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Orbital pseudotumor can be a localized form of granulomatosis with polyangiitis as revealed by gene expression profiling

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Author Contributions:

JTR conceived of the study, recruited study centers, supervised the study, reviewed the clinical diagnoses, and participated in the study design, data analysis and interpretation, and drafted the manuscript. DC helped with the study design, performed the statistical analysis, participated in data interpretation, and helped prepare the manuscript. DJW evaluated stained sections, reviewed the clinical diagnoses, and helped prepare the manuscript. HEG evaluated stained sections and helped prepare the manuscript. CAH helped with study design, supervised the RNA extraction and microarray assays, participated in data analysis, interpretation, and critical revision of the manuscript. CHS assisted in data interpretation and critical revision of the manuscript. RAD, JDN, and EAS obtained samples for analysis, gathered clinical and demographic data, and helped prepare the manuscript. CNC, JAF, DT, CA, GIH, MK, PJP, BSK, DOK, DPE, HMA, HaH, and RPY obtained IRB approval, obtained samples for analysis, gathered clinical and demographic data, and helped prepare the manuscript. SD, PP obtained samples for analysis, gathered clinical and demographic data, and helped prepare the manuscript. VAW, PJD, and DS obtained ethics committee approval, obtained samples for analysis, gathered clinical and demographic data, and helped prepare the manuscript. PS managed tissue sectioning and storage, provided administrative support, stained slides, and helped prepare the manuscript. SRP assisted in the study design and supervision, coordinated the study centers, obtained IRB approval, participated in data acquisition, analysis and interpretation, and critically revised the manuscript. All authors read and approved the manuscript.

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Abstract

Biopsies and ANCA testing for limited forms of granulomatosis with polyangiitis (GPA) are frequently nondiagnostic. We characterized gene expression in GPA and other causes of orbital inflammation. We tested the hypothesis that a subset of patients with nonspecific orbital inflammation (NSOI, also known as pseudotumor) mimics a limited form of GPA. Formalin-fixed, paraffin-embedded orbital biopsies were obtained from controls (n=20) and patients with GPA (n=6), NSOI (n=25), sarcoidosis (n=7), or thyroid eye disease (TED) (n=20) and were divided into discovery and validation sets. Transcripts in the tissues were quantified using Affymetrix U133 Plus 2.0 microarrays.

Distinct gene expression profiles for controls and subjects with GPA, TED, or sarcoidosis were evident by principal coordinate analyses. Compared to healthy controls, 285 probe sets had elevated signals in subjects with GPA and 1472 were decreased (>1.5-fold difference, false discovery rate adjusted $p < 0.05$). The immunoglobulin family of genes had the most dramatic increase in expression. Although gene expression in GPA could be readily distinguished from gene expression in TED, sarcoidosis, or controls, a comparison of gene expression in GPA versus NSOI found no statistically significant differences.

Thus, forms of orbital inflammation can be distinguished based on gene expression. NSOI/pseudotumor is heterogeneous but often may be an unrecognized, localized form of GPA

Keywords

Granulomatosis with polyangiitis; orbital pseudotumor; orbit pathology; molecular pathology; gene expression profiling; microarray analysis

INTRODUCTION

Limited forms of granulomatosis with polyangiitis (GPA) are challenging to diagnose. The antineutrophil cytoplasmic antibody test is often negative in patients with limited disease.[1] Biopsy of sites such as nasal mucosa, sinus, orbit, or subglottis often fails to produce definitive diagnostic information.[1-5] Thus, in theory many patients might have a limited form of GPA that cannot be confidently diagnosed unless the inflammation extends to additional sites.

Orbital inflammation is a challenging cause of vision loss, pain, diplopia, and even blindness. The differential diagnosis is extensive and includes GPA, thyroid eye disease (TED), sarcoidosis, lymphoma, metastatic disease, histiocytosis, xanthogranuloma, and infection.[6] Many patients with orbital inflammation have a disease that defies categorization despite biopsy. Terms to describe this latter condition include orbital pseudotumor, idiopathic orbital inflammatory disease, and nonspecific orbital inflammation (NSOI).[7-13]

Transcriptomics or gene expression profiling is rapidly changing the diagnostic approach to several malignant diseases. For example, lymphomas which cannot be distinguished on the basis of histology can be differentiated on the basis of gene expression.[14-16]

In order to elucidate the pathogenesis of GPA in relation to other forms of orbital inflammation, we assembled an international consortium of ocular pathologists and orbital surgeons to collect and analyze tissues from orbital biopsies. Our data show that GPA, sarcoidosis, TED, and healthy tissue each display a distinctive pattern of gene expression. The pattern from NSOI indicates more heterogeneity, but many tissues from NSOI cannot be distinguished from GPA on the basis of gene expression.

