on 172 curated datasets (<https://gxb.benaroyaresearch.org/dm3/geneBrowser>) which makes is a practical resource.

All sections of the article is extremely well written and I strongly recommend the article be indexed subject to the following comments.

1. **Introduction:** The introduction starts with the Refseq definition of ADAM9 and a thorough review of existing literature on gene function of ADAM9. It left me wondering what motivated them to ADAM9 until the first section of Results (Knowledge gap assessment). It would be useful to the reader if a brief sentence or two on the motivation to study this gene was at the beginning of the Introduction section.
2. **Figure 1:** I find it very difficult to color match the Cell type on the x-axis of Figure 1 especially when it appears legend colors are sorted differently. A plot with seven smaller panels (one for each cell type) or even just 2 panels (neutrophils and monocytes) might be clearer. Can you add GSE60424 to title of Figure 1?
3. General comment on **Figures 2 - 5** and **Tables 1 - 4:**
 - a) There is an inconsistency in the number of datasets stated in text and demonstrated in the figure. E.g. the text for Figure 2 talks about seven datasets but figure only shows three and Table 1 also talks about three datasets but includes SOJIA vs Control and HIV vs Control from GSE29536.
 - b) I find the process diagrams (top half of figures) distracting and redundant with text and legend. This space could be used to incorporate the other studies. I suggest incorporating the cell type and measurement type after the study names on plot (e.g. GSE34205 \n microarray on whole blood; GSE29536 \n RNA-seq on neutrophils). Legend is well described.
 - c) The column for "Avg A - Avg B" is meaningless especially when comparing different platforms. The fold change (Avg A / Avg B) is more meaningful and would be worth stating to two decimal points.
 - d) If possible, combine Tables 1 - 4 into one page, possibly a large table with subheadings for during infection, after treatment with PAMPs, during tissue remodelling etc ... T
4. **Forest plot:** An alternative/additional suggestion to 3d is to present the data visually in the form of a forest plot with subheadings (e.g. https://www.nichd.nih.gov/cochrane_data/mcguirew_13/fig2019799225306621155.png; test of heterogeneity might not be necessary). This allows the readers to visually scan all of the in one page. Many ways of doing this but I suggest calculating the Glass effect size (see R codes below) followed by forestplot function from R package rmeta or forestplot.

The authors might also find such a plot on their webtool useful in the long run but this is beyond the scope of current paper.

```
effectSize <- function(baseline, posttest){
```

```
## Source
```

<http://www.meta-analysis.com/downloads/Meta-analysis%20Effect%20sizes%20based%20on%20>

```
stopifnot( length(posttest)==length(baseline) ) ## assume the data is in same patient order
```

```
w <- which( !is.na(posttest) & !is.na(baseline) )
```

```
posttest <- posttest[w]
```

```
baseline <- baseline[w]
```

```
r <- cor( posttest, baseline )
```

```
diff <- posttest - baseline
```

```
n <- length(diff)
```

```
S.diff <- sqrt( var(baseline) + var(posttest) - 2*cov(baseline, posttest) )
```

```
# note: var(x - y) = var(x) + var(y) - 2cov(x, y)
```

```
S.within <- S.diff/sqrt(2*(1-r)) # same as S.pooled
```

```
## Cohen's d ##
```

```
d <- mean(diff)/S.within
```

```
var.d <- ( 1/n + (d^2)/(2*n) ) * 2 * (1-r)
```

```
## Hedge's g estimate ##
```

```
cf <- 1 - 3/(4*n - 5)
```

```
g <- cf*d
```

```
var.g <- cf^2 * var.d
```

```
se.g <- sqrt(var.g)
```

```
rm(posttest, baseline, w, r, diff, n, S.diff, S.within, d, var.d, cf, var.g)
```

```
return( c( g=g, se.g=se.g, LCL=( g - 1.96*se.g), UCL=( g + 1.96*se.g) ) )
```

```
}
```

5. I am unclear what Supplementary Figures 2, 3, 4 and 5 adds to the paper.
6. There is emerging evidence that the monocyte to lymphocyte ratio has relevance to susceptibility to infectious diseases (e.g. [Wang et al., 2015](#); [Naranbhai et al., 2014](#); [Warimwe et al., 2013](#)). Could you speculate/demonstrate how you could potentially use your resource to test for this hypothesis? Perhaps using cell deconvolution methods on whole blood?

