



BIOINTERPRETER

New Biological Perspective to your Differentials

Case Study

An alternate scientific analysis of the article by Reghunanathan et al (PMID: 15655079). A case study for the demonstration of Biointerpreter

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Case Study Abstract

In this information era where rapid parallel advances in computing and biology yields huge amounts of raw data within a short period of time, validating and importantly interpreting them is a huge challenge, especially in the form of multidimensional biological models and inter-linking them to give life to meaningful concepts- without risking the fear of getting lost in what we can't readily see and comprehend. Our attempt to meet this challenge and offer a solution- Biointerpreter, is a web based versatile tool to address these challenges confidently. Here, we have assembled a case study to demonstrate all the tools, the versatility, efficiency, scientific value and advantage of using Biointerpreter over a scenario in which it was not used as in this model publication "Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome", by Renji Reghunathan et al PMID: 15655079. The authors sincerely do not feel or wish to indicate that all the arduous scientific efforts and the strong, lucid analyses of the original authors are inadequate or incomplete. We simply wish to portray that our tool has far reaching overall potential in the scope of analysis of microarray data achievable in a much shorter time period through a more complex and thorough systems biology approach and wish to in fact, thank them for making their publication available in the public domain for critical review.

About Biointerpreter

Meeting the challenges in biological interpretation of Genomics data

- How to make sense out of so many gene lists?
- How to speed up biological analysis?
- How to quickly get updated and comprehensive annotations
- Is there a simple, quick and effective solution for interpreting your microarray data?

Genotypic's Biointerpreter

Biointerpreter is a user-friendly web-based Biological interpretation tool for microarray data. It significantly reduces the biological analysis time from weeks to hours. Biointerpreter is provided for users as annual subscription.

Publication Abstract

Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome

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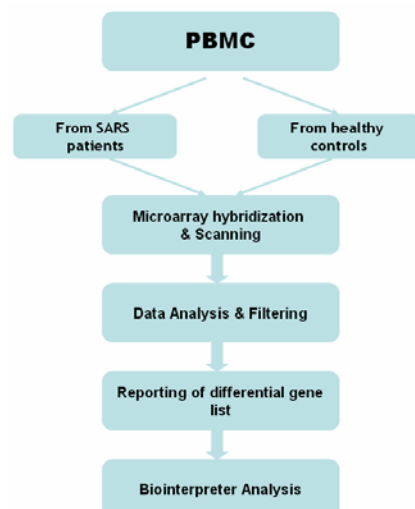
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We investigated the gene expression profiles of peripheral blood mononuclear cells (PBMCs) derived from SARS patients, and compared with healthy controls. The number of differentially expressed genes was found to be 186 under stringent filtering criteria of microarray data analysis. Several genes were highly up-regulated in patients with SARS, such as, the genes coding for Lactoferrin, S100A9 and Lipocalin 2. The real-time PCR method verified the results of the gene array analysis and showed that those genes that were up-regulated as determined by microarray analysis were also found to be comparatively up-regulated by real-time PCR analysis. This differential gene expression profiling of PBMCs from patients with SARS strongly suggests that the response of SARS affected patients seems to be mainly an innate inflammatory response, rather than a specific immune response against a viral infection, as we observed a complete lack of cytokine genes usually triggered during a viral infection. Our study shows for the first time how the immune system responds to the SARS infection, and opens new possibilities for designing new diagnostics and treatments for this new life-threatening disease.

Publication Link: [Pubmed Central](#) [BMC Immunology](#)

Experimental Overview



Differentially regulated genes

Immune-response related genes which were found to be significantly up-regulated in PBMCs of SARS patients. Level of expression is expressed in Fold change (average of fold changes of ten patients, S1-S10) as compared to that of control samples from normal human subjects (C1-C4). ([Table 1](#))

Immune-response related genes which were found to be significantly down-regulated in PBMCs of SARS patients. Level of expression is expressed in Fold change (average of fold changes of ten patients, S1-S10) as compared to that of control samples from normal human subjects (C1-C4). ([Table 2](#))

Genes involved in homeostasis and cell growth, which were found to be significantly up-regulated in PBMCs of SARS patients. Level of expression is expressed in Fold change (average of fold changes of ten patients, S1-S10) as compared to that of control samples from normal human subjects (C1-C4). ([Table 3](#))

[Get the Gene list used in this analysis](#)