MATERIALS AND METHODS

Centers, Biopsies, Database

This study was approved by Institutional Review Boards or Research Ethics Committees from Oregon Health & Science University, Columbia University, University of California San Diego, Wake Forest University, Medical College of Wisconsin, Mount Carmel Health System (Ohio), University of Miami, University of British Columbia, Royal Adelaide Hospital, and the King Khaled Eye Specialist Hospital. Informed consent was obtained where required by the local review board. The research adhered to the tenets of the Declaration of Helsinki. Formalin-fixed, paraffin-embedded (FFPE) samples and relevant demographic and clinical data were obtained from the participating institutions. The diagnoses of NSOI, sarcoidosis, GPA, TED, and normal were based on the clinical and histopathological information submitted by physicians from their respective institutions.

Biopsies of the orbital adipose tissue from a total of 83 subjects were studied. The age, gender, and diagnoses for subjects are summarized in Table 1. Among the 6 subjects with a diagnosis of GPA, the antineutrophil cytoplasmic antibody (ANCA) test was positive in four of five and unknown in one. Of those with a positive test, 3 had a cytoplasmic ANCA pattern. The subject with a negative ANCA had pulmonary disease in addition to the orbital disease. One other subject with GPA had renal disease, but the others had a limited form of GPA. Among the 7 subjects diagnosed with sarcoidosis, all had noncaseating granulomata present on the orbital adipose biopsy. Adenopathy was present on chest CT scan in three for whom the test was reported. In a fourth patient, granulomas were found in a confirmatory lip biopsy. In another subject, the serum angiotensin converting enzyme level was elevated, but a chest CT scan was not obtained. In 2 subjects, a clinical diagnosis of sarcoidosis was made but the details of the diagnostic work up were not available. ANCA results were available for 12 of the subjects diagnosed with NSOI. All 12 were negative. The control tissue was obtained during surgeries, such as blepharoplasty and enucleation on patients with non-inflamed orbits.

Pathology Review

Two ocular pathologists (D.J.W. and H.E.G.) independently evaluated hematoxylin and eosin stained slides of all samples without reference to the indications for biopsy or other clinical information. After rendering a diagnosis in a masked fashion, the pathologists were informed of the diagnosis from the institution where tissue had been obtained. In some cases, additional stains were requested or additional clinical information was reviewed. A few cases with an ambiguous diagnosis were excluded. In all cases included in this study, a consensus diagnosis was determined by Drs. Wilson and Rosenbaum, and the contributing center's diagnosis was accepted. Thus, a diagnosis which combined clinical and histopathological information was considered the gold standard for the purposes of this study.

Tissue Preparation and Gene Expression Profiling

RNA extraction and microarray assays were performed in the OHSU Gene Profiling Shared Resource. The tissue samples, designated as set 1 (discovery set) and set 2 (validation set), were processed at two different time points. Between the two sample sets, the RNA amplification and labeling kit was modified by the vendor (Affymetrix P/N 703088 Rev1 vs P/N 703088 Rev 3). For each specimen, multiple 10-20 μm sections were collected, and total RNA was extracted with the miRNeasy FFPE kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. RNA was amplified and labeled with SensationPlus FFPE Amplification and 3' IVT Labeling kits (Affymetrix, Santa Clara, CA) for microarray analysis. For the majority of samples, an input of 50 ng of RNA was used, and a minimum input of 20 ng RNA was used for samples with limited RNA recoveries. Biotin-labeled cDNA targets were hybridized with a GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA), processed, and scanned according to vendor recommendations. The Human U133 Plus 2.0 array contains over 54,000 probe sets for 47,000 human transcripts and variants. Affymetrix GeneChip Command Console (AGCC) v. 3.1.1 and Affymetrix Expression Console v. 1.1 software were used for image processing and expression analysis for initial quality control, respectively. Raw and normalized gene

expression microarray data are available from the GEO database (accession numbers GSE58331 and GSM1407182 through GSM1407356).

RNA quality

RNA extracted from FFPE specimens was highly fragmented as is typical of this sample type. The RNA Quality Scores (RQS) from Caliper LabChip GX (Perkin-Elmer, Waltham, MA) HT RNA assays ranged from 1.8 to 6.5 with an average of 3.0 for Set 1 samples and from 1.7 to 7.4 with an average of 2.7 for Set 2. In both sample groups the majority of the samples had an RQS between 2 and 3 with RNA fragment sizes distributed between 25 and approximately 500 bases.

Statistical Analysis

The two sets of array data were analyzed independently because samples were processed at two different time points and the amplification and labelling kit had changed. Affymetrix CEL files were normalized by the Robust Multiarray Analysis.[17] Principal coordinate analysis (PCA) was used to visualize the dissimilarity in gene expression profiles across the disease groups.[18] Linear models were fitted to test potential differences in gene expression across disease groups after controlling for sex and age at biopsy. A probe set was considered statistically significantly changed when it had at least 1.5-fold change with a false discovery rate (FDR) adjusted p-value[19] less than 0.05 in both sets. All computations were done with the R project[20] and its add-on packages: “affy”, “limma”, and “MASS”.