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 11 Oct 2016

Damien Chaussabel, Sidra Medical and Research Center, Qatar

We would like to thank reviewer for kindly comments and suggestions to improve our manuscript.

Rinchai *et al.* suggest a novel role for ADAM9 by mining existing dataset. This clever re-use of existing dataset is a demonstration on how scientists can test new hypothesis quickly, inexpensively and with more robustness. They also provide a web tool based on 172 curated datasets (<https://gxb.benaroyaresearch.org/dm3/geneBrowser>) which makes is a practical resource.

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Authors: *Thank you for raising this point, we agree that it would be better to start with such a description. So we have now added a paragraph explaining the “Collective data to knowledge” approach as the first paragraph of the introduction section.*

2. **Figure 1:** I find it very difficult to color match the Cell type on the x-axis of Figure 1 especially when it appears legend colors are sorted differently. A plot with seven smaller panels (one for each cell type) or even just 2 panels (neutrophils and monocytes) might be clearer. Can you add GSE60424 to title of Figure 1?

Authors: *We agree with this suggestion. We initially wanted to use the plot as it would appear to the reader when accessing the GXB via the link provided, but it is rather difficult to interpret without the interactive features built into the software tool (overlay of sample information, sample sorting, pop ups etc...). Also per your and another reviewer’s suggestion we changed the plot of Figure 1 showing only neutrophil and monocyte data.*

3. General comment on **Figures 2 - 5** and **Tables 1 - 4:**

a) There is an inconsistency in the number of datasets stated in text and demonstrated in the figure. E.g. the text for Figure 2 talks about seven datasets but figure only shows three and Table 1 also talks about three datasets but includes SOJIA vs Control and HIV vs Control from GSE29536.

Authors: *Thanks for pointing this out. Not all dataset analyzed were plotted on the figures but all are now listed in the tables, in the textual figure legends, are represented on the graphical figure legends and can be accessed via the links provided.*

b) I find the process diagrams (top half of figures) distracting and redundant with text and legend. This space could be used to incorporate the other studies. I suggest incorporating the cell type and measurement type after the study names on plot (e.g. GSE34205 \n microarray on whole blood; GSE29536 \n RNA-seq on neutrophils). Legend is well described.

Authors: *This point has been raised by another reviewer as well and is obviously important. We did not properly communicate the purpose of these diagrams which are meant as “graphical figure legends”. We aimed at structuring the information communicated and also help readers navigate the many finding that are reported while providing links to interactive figures and make details regarding study design more readily accessible (which a third reviewer deemed particularly important). In addition to providing the rationale for including those graphical figure legends we also moved them at the bottom of each figure, which is really the most logical spot for them to be.*

Rationale: Diagrams have been incorporated within each figure. These have dual purpose, first providing readers with a graphical summary of the findings and second constitute an attempt a structuring information for future computational applications. Indeed, an important limitation of communicating biomedical knowledge in the form of research articles is that it consists in unstructured information (free text). This type of information is notoriously difficult to extract by computational means [Chaussabel D. Am J Pharmacogenomics 2004; 4: 383-93]. Standardized graphical summaries such as the ones provided in this manuscript constitutes structured information that is both human readable and computationally tractable. The need for such solutions will become more pressing as the biomedical literature continues to grow exponentially to such scales that it can only be very narrowly apprehended by research investigators.

c) The column for "Avg A - Avg B" is meaningless especially when comparing different platforms. The fold change (Avg A / Avg B) is more meaningful and would be worth stating to two decimal points.

