Published in final edited form as:

Mol Cell Endocrinol. 2018 August 15; 471: 97–104. doi:10.1016/j.mce.2017.07.037.

Differentially Expressed Genes in PPAR γ -deficient MSCs

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Abstract

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a key regulator of adipogenesis. It is also a central player in energy metabolism, inflammation and immunity. As an important nuclear transcription factor, PPAR γ can regulate the expression and function of genes or biological processes directly or indirectly via association with other factors and thus modulate their activities. To better understand the impact of PPAR γ on the global gene expression profile, we evaluated the bioinformatic data, which revealed the changes that occurred in genes and their pathways in the absence of PPAR γ . In brief, we performed RNA deep sequencing (RNA-Seq) analysis using RNA samples isolated from multipotent mesenchymal stromal cells (MSCs) of PPAR γ knockout and wild type control mice. The RNA-Seq data sets were then subjected to bioinformatic analyses from various angles to better reveal the breadth of PPAR γ function in different biological processes. Our results reveal novel genes and networks modulated by PPAR γ and provides new insights into our understanding of the physiologic and pathophysiologic role this nuclear receptor plays in health and disease.

Keywords

RNA-Seq; differential expression; PPAR γ ; inflammation; MSC; adipocyte; bone marrow

1. Introduction

The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ) is a key transcription factor regulating not only adipocyte differentiation (Barak et al., 1999; Rosen et al., 1999), but also glucose metabolism and energy balance (Rosen and Spiegelman, 2001; Sharma and Staels, 2007). The mesenchymal stromal/stem cells (MSCs) are multipotent and

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capable of differentiating into several distinct cell lineages, including osteoblasts, adipocytes, chondrocytes, muscle cells, and even neuronal cells (Kopen et al., 1999; Minguell et al., 2001; Pittenger et al., 1999; Prockop, 1997). In the bone marrow microenvironment, the MSC is the common progenitor for osteoblasts and marrow adipocytes, and these two differentiation pathways have a reciprocal relationship (Justesen et al., 2001; Meunier et al., 1971; Verma et al., 2002). With advancing age or under the influence of pharmacological agents (anti-diabetic thiazolidinediones or TZDs, or anti-inflammatory glucocorticoids), the expression and activity of PPAR γ increases and drives MSC into becoming adipocytes at the expense of osteoblasts, resulting in bone loss and increased risk of fractures (Almeida et al., 2009; Imai et al., 2004; Jiang et al., 2008). Indeed, animal studies have shown that PPAR γ haploinsufficient mice (PPAR $\gamma^{+/-}$) exhibit increased bone formation, decreased body fat, and are protected from high-fat diet-induced insulin resistance (Akune et al., 2004; Kubota et al., 1999). Furthermore, increased marrow fat produces large quantities of inflammatory cytokines such as IL-6 and TNF α (Abrahamsen et al., 2000; Filková et al., 2009; Khosla et al., 1994; Manolagas and Jilka, 1995; Nanes, 2003; Trujillo and Scherer, 2006). These cytokines not only inhibit the activity of bone-forming osteoblasts (Abbas et al., 2003; Ding et al., 2008; Filková et al., 2009; Kaneki et al., 2006; Khosla et al., 1994; Yamazaki et al., 2009) but also stimulate the differentiation of bone-resorbing cells, the osteoclasts (Fried et al., 1998; Maurin et al., 2000). Since PPAR γ is a key regulator of the marrow fat vs bone balance, and because there was no bone-specific PPAR γ conditional knockout (cKO), our laboratory generated PPAR γ cKO mice (Cao et al., 2015). In the present study, we wanted to explore the expression profiles of genes in PPAR γ -deficient MSCs to gain further insight into possible PPAR γ regulated pathways.

2. Materials and methods

2.1. Animals

PPAR γ cKO mice were created by breeding Col3.6-Cre transgenic mice (Liu et al., 2004) with PPAR γ floxed (PPAR $\gamma^{fl/fl}$) mice (Akiyama et al., 2002) as previously described (Cao et al., 2015).

2.2. Ethics Statement

Animals were maintained in a centralized barrier facility and all experimental procedures were approved by the Augusta University Institutional Animal Care and Use Committee (IACUC).

2.3. Cell culture and RNA isolation

Bone marrow mesenchymal stem/progenitor cells (MSCs) were isolated from long bones of PPAR γ cKO and PPAR $\gamma^{fl/fl}$ mice using a negative immuno-depletion (magnetic beads conjugated with anti-mouse CD11b, CD45R/B220, and Pan DC) and positive immuno-selection (anti-Sca-1 beads) as previously described (Zhang et al., 2008). Total cellular RNAs were isolated using TRIzol reagent according to the manufacturer's instructions (Invitrogen Corp., Grand Island, NY, USA). The RNA samples were cleaned up with the

Qiagen RNeasy® Mini Kit and submitted to the Otogenetics Corporation (Atlanta, GA) for RNA-Seq analysis.