Discussion by authors in publication

Several genes were highly upregulated in patients with SARS, such as the genes coding for Lactoferrin, S100A9 and Lipocalin. Upregulation of expression of genes such as [Bacterial Permeability Increasing Protein \(BPI\) and carcinoembryonic antigen related cell adhesion molecule 8 \(CECAM8 or CD66b\)](#). CEACAM 8 is expressed by activated monocytes and granulocytes, and significant up-regulation of this gene indicates the [involvement of innate immune cells in SARS](#). Other genes which encode proteins like Leukotrien-B4 receptor (LTB4R), Leukotrien - A4 hydrolase, IL-8 receptor (IL-8RA), anaphylatoxin C3a receptor-1 (C3aR1), Neutrophil Cytosolic Factor 1 (NCF 1), S100 calcium binding protein A9, Defensin, (DEFA 1/4), LPS binding protein CAP18 (CAMP), and Peptidoglycan Recognition Protein (PGLYRP) are involved in [chemotaxis, inflammatory reaction and superoxide metabolism](#). Similarly, [Formyl Peptidase Receptor \(FPR\) genes are expressed by activated neutrophils](#). Up-regulation of CD24 and FcR3A indicates some degree of neutrophil, B cell and NK cell activity. FcR3a, a low affinity receptor for IgG, expressed in activated macrophages and NK cells. There is moderate up-regulation of the kappa light chain of the Nuclear Factor (NFB 1A) and the B- cell lymphoma 3-encoded protein (BCL3) (Table 1). In SARS patients there is down-regulation of genes (Table 2) which regulate proliferation and differentiation of T-cells, such as the [Lymphocyte-specific protein tyrosine kinase \(LCK\)](#), which is [required for phosphorylation of CD3](#).

There is down-regulation of genes coding for the epsilon polypeptide of the TCR (CD3E), IL-2 induced T-cell kinase (ITK), the Zeta chain of the TCR (CD3Z), the Alpha 4 subunit of VLA-4 receptor (ITGA4), the Chemokine (C-C motif) Receptor 7 (CCR7), and the Interleukin 10 receptor alpha (IL10 RA). In our study, there is up-regulation of genes involved in [homeostasis and cell growth](#) (Table 3); for example, genes involved in DNA synthesis, nucleosome assembly and protein synthesis, such as, genes encoding Translation Initiation Factor 1A (EIF1 AY), and histone proteins. There is down-regulation of genes coding for [negative regulators of the cell cycle](#), such as, [Retinoblastoma-like 2 protein \(RBL2\)](#), Cyclin-Dependent Kinase Inhibitor 1B (CDKN1B) and anti-apoptotic protein TNF induced protein GG2-1 etc (data not shown). The observed results, in figure 2, showed that in the influenza patients the mRNA for type I interferons (IFN α and IFN β), TNF α and IL-12-p40 were upregulated, whereas genes upregulated in the SARS patients (LTF, Lipocalin, S100P, FGR3A and TLR2), were not upregulated in the influenza patients.

Biointerpreter Analysis

- ☛ Significant representation from one location, **15 genes cluster in chromosome 1, arm "q" was found to be up regulated- Possible amplification event driven by SARS virus infection or mere co-regulation**. Viral infection susceptible region?
- ☛ Publication reported around 50 genes related to immune response being up regulated. **Biointerpreter found out an additional 12 genes related to immune response being activated upon viral infection**. Candidates for studying infection inducible gene expression?
- ☛ Many other significant biological processes that were not reported were found to be up regulated by Biointerpreter – **Molecular events that were co-regulated upon viral infection which were not reported and significant class of genes that were activated were also reported by Biointerpreter**. **These events and gene classes' information might have helped the investigators hypothesize in depth changes that were induced as a result of viral pathogenesis**.

☛ Significant number of up regulated genes were found to be localized in the cytoplasm, plasma membrane and outside which were not reported – **Statistically significant number of genes was found to be localized extra cellular or secreted and in cytoplasm.** These genes can be screened for groups which can be used as potential diagnostic markers of SARS infection using PCR based methods.

☛ More than 1/3rd of the up regulated genes were known to be associated with major viral immune related diseases like AIDS and Influenza. ~ 20genes were previously found to be altered upon HIV and Influenza virus infection. These genes could provide vital clues to gene regulation upon viral infection.

☛ Biological processes which were mentioned to be altered by up and down regulated genes after in-depth analysis were reported in just a click of mouse – **Biointerpreter was able to find more number of genes related to the key molecular events behind the viral pathogenesis was reported by Biointerpreter adding significance to the hypothesis.** Lung tumor specific up regulation was observed indicating tissue specific gene activation response by virus infection. More than a 12 genes were up regulated in lung tumor samples indicating cell or tissue specific induction of gene expression response.