The Gene Set Enrichment Analysis (GSEA) program[21, 22] was used as an independent means to identify genes with enriched expression in the clusters for microarray set 2 described above. GSEA rank order analysis was based on the 40,450 probe sets that remained after excluding probe sets that had not been associated in the Affymetrix annotation with a specific gene, i.e., annotated as unknown locus, open reading frame, etc. For genes with transcripts detected by more than one probe set, the probe set with the highest signal was used for the analysis. In addition, NIH DAVID[23] was used to estimate the number of unique genes in lists of probe sets.

RESULTS

We collected FFPE orbital adipose biopsies from 83 subjects from ten centers from North America, Saudi Arabia, and Australia (Table 1). As described in the methods section, set 1 and set 2 were processed and hybridized to arrays at two different times (about 1 year apart). Therefore, we analyzed the sets independently to minimize the influence of batch variation. The two sets are also called a discovery set and a validation set, respectively, for convenience throughout the manuscript.

To visualize NSOI heterogeneity in gene expression profiles and to compare these profiles to those of other orbital inflammatory diseases, we employed a principal coordinate analysis (PCA) that allows a comparison of complex data sets. In a PCA, the relative similarity of RNA patterns of two samples is indicated by their proximity on a graph. PCA plots based on all probe sets that showed a significant difference (FDR $p<0.05$ and 1.5-fold difference) between at least one disease group and the normal control group are shown in Figure 1 for

both data sets. Supplemental videos show these data in 3 dimensions (see Set1_PCA and Set2_PCA). The clustering of samples representing normal tissue indicates the relative similarity of the RNA patterns in the biopsies. The samples from subjects with TED are relatively homogeneous and have the closest proximity to normal samples among the four disease groups. The gene expression profiles from subjects with either sarcoidosis or GPA also tend to cluster. The gene expression profile for patients with NSOI shows more heterogeneity than the other four categories, consistent with the hypothesis that NSOI is not a single disease entity.

The GSEA program[21, 22] was used to interrogate the gene expression patterns further. GSEA provided a means to identify expression differences in predetermined sets of genes without reliance on the FDR p-values and fold-differences used to select the probe sets employed in the creation of the PCA plots. A GSEA-generated heat map (Figure 2) shows cytokine expression profiles for orbital adipose tissues from each experimental group. Consistent with the PCA plots, a subset of the NSOI profiles are very similar to the GPA profile.

Disease duration might markedly affect gene expression. We divided NSOI tissues from each set into samples biopsied less than 6 months after diagnosis or greater than 18 months after diagnosis, and we were unable to identify differences based on this variable. Similarly we hypothesized that corticosteroid use would markedly affect the detection of transcripts, but we could not identify differences in gene expression based on use of corticosteroid at the time of biopsy.

Table 2 and Supplemental Table 1 (see Supplementary Data file) contain data on specific gene expression in tissue from subjects with GPA based on both the validation and discovery sets. They show 285 probe sets (for about 212 genes) with signals that were at least 1.5-fold higher, with FDR adjusted $p<0.05$, in tissue from the orbital fat compared to tissue from orbital adipose tissue from subjects with no known orbital disease in both sets. Most of the transcripts with the highest fold increases in expression have immunological functions including the production of antibodies and cell signaling. Table 3 and Supplemental Table 2 (see Supplementary Data file) show a similar analysis except that the supplemental table lists 1,472 probe sets (about 1,054 genes) in common to both discovery and validation sets with lower signals in GPA in the orbit compared to uninflamed controls.

The expression profiles of orbital adipose tissues from the two granulomatous diseases, GPA and sarcoidosis, were also compared. Based on the same threshold of a least a 1.5-fold difference with FDR adjusted $p <0.05$ in both sets, we noted 54 probe sets (about 42 genes) with higher signals in GPA (Supplemental Table 3, see Supplementary Data file) and 237 probe sets (about 200 genes) with lower signals in GPA (Supplemental Table 4, see Supplementary Data file). The GPA-rich group has several cytokines expressed at relatively high levels, while the sarcoidosis group has a smaller, mostly different set of cytokine and cytokine receptor genes expressed at higher levels.

Additionally, we used Venn diagrams to depict the similarity or distinctiveness of gene expression in GPA in comparison with gene expression in healthy controls or other orbital

diseases (Figure 3). Although individual patients with NSOI clearly differed from GPA, as a group, there were no probe sets that were consistently, significantly different between GPA and NSOI.

Although no prior study has analyzed gene expression in orbital tissue from patients with GPA, we were able to compare our results to publications on either leukocytes or nasal brushings. One group has analyzed gene expression in GPA based on isolated leukocytes. [24, 25] Seven of the probe sets that we find upregulated in orbital adipose tissue from patients with GPA relative to controls were also upregulated in leukocytes from patients with GPA. These 7 mRNAs are matrix metalloproteinase 9 (MMP9), interleukin-7 receptor (IL-7R), CD64, interleukin-1 receptor antagonist (IL-1RN), T cell receptor 1 β (TCR 1β), solute carrier protein 11A1 (SLC11A1) (also known as natural resistance associated macrophage protein-1), and Toll like receptor 2 (TLR2). In addition we noted 21 probe sets that were down regulated in both our orbital tissue specimens and the leukocytes from patients with GPA (Supplemental Table 5, see Supplementary Data file 3).