2.4. RNA-Seq analysis

The RNA QC, polyA cDNA preparation and QC, Illumina library preparation and QC were all performed at the Otogenetics Corporation. RNA-Seq were performed on a HiSeq2000/2500 sequencing machine. A minimum of 20 million reads (2 reads x 10 million fragments) were guaranteed. Basic bioinformatics and differential expression analyses were performed on DNAnexus platform. Raw data FASTq and bioinformatic reports were delivered via DNAnexus and a secure Google Cloud Drive. The RNA-Seq data have been deposited in the Gene Expression Omnibus at NCBI (GEO; available on the World Wide Web at www.ncbi.nlm.nih.gov/geo) with GEO accession number GSE101925.

2.5. Data analysis workflow

1. The sequencing data sets from illumina HiSeq2000 (fastq.gz) were first mapped with *Tophat* (v2.0.5) against reference assembly UCSC mm9 downloaded from illumina iGenome and then the mapped files (accepted_hits.bam) from each sample were input into *cufflinks* (v2.0.2) to locate genomic regions with expression under guide of 'genes.gtf' (annotation file from illumina iGenome). Expression information were recorded in 'transcript.gtf'.
2. Cufflinks.cuffmerge were called to combine all 'transcripts.gtf' into a single 'merged.gtf', and mapping files generated by Tophat were input into *cufflinks.cuffdiff* (v2.0.2) to measure expression level on regions (genes, transcripts, CDS, etc.) defined in 'merged.gtf'.
3. Expression levels were measured with FPKM (Fragments Per Kilobase per Million mapped), which is located in. fpkm_tracking files in cuffdiff folder. Statistical tests on the difference between samples were located in.diff files. q-value less than 0.05 were considered statistically significant.
4. Cuffdiff analysis results were implemented at different levels, including gene, isoform and CDS. All result files were tab-delimited plain text, and could be opened with Excel. For definition of each field in files, please refer to cufflinks manual at <http://cufflinks.cbc.umd.edu/manual.html>.

2.6. Construction of human gene association network

Gene association network links genes or encoded proteins by their functional interplays, including direct physical binding and indirect interaction such as their participation in a common cellular process. In this study, the network was constructed from version 9.05 of the STRING database (von Mering et al., 2003). Such a construction results in a weighted network where the weight of each link is a confidence score of the corresponding interaction and the score has been normalized to the interval of [0, 1]. In order to construct a gene association network with high confidence edges, we first filtered out the STRING with a threshold of 0.9. Only interactions whose weights were above the threshold of 0.9 were chosen to enter the new network. Then we checked whether the 78 genes under study (refer

to Section 3.1 for details) were included in the new network or not. If some genes under our study were included in the STRING but not in the new network, we lowered the threshold for these genes and their interactions, until all these genes were included in the background network. Finally, a high-confidence weighted human gene association network with 9178 nodes was constructed.

2.7. Scoring the impact of seed nodes in a network

We utilized the random walk with restart (RWR) algorithm to score the impact of a group of seed nodes on all the nodes in a network (Tong et al., 2008). Here the network was the high-confidence weighted human gene association network, and the seeds were the 78 human ortholog genes corresponding to the differentially expressed mouse genes. The RWR algorithm mimics the process of a walker randomly going from one node to a neighbors along a network. When the algorithm converges, the converged probability vector can measure the proximity of two nodes in a weighted graph. Thus, it provides a good relevance score between two nodes.

The RWR algorithm utilizes the following equation:

$$x^{t+1} = (1 - r)Px^t + rx^0 \quad (1)$$

where P is the normalized adjacency matrix of the weighted network; $r \in (0,1)$ is a parameter denoting the probability that the walker jumps back to its start node at any position; x^t is a vector in which $x^t(v)$ denotes the probability that the walker will be at node v at time t ; x^0 is an initial vector representing the strength of the seed nodes. After a sufficiently long time, the algorithm will converge and get a steady-state probability vector x^∞ . This vector is considered as the impact score of the seeds to each node in the network.

2.8. Real-time qRT-PCR analysis

The RNA-Seq results were confirmed by real-time qRT-PCR analysis of representative candidate genes that were differentially expressed in MSCs of control (PPAR $\gamma^{fl/fl}$) and PPAR γ cKO mice. qRT-PCR was performed as described previously using TaqMan Reverse Transcription Reagents and a StepOnePlus Real-Time qPCR System (Thermo Fisher Scientific). The mRNA levels were normalized to β -actin (internal control) and gene expression was presented as fold change relative to control (PPAR $\gamma^{fl/fl}$) which was set as 1. The primer sequences used in the PCR reactions are as follows: Adiponectin (NM_009605): 5'-TGAGACAGGAGATGTTGGAATG-3' (forward), 5'-ACGCTGAGCGATACACATAAG-3' (reverse); Aldh1a1 (NM_013467): 5'-GCAGCAGGACTCTTCACTAAA-3' (forward), 5'-CACTGGGCTGACAACATCATA-3' (reverse); Ifi202b (NM_008327): 5'-GTCTGAGGTGAAGAGCCAAA-3' (forward), 5'-CTCCATCAACCAGCCTTTCT-3' (reverse); Ankrd1 (NM_013468): 5'-GCCTACAAGAACTCTCGCATAG-3' (forward), 5'-GTTGCTCTTCTGTTGGGAAATG-3' (reverse); PLAC8 (NM_139198): 5'-ACTCTCTACCGAACCCGATAC-3' (forward), 5'-CATGGCTCTCCTCCTGTTAATG-3' (reverse); Ncoa7 (NM_001111267): 5'-CACCCAAGTTCTCCCATTGA-3' (forward), 5'-