Authors: *We agree that it indeed cannot be compared across platforms, which we did not intend to do since rather than a meta-analysis our approach consists in a “meta-interpretation” across publicly available datasets. However, it is a good indication of robustness of the changes that are measured. We have used this criterion for many years to weed out genes that show high fold change but for expression levels that are close to background levels, which we have found to be poorly reproducible. For example, in case where fold change = 3 difference if A=30 and B=10 will be 20 which might be about twice the background intensity of the chip; whereas if A=300 and B=100, A/B is still = 3 but A-B is 200 or twenty time the background intensity. So having this information can help decide whether the changes that are observed are likely to be robust.*

d) If possible, combine Tables 1 - 4 into one page, possibly a large table with subheadings for during infection, after treatment with PAMPs, during tissue remodelling etc ... T

Authors: *Thank you for raising this point, we initially considered this possibility but found that It would be too much information in one table. We were also concerned that it would make it too difficult for the reader to locate this information.*

4. **Forest plot:** An alternative/additional suggestion to 3d is to present the data visually in the form of a forest plot with subheadings (e.g.

https://www.nichd.nih.gov/cochrane_data/mcguirew_13/fig2019799225306621155.png; test of heterogeneity might not be necessary). This allows the readers to visually scan all of the in one page. Many ways of doing this but I suggest calculating the Glass effect size (see R codes below) followed by forestplot function from R package rmeta or forestplot.

The authors might also find such a plot on their webtool useful in the long run but this is beyond the scope of current paper.

```
effectSize <- function(baseline, posttest){
  ## Source
  http://www.meta-analysis.com/downloads/Meta-analysis%20Effect%20sizes%20based%20on%20me
```

```
stopifnot( length(posttest)==length(baseline) ) ## assume the data is in same patient order
w <- which( !is.na(posttest) & !is.na(baseline) )
```

```
posttest <- posttest[w]
```

```
baseline <- baseline[w]
```

```
r <- cor( posttest, baseline )
```

```
diff <- posttest - baseline
```

```
n <- length(diff)
```

```
S.diff <- sqrt( var(baseline) + var(posttest) - 2*cov(baseline, posttest) )
```

```
# note: var(x - y) = var(x) + var(y) - 2cov(x, y)
```

```
S.within <- S.diff/sqrt(2*(1-r)) # same as S.pooled
```

```
## Cohen's d ##
```

```
d <- mean(diff)/S.within
```

```
var.d <- ( 1/n + (d^2)/(2*n) ) * 2 * (1-r)
```

```
## Hedge's g estimate ##
```

```
cf <- 1 - 3/(4*n - 5)
```

```
g <- cf*d
```

```
var.g <- cf^2 * var.d
```

```
se.g <- sqrt(var.g)
```

```
rm(posttest, baseline, w, r, diff, n, S.diff, S.within, d, var.d, cf, var.g)
```

```
return( c( g=g, se.g=se.g, LCL=( g - 1.96*se.g), UCL=( g + 1.96*se.g) ) )
```

```
}
```

Authors: We thanks reviewer for this valuable suggestion and sharing the R code! Our GXB tool is still in development and we hope to add additional options for data visualizations in the future so this is perfect.

5. I am unclear what Supplementary Figures 2, 3, 4 and 5 adds to the paper.

Authors: We intended to show in Supplement Figures 2-5 the data exactly how they can be visualized interactively in the GXB. We could remove these figures but since this information is merely added as a supplement we felt that there is no harm in leaving it as is.

6. There is emerging evidence that the monocyte to lymphocyte ratio has relevance to susceptibility to infectious diseases (e.g. [Wang et al., 2015](#); [Naranbhai et al., 2014](#); [Warimwe et](#)

al., 2013). Could you speculate/demonstrate how you could potentially use your resource to test for this hypothesis? Perhaps using cell deconvolution methods on whole blood?

Authors: *This is a very interesting hypothesis but perhaps beyond the scope of this manuscript; it might be possible to identify relevant dataset but such outcome measure is not easily ascertained at least in humans (i.e. susceptibility to infection)*

Competing Interests: No competing interests were disclosed.