A recent report on 10 subjects with active GPA characterized gene expression in cells obtained by brushing of nasal mucosa [26]. Although this study examined a different tissue and analyzed tissue obtained by brushing rather than biopsy [26], the two analyses again offer several concordant results. Both studies implicated contributors to innate immunity including SLC11, TLR2, and TLR8. Both SLC11 and TLR2 have now been implicated in leukocytes (24, 25), nasal mucosa [26], and orbit in the pathogenesis of GPA. The nasal study found up regulation of TREM1 (triggering receptor expressed on myeloid cells) and up regulation of interleukin-1 beta and the type II IL-1 receptor, whereas our study on orbital tissue implicated TREML2 (TREM like molecule 2) and the interleukin-1 receptor antagonist. Both studies discovered an up regulation of SERPINA1 (also known as alpha 1 anti-trypsin). The nasal study found many transcripts to be down regulated but included only ten in its publication [26]. One of these, SPARCL1 (secreted protein acid rich in cysteine-like 1) or hevin is also down regulated in orbital tissue affected by GPA.

DISCUSSION

To our knowledge, this report is the first analysis of gene expression in biopsies from patients with GPA and the first report to suggest that many patients with what was once called orbital pseudotumor actually have a limited form of GPA. The analysis of gene expression strongly supports the hypothesis that NSOI is a collection of diseases with the gene expression profile often overlapping with other known entities, namely TED, sarcoidosis, or most commonly GPA.

Gene expression profiling is providing insights into other inflammatory diseases.[27] For example, gene expression profiling from peripheral blood indicates that a subset of patients with systemic lupus erythematosus express several genes regulated by type I interferons.[28] Analysis of transcripts from solid tissue has been applied to eosinophilic esophagitis[29] and giant cell myocarditis.[30] Synovium from rheumatoid arthritis, spondyloarthritis, or osteoarthritis displays distinct patterns of mRNA or protein expression.[31, 32] This study

shows that a form of nonspecific inflammation can be classified on the basis of gene expression.

Our data indicate that GPA and sarcoidosis gene expression can be distinguished, but many of the tissues from subjects with NSOI have a gene expression profile indistinguishable from GPA. We did not detect a positive ANCA among these subjects with NSOI. Even among patients who are eventually diagnosed with GPA, biopsy of certain tissue sites such as the orbit, sinus, nasal mucosa, or subglottis provides definitive diagnostic information in a minority of cases.[1-5] We reported that an algorithm based on our data is more accurate than histopathology at identifying the cause of orbital inflammation.[33] We predict that molecular diagnosis will enhance the ability to diagnose GPA in mucosal sites.

Our sample population was adequate to analyze potential confounders including age, gender, disease duration, and use of corticosteroids. None of these factors could account for our findings (data not shown.)

Our report has limitations. Although we analyzed 83 tissues, they were divided among 5 separate conditions so that the diagnosis of GPA is represented by only six biopsies. The sample size, however, still allowed us to divide tissues into a discovery and validation set to demonstrate consistency in our findings. The GPA sample size was adequate to distinguish GPA clearly from sarcoidosis, TED, or controls, but not from NSOI. Furthermore, our tissue observations correlated with independent results reported for leukocytes [24, 25] or nasal brushings [26]. In addition, the upregulated transcripts which we detect are predominantly related to inflammation or response to infection. The transcripts identified by our study and previous analyses of RNA expression including TLR2 and SLC11 are especially intriguing. Many believe that bacteria such as *Staphylococcus aureus* act as a trigger for GPA. [34] Accordingly, the up regulation of TLR2, a receptor that recognizes bacterial cell wall, can easily be related to pathogenesis. Similarly SLC11, a molecule that transports iron, is also involved in host response to infection.[35, 36] Both our study and the report on nasal brushings from patients with GPA [26] found SPARCL1 to be down regulated. SPARCL1 deficiency has been implicated in cancer growth [37] and in abnormal wound healing [38], so it is easy to relate its relative lack to abnormal tissue repair.