☛ Majority of the differentially regulated genes belonged to immune and hematopoietic phenotype. **Biointerpreter reported immune response and hematopoietic phenotype to be significantly induced in a single batch query submission, which was in conjunction with the findings of the investigators.**

Biological processes which were found to be up regulated by Biointerpreter in addition

- Gene regulation
- Metabolism
- Antigen processing & presentation
- Growth factors & regulators
- Cell cycle
- Cell proliferation

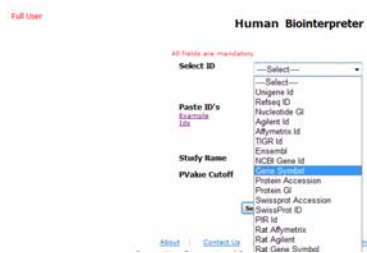
Conclusion

Using Biointerpreter, given a gene list one can confidently decipher the biologically significant events, without missing the obvious

Biointerpreter Login Page



Selecting Gene identifier



Pasting Gene List



Results & Discussion

Chromosome

	Chromosome	No of genes	P value
<input type="checkbox"/>	1[2263]	15	1E-09
<input type="checkbox"/>	4[883]	6	1E-09
<input type="checkbox"/>	12[1128]	9	1E-09
<input type="checkbox"/>	14[683]	6	1E-09
<input type="checkbox"/>	Y[57]	4	1E-09
<input type="checkbox"/>	8[770]	5	0.0016
<input type="checkbox"/>	19[1374]	7	0.0028
<input type="checkbox"/>	20[590]	4	0.0034
<input type="checkbox"/>	6[1173]	6	0.0055
<input type="checkbox"/>	3[1211]	6	0.0072

[Select All](#) [Clear](#) [TableView](#) [Pie Chart](#)

15 genes cluster in chromosome 1, arm "q" was found to be up regulated.

Query id	Unigene ID	Gene Symbol	Gene Name	Chromosome	Cytoband
ACTN1	Hs.509765	ACTN1	Actinin, alpha 1	1	1q32.1 ; 14q24.1-q24.2
HIST2H2AA4	Hs.647733	HIST2H2AA4	Histone cluster 2, H2aa4	1	1q21.2
TROVE2	Hs.208176	TROVE2	TROVE domain family, member 2	1	1q31
MTX1	Hs.490074	MTX1	Metaxin 1	1	1q21
VAMP3	Hs.66708	VAMP3	Vesicle-associated membrane protein 3 (cellubrevin)	1	1p36.23
FCGR3A	Hs.372670	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	1	1q23
MNDA	Hs.153837	MNDA	Myeloid cell nuclear differentiation antigen	1	1q22
RAB13	Hs.151536	RAB13	RAB13, member RAS oncogene family	1	1q21.2
S100A9	Hs.112405	S100A9	S100 calcium binding protein A9	1	1q21
STIL	Hs.525190	STIL	SCL/TAL1 interrupting locus	1	1q32 ; 1p32
HIST2H2BE	Hs.2178	HIST2H2BE	Histone cluster 2, H2be	1	1q21 ; 1q21-q23
S100A12	Hs.19413	S100A12	S100 calcium binding protein A12	1	1q21
PADI4	Hs.522969	PADI4	Peptidyl arginine deiminase, type IV	1	1p36.13
CD247	Hs.156445	CD247	CD247 molecule	1	1q22-q23
LCK	Hs.470627	LCK	Lymphocyte-specific protein tyrosine kinase	1	1p34.3

Possible amplification event driven by SARS virus infection. Viral infection susceptible region?

Functions

Function	No of genes	P value
Activation[4856]	65	1E-09
Ageing[379]	8	1E-09
Angiogenesis[682]	17	1E-09
Antigen Processing And Presentation [1554]	41	1E-09
Apoptosis[2028]	35	1E-09
Biosynthesis[1299]	11	1E-09
Cell Adhesion[1915]	30	1E-09
Cell Cycle[2387]	28	1E-09
Cell Differentiation[1363]	23	1E-09
Hemostasis[222]	8	1E-09
Homeostasis[602]	13	1E-09
Immune Response[5232]	79	1E-09

BioInterpreter found out an additional 12 genes related to immune response being activated upon viral infection.

Cellular Localization

Localization	No of genes	P value
Cytoplasm[3802]	36	1E-09
Endosome[227]	3	1E-09
Extracellular[1332]	18	1E-09
Lysosomes[228]	4	1E-09
Plasma Membrane[2092]	24	1E-09
Secreted[1397]	20	1E-09
Vesicles[873]	6	1E-09

Select All Clear And Or(Default) TableView Pie Chart

Significant number of up regulated genes were found to be localized in the cytoplasm, plasma membrane and secreted.