We studied FFPE tissues. RNA in the blocks stored at room temperature degrades over time, and the quality of RNA that can be recovered decreases. Consequently we chose a cDNA synthesis protocol optimized for partially degraded RNA that would amplify relatively short sequences across the gene transcripts. We limited the duration that tissue could be stored until analysis. Still fresh tissue, frozen tissue, or an alternative fixation method might have yielded different results due to increased sensitivity. We limited our study to tissue from the orbit, which is composed mostly of adipose tissue in a healthy adult. NSOI or GPA can also affect the lacrimal gland (dacryoadenitis) and extra-ocular muscles (myositis). Additional studies are required to determine how results from the orbital adipose tissue compare to results from other sites. We did not validate individual levels of gene expression by a technique such as RT-PCR because we used a second data set for validation and because this report is based on patterns of gene expression, not on individual transcripts. Furthermore, we previously reported a correlation coefficient of >0.7 when we compared relative expression

levels determined by our microarray procedure with levels obtained from quantitative RT-PCR.[39]

On the basis of our experience to date, we predict that molecular profiling will eventually become a routine component in the evaluation of patients with orbital inflammatory disease. B cell depletion is now accepted as an approved form of therapy for GPA[40], but B cell depletion is not effective in the treatment of TED.[41] We and others have noted success in treating orbital inflammatory disease with rituximab therapy.[42, 43] The pronounced increased expression of transcripts relating to immunoglobulins adds rationale to this approach to treatment.

Additional experience in analyzing gene expression from orbital tissues may allow even greater diagnostic subdivision. Transcriptional profiling cannot be recommended as a routine diagnostic test, but this approach shows promise in defining a small set of transcripts with implications for both diagnosis and choice of therapy.

CONCLUSIONS

In summary, this study has resulted in several novel findings. Gene expression profiling can distinguish the major causes of orbital inflammation. Although NSOI is a heterogeneous collection of diseases, some patients labeled as having NSOI may actually have a limited form of GPA. Immunoglobulin gene expression characterizes orbital tissue from patients with orbital GPA in comparison to healthy controls. Some of the transcripts with increased expression in orbital tissue from patients with GPA have also been reported to be increased in leukocytes or nasal brushings from patients with GPA. An extrapolation from this study is that the diagnostic yield from biopsies to assess the likelihood of GPA in several sites will be improved by analysis of the genes expressed in the biopsied tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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JTR has in the past consulted for Genentech and was a co-investigator on a studied funded by Genentech to evaluate the use of rituximab for orbital inflammatory diseases.

List of abbreviations

GPA	granulomatosis with polyangiitis
TED	thyroid eye disease

NSOI	nonspecific orbital inflammation
GSEA	gene set enrichment analysis
FFPE	formalin fixed, paraffin embedded
PCA	principal coordinate analysis
ANCA	anti-neutrophil cytoplasmic antibody
NRAMP	natural resistance associated macrophage protein
MMP	matrix metalloproteinase

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Highlights

- Some types of orbital inflammation are distinguishable by gene expression profiles.
- Nonspecific orbital inflammation can be subdivided by analysis of gene expression.
- Some NSOI gene expression profiles are indistinguishable from those of GPA.
- Limited GPA in the orbit requires gene expression profiling to be identified.

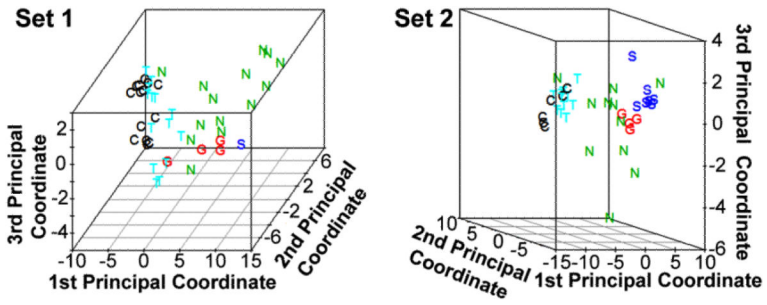

Figure 1. PCA reveals clustering of expression profiles of samples within each experimental group except NSOI

In both data sets, the NSOI samples were much more heterogeneous and not clustered. The distance between points on the plots is indicative of the difference between gene expression profiles. Rotatable views of the 3-dimensional plots are in online supplemental files (Set1_PCA and Set2_PCA). C: Control; G: GPA; N: NSOI; S: Sarcoidosis; T: TED.

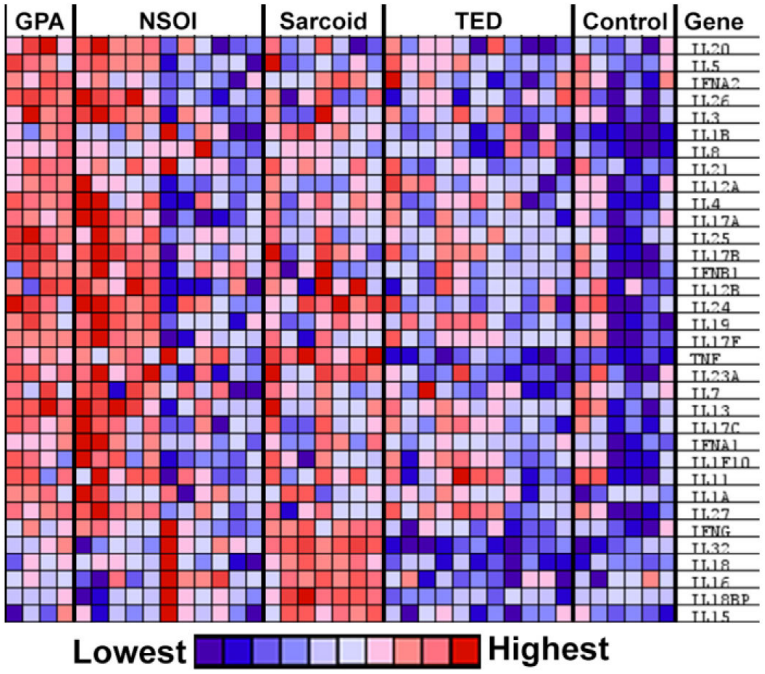

Figure 2. Orbital adipose tissues from some NSOI patients have cytokine expression profile similar to GPA patients

The GPA profile differs from the sarcoidosis, TED, and control profiles. A heat map of relative transcript levels was generated by GSEA from data set 2.

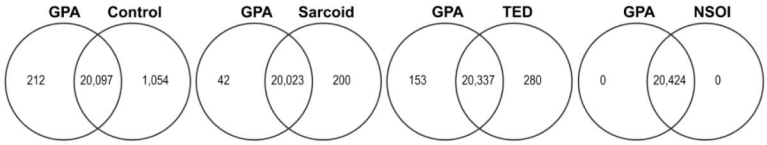

Figure 3. Venn diagrams comparing gene expression in orbital adipose from GPA patients with the other experimental groups

Expression levels in tissues from subjects with GPA were compared to levels in uninflamed control, sarcoidosis, NSOI, and TED orbital adipose samples. Probe sets were considered to indicate a significant difference between groups when their signals had at least a 1.5-fold change with a FDR adjusted p-value less than 0.05 in both sets. Shown are the numbers of unique genes identified by NIH DAVID from probe set lists for each category.

Table 1

Ages and genders for each experimental group

	Set 1			Set 2		
Diagnosis	**N**	**Mean age at biopsy**	**Female %**	**N**	**Mean age at biopsy**	**Female %**
NSOI	14	44.0 ± 21.8	64.3	11	58.5 ± 24.1	63.6
Sarcoid	1	60.6	100	7*	48.8 ± 14.7	71.4
TED	14	54.0 ± 14.7	78.6	11	48.6 ± 13.0	72.7
GPA	4	43.4 ± 14.3	75.0	4**	40.0 ± 13.9	50.0
Normal	14	61.0 ± 15.6	64.3	6	69.7 ± 9.8	83.3

* one repeated from set 1;

** two repeated from set 1

Table 2

Probe sets indicating increased expression of genes in orbital adipose tissues from subjects with GPA.

		Set 1		Set 2	
Probe Set	**Gene Title**	**Fold Difference**	**FDR P-value**	**Fold Difference**	**FDR P-value**
209875_s_at	secreted phosphoprotein 1	56.68	7.04E-04	54.74	2.51E-03
214677_x_at	immunoglobulin λ constant 1 (Mcg marker)	9.35	9.63E-03	58.44	1.11E-03
216491_x_at	immunoglobulin heavy constant mu	15.85	1.30E-03	41.14	3.77E-04
214669_x_at	immunoglobulin κ constant	13.28	3.77E-04	41.10	7.07E-04
215379_x_at	immunoglobulin λ variable 1-44	8.33	3.30E-03	36.77	1.38E-03
209396_s_at	chitinase 3-like 1 (cartilage glycoprotein-39)	20.75	2.12E-04	10.78	2.16E-02
214973_x_at	immunoglobulin heavy constant delta	12.44	3.35E-04	15.87	6.54E-03
202834_at	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	12.04	1.18E-03	8.18	4.06E-02
1555745_a_at	lysozyme	9.04	2.47E-03	10.53	1.83E-02
1555756_a_at	C-type lectin domain family 7, member A	10.12	1.38E-02	8.19	3.73E-02
211429_s_at	serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1	11.48	1.75E-04	6.31	3.80E-02
205267_at	POU class 2 associating factor 1	10.59	1.47E-03	7.01	1.47E-02
219386_s_at	SLAM family member 8	11.25	8.00E-06	5.80	3.41E-02
211919_s_at	chemokine (C-X-C motif) receptor 4	8.67	1.13E-02	7.30	7.46E-03
203936_s_at	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	10.40	2.16E-05	4.75	1.54E-02
204116_at	interleukin 2 receptor, γ	8.62	9.70E-04	5.94	1.70E-02
217235_x_at	immunoglobulin λ-like polypeptide 5	6.24	7.73E-03	8.23	1.51E-02
219890_at	C-type lectin domain family 5, member A	7.85	1.70E-06	5.68	6.26E-03
210072_at	chemokine (C-C motif) ligand 19	8.99	1.37E-04	4.07	4.51E-02
224342_x_at	BMS1 homolog, ribosome assembly protein (yeast) pseudogene	6.29	2.66E-03	5.59	4.79E-02
222838_at	SLAM family member 7	5.30	4.72E-03	6.46	3.09E-02
1555349_a_at	integrin, β 2 (complement component 3 receptor 3 and 4 subunit)	6.57	2.61E-04	5.06	7.82E-03
1558199_at	fibronectin 1	4.89	2.41E-02	6.07	6.71E-03
205798_at	interleukin 7 receptor	6.15	1.21E-02	4.42	1.78E-02
213193_x_at	T cell receptor β constant 1	4.96	3.31E-03	5.37	1.81E-02
220306_at	family with sequence similarity 46, member C	4.28	3.16E-02	6.01	2.99E-02
205997_at	ADAM metallopeptidase domain 28	6.21	4.19E-03	3.80	4.67E-02
1552806_a_at	sialic acid binding Ig-like lectin 10	5.43	8.53E-05	3.35	4.11E-02
233510_s_at	parvin, γ	5.44	5.90E-04	3.20	5.80E-03
211881_x_at	immunoglobulin λ joining 3	3.10	4.14E-02	5.53	2.30E-02
211908_x_at	immunoglobulin κ locus	3.52	1.58E-02	5.11	8.75E-04
205213_at	ArfGAP with coiled-coil, ankyrin repeat	4.85	6.72E-03	3.48	1.37E-03