Diseases

Disease	No of genes	P value
Aids[1369]	27	1E-09
Alzheimer Disease[360]	9	1E-09
Amoebiasis[10]	1	1E-09
Anal Cancer[4871]	2	1E-09
Bladder Cancer[179]	4	1E-09
Breast Cancer[923]	18	1E-09
Influenza[1391]	27	1E-09

More than 40 up regulated genes were already known to be associated with major viral immune related diseases like AIDS and Influenza.

BioInterpreter is Biologist friendly: Advanced Excel and database skills not required

Cancer Gene Expression

Cancer Type	Up	Down	Both	P value
Blood Tumor[253]	3	0	0	0.0001
Pancreas Tumor[361]	2	0	1	0.0029
Breast Tumor[61]	0	1	0	0.0067
Prostate Tumor[83]	1	0	0	0.0256
Adrenal Tumor[20]	0	1	0	1E-09
Bone Tumor[190]	4	0	0	1E-09
Cervix Tumor[59]	1	2	0	1E-09
Colon Tumor[1033]	8	0	1	1E-09
Germcell Tumor[905]	5	2	0	1E-09
Head&Neck Tumor[205]	0	4	0	1E-09
Liver Tumor[192]	3	0	0	1E-09
Lung Tumor[1259]	12	1	0	1E-09
Ovary Tumor[963]	4	2	4	1E-09
Uterus Tumor[940]	4	2	1	1E-09

Select All Clear And Or(Default) TableView Pie Chart Up

Lung tumor specific up regulation was observed indicating tissue specific gene activation response by virus infection

Phenotype

Phenotype	No of genes	P value
adipose tissue phenotype[198]	5	1E-09
cellular phenotype[630]	11	1E-09
hematopoietic system phenotype[928]	21	1E-09
immune system phenotype[1204]	35	1E-09
life span-post-weaning/aging[651]	12	1E-09
other phenotype[82]	2	1E-09
pigmentation phenotype[148]	3	1E-09
renal/urinary system phenotype[435]	7	1E-09
tumorigenesis[323]	4	1E-09

Select All Clear And Or(Default) TableView Pie Chart

Majority of the differentially regulated genes belonged to immune and hematopoietic phenotype.

BioInterpreter is available for:

- ✓ Human
- ✓ Mouse
- ✓ Rat
- ✓ Arabidopsis
- ✓ Drosophila
- ✓ Rice
- ✓ M.tuberculosis
- ✓ Yeasts and other plant species coming soon

Human BioInterpreter Statistics

Number of functions annotated by Genotypic: **57**
 Number of disease annotated by Genotypic: **122**
 Number of sub-cellular localization annotated by Genotypic: **20**
 Number of Gene expression conditions curated by Genotypic: **1576**
 Number of Gene expression data points available in the database: **84850**
 Number of research articles curated: **417**
 Number of Pubmed abstracts curated: **> 500,000**

Using BioInterpreter, we arrived to the same conclusion as reported in the publication and server other interesting relationships were also found within few hours!

Testimonials

• BioInterpreter allowed me to see clear functional difference of 3 categories of ovarian tumors. I got a better view of the Biology behind the differential expression.

James Cherry, SAIC-Frederick, NCI at Frederick. Gene Expression Laboratory, MD, USA

• These are not ME TOO tools; these address the unmet need of biological interpretation of Microarray data.

Preveen Ramamoorthy, Medimmune vaccines Inc, CA USA.

• The software is very useful in microarray data interpretation and in predicting mechanism of action.

Dr. Abhijit Chatterjee, Zydus Research Centre, India

• Genotypic has developed an essential tool for analysis of microarray data. This tool helps even a beginner to achieve better interpretation of microarray data. I hope Genotypic will continue to develop such tools to assist the microarray-gene expression community.

Dr. Sanjeev Galande, National Centre for Cell Science, India

• I have been using your BioInterpreter tool a lot recently. It is absolutely fabulous! A real gold mine for microarray analysis!

Claire Bonfils, Senior Research Scientist, Methylgene Inc, Canada

BioInterpreter Users worldwide

Quark Biotech - USA and Israel, EMBL - Heidelberg Germany, Yale University - USA, NCI at Frederick. Gene Expression Laboratory, MD, USA, Medimmune vaccines Inc, CA USA, Methylgene - Canada, Hellenic Pasteur Institute - Greece, Astra Zeneca Research Foundation - India, Indian Institute of Science - India, Indian Institute of Technology Kanpur - India, National Centre for Biological Science - India, National Centre for Cell Sciences - India, Zydus Research Centre, India, Unilever - India.

Try BioInterpreter today!

BioInterpreter is currently available on a free, limited time, full-functionality access to users who have significant research interests. Write to biointerpreter@genotypic.co.in for your trial login or fill up the form available at http://genotypic.co.in/biointerpreter_register.html

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