		Set 1		Set 2	
Probe Set	**Gene Title**	**Fold Difference**	**FDR P-value**	**Fold Difference**	**FDR P-value**
	and PH domains 1				
207794_at	chemokine (C-C motif) receptor 2	4.38	1.22E-02	3.40	1.82E-02
212657_s_at	interleukin 1 receptor antagonist	4.06	8.97E-03	3.69	4.68E-03
229041_s_at	ITGB2 antisense RNA 1	4.05	1.73E-03	3.68	3.71E-02
215946_x_at	immunoglobulin λ-like polypeptide 3, pseudogene	3.68	4.55E-02	3.78	2.39E-02
226658_at	podoplanin	3.28	1.54E-02	4.13	2.46E-02
222858_s_at	dual adaptor of phosphotyrosine and 3-phosphoinositides	4.07	1.40E-03	3.32	3.07E-02
204852_s_at	protein tyrosine phosphatase, non-receptor type 7	3.90	2.36E-04	3.44	3.27E-03
1552960_at	leucine rich repeat containing 15	3.66	2.53E-02	3.64	1.51E-02
211991_s_at	major histocompatibility complex, class II, DP α 1	3.21	2.22E-02	4.08	5.08E-03
229721_x_at	derlin 3	3.87	8.11E-03	3.21	2.40E-02
1552497_a_at	SLAM family member 6	3.30	4.60E-03	3.75	1.87E-03
224310_s_at	B-cell CLL/lymphoma 11B (zinc finger protein)	3.65	4.56E-02	3.25	8.32E-04
209696_at	fructose-1,6-bisphosphatase 1	3.32	2.01E-02	3.57	2.58E-02
210423_s_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	3.48	3.64E-03	3.32	1.89E-02
1553863_at	WDFY family member 4	2.98	5.10E-03	3.68	3.86E-05
219971_at	interleukin 21 receptor	2.88	4.59E-02	3.65	1.61E-03
228658_at	myocardial infarction associated transcript (non-protein coding)	3.25	2.39E-02	3.21	8.95E-03
202856_s_at	solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	2.76	8.22E-03	3.46	1.55E-03

Shown here are 50 probe sets with the highest fold differences compared to uninflamed control tissues and with adequate annotation. For genes interrogated by multiple probe sets, only the one with the highest fold difference is listed here. The entire list of probe sets with significantly higher signals is in Supplemental Table 1.

Table 3

Probe sets indicating decreased expression of genes in orbital adipose tissues from subjects with GPA.

Probe Set	Gene Title	Set 1		Set2	
		Fold Difference	FDR P-value	Fold Difference	FDR P-value
205913_at	perilipin 1	−143.00	1.25E-04	−121.91	1.11E-05
209612_s_at	alcohol dehydrogenase 1B (class I), β polypeptide	−105.20	5.19E-05	−110.27	8.66E-06
207175_at	adiponectin, C1Q and collagen domain containing	−91.04	2.35E-04	−67.65	2.89E-05
205478_at	protein phosphatase 1, regulatory (inhibitor) subunit 1A	−54.07	5.49E-05	−69.20	3.00E-06
1565162_s_at	microsomal glutathione S-transferase 1	−37.42	1.95E-04	−69.65	8.09E-07
219398_at	cell death-inducing DFFA-like effector c	−47.89	3.18E-05	−52.73	1.65E-05
226304_at	heat shock protein, α-crystallin-related, B6	−13.64	1.57E-03	−80.10	9.01E-06
203980_at	fatty acid binding protein 4, adipocyte	−43.92	4.28E-04	−44.16	9.18E-05
225420_at	glycerol-3-phosphate acyltransferase, mitochondrial	−48.61	8.73E-05	−38.56	9.04E-05
201540_at	four and a half LIM domains 1	−25.32	6.97E-04	−55.33	2.54E-05
209699_x_at	aldo-keto reductase family 1, member C2	−33.58	5.20E-06	−42.33	1.65E-05
211356_x_at	leptin receptor	−24.20	4.22E-04	−37.59	4.87E-06
209555_s_at	CD36 molecule (thrombospondin receptor)	−34.54	1.22E-03	−27.21	2.79E-04
202036_s_at	secreted frizzled-related protein 1	−31.23	1.21E-05	−27.05	2.42E-07
204151_x_at	aldo-keto reductase family 1, member C1	−17.12	3.04E-05	−40.77	1.76E-05
209283_at	crystallin, αB	−10.13	2.11E-03	−46.48	4.25E-05
212097_at	caveolin 1, caveolae protein, 22kDa	−14.11	7.32E-04	−39.56	6.29E-05
204894_s_at	amine oxidase, copper containing 3	−30.59	2.46E-04	−22.59	2.55E-05
219140_s_at	retinol binding protein 4, plasma	−23.29	6.10E-06	−27.16	1.87E-05
222124_at	hypoxia inducible factor 3, α subunit	−18.41	5.96E-03	−31.88	6.02E-05
221796_at	neurotrophic tyrosine kinase, receptor, type 2	−18.69	2.24E-04	−30.75	1.31E-05
229476_s_at	thyroid hormone responsive	−20.58	2.24E-04	−28.73	4.43E-05
203324_s_at	caveolin 2	−12.07	5.31E-03	−35.52	2.89E-06
203407_at	periplakin	−17.08	8.92E-06	−27.81	1.11E-06
222513_s_at	sorbin and SH3 domain containing 1	−10.64	1.67E-03	−34.00	8.86E-05
205498_at	growth hormone receptor	−19.66	1.40E-04	−24.45	7.97E-06
202016_at	mesoderm specific transcript	−19.57	2.23E-04	−21.60	9.30E-06
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	−21.77	7.39E-04	−18.77	8.67E-06
1555740_a_at	melanocortin 2 receptor accessory protein	−19.05	3.34E-04	−20.58	4.22E-05
212230_at	phosphatidic acid phosphatase type 2B	−13.57	1.30E-03	−26.03	7.54E-06
210963_s_at	glycogenin 2	−20.66	4.64E-04	−18.21	6.38E-05
213524_s_at	G0/G1switch 2	−13.65	4.73E-03	−24.89	1.41E-04
222835_at	thrombospondin, type I, domain	−13.50	2.27E-03	−24.22	6.56E-05

Probe Set	Gene Title	Set 1		Set2	
		Fold Difference	FDR P-value	Fold Difference	FDR P-value
	containing 4				
201432_at	catalase	−10.70	1.20E-03	−25.30	2.14E-05
209763_at	chordin-like 1	−16.42	9.03E-04	−19.39	6.03E-06
225207_at	pyruvate dehydrogenase kinase, isozyme 4	−13.50	1.04E-02	−21.80	8.69E-05
221748_s_at	tensin 1	−9.92	2.77E-04	−25.36	9.14E-04
225975_at	protocadherin 18	−16.49	1.15E-04	−18.31	3.06E-06
212741_at	monoamine oxidase A	−16.69	1.37E-04	−18.01	2.38E-06
243585_at	ATPase type 13A5	−14.16	3.23E-04	−19.16	6.14E-06
219789_at	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	−15.40	4.13E-04	−17.67	7.73E-07
203851_at	insulin-like growth factor binding protein 6	−9.38	1.10E-03	−20.16	3.77E-06
222484_s_at	chemokine (C-X-C motif) ligand 14	−5.60	4.36E-02	−23.53	1.30E-03
228224_at	proline/arginine-rich end leucine-rich repeat protein	−6.57	1.93E-02	−21.02	7.12E-06
211959_at	insulin-like growth factor binding protein 5	−9.01	9.98E-03	−18.21	1.56E-04
206243_at	TIMP metallopeptidase inhibitor 4	−9.02	4.52E-03	−18.13	5.55E-05
227646_at	early B-cell factor 1	−9.45	2.29E-03	−17.15	1.28E-05
212071_s_at	spectrin, β, non-erythrocytic 1	−6.58	2.51E-03	−19.02	4.42E-04
203571_s_at	adipogenesis regulatory factor	−7.67	2.98E-04	−16.93	3.89E-05
201525_at	apolipoprotein D	−4.64	7.20E-03	−18.85	1.02E-03

Shown here are 50 probe sets with the largest negative fold differences compared to uninflamed control tissues and with adequate annotation. For genes interrogated by multiple probe sets, only the one with the largest negative fold difference is listed here. The entire list of probe sets with significantly lower signals is in Supplemental Table 2.