TCATCACCACACGACATTCC-3' (reverse); Xaf1 (NM_001291153): 5'-CTTCTGGACAAGACAGGTTAGG-3' (forward), 5'-CCTCTCCTTCTCTTTCCCTTTC-3' (reverse); Lpl (NM_008509): 5'-TTAACTACCCCCTAGACAACGTCCA-3' (forward), 5'-AAGAGATGAATGGAGCGCTCG-3' (reverse); Hsd11b1 (NM_008288): 5'-TAGAGAGGAGGAGAGAGAGAGA (forward), 5'-GCCCTTGAGGCTTCAGATAA-3' (reverse); and b-actin (NM_007393): 5'-ATGGAGGGGAATACAGCCC-3' (forward), 5'-TTCTTTGCAGCTCCTTCGTT-3' (reverse).

3. Results

3.1. Differentially expressed genes and the biological processes they participate in

We compared the expression levels of all genes in the mouse PPAR γ knockout (KO) MSCs with the expression levels in the PPAR γ -floxed (WT) control MSCs. A Gene whose expression in KO vs WT was larger than 3 and whose q-value in KO vs WT was less than 0.05 was considered as differentially expressed. We found 93 differentially expressed genes, 79 of which have known mouse gene symbols.

Since the mouse and human genes are highly conserved, we mapped the differentially expressed genes identified in mouse PPAR γ KO and WT control MSCs to a human data base (Sonnhammer and Östlund, 2015) to establish potential biological implications of PPAR γ deficiency. The mapping results showed that these 93 differentially expressed mouse genes corresponded to 78 distinct human genes.

To explore the biological processes in which these differentially expressed genes participated, we conducted gene ontology (GO) analysis. We used the p-value to quantitatively measure whether this group of differentially expressed genes was statistically significantly enriched with genes of a specific Gene ontology (GO) term. The GO database of human biological processes includes 45240 genes, and our GO analysis results showed that 74 of the 78 differentially expressed genes are included in GO terms. Using a p-value of less than 0.01, we obtained 95 significantly enriched GO terms in biological process. The GO vocabularies are organized in a hierarchical way. The deeper the level of a GO term, the more specific the corresponding function will be.

We therefore filtered out GO terms with depths smaller than 4 and then had 52 terms remaining for further analysis. We ranked these 52 GO terms according to their levels and listed the GO terms with deepest levels in Table 1. These terms suggest that the most specific biological processes in which the differentially expressed genes participate are the regulation of the renin-angiotensin system and blood pressure (Table 1).

We also ranked the GO terms according to their p-values and listed those with smallest p-values in Table 2. These terms indicate that the biological processes that are most significantly enriched and associated with the differentially expressed genes play important roles in the regulation of inflammation and immunity (Table 2).

3.2. Genes significantly influenced by the differentially expressed genes in the network

A group of differentially expressed genes can exert their effects on other genes through network links. Using the 78 human ortholog genes as seed genes, we applied a random walk with restart (RWR) algorithm to score the influence of these genes to all the genes in the human gene association network. 8801 genes in the network had scores larger than zero, suggesting that they may be influenced by the differentially expressed genes. However, most of the scores were close to zero, which indicates that only a very small number of genes could be significantly affected. The higher the score is, the greater the likelihood that the gene is influenced by the group of differentially expressed genes. The highest score on the list was 0.602. We set 10% of the highest score as a threshold and got 204 genes whose score was larger than 0.0602, which was about 2% of all the 8801 affected genes. This set of 204 genes includes all of the 76 seed genes. Thus, we considered these 204 genes as significantly influenced by the differentially expressed genes and assumed that they are associated with the biological functions of PPAR γ gene.

Similar to the process described in the last section, we also conducted GO analysis for these 204 affected genes. We listed the most specific biological processes in which these genes participate and those most significantly enriched processes affected by these genes in Table 3 and 4, respectively. Taken together these data suggest that the most specific biological processes in which the differentially expressed genes are involved is in the regulation of energy metabolism (Table 3), and the genes most significantly influenced by the differentially expressed genes are the biological processes regulating inflammation and immunity (Table 4).

3.3. Network affected by the differentially expressed genes

We took all of the 204 genes identified as affected by the differentially expressed genes, together with all the links between them from the human gene association network and constructed a sub-network. This sub-network is spanned by the 204 most affected genes identified and thus could be considered as associated with PPAR γ functions. Excluding 6 isolated nodes, this network includes 198 nodes and 740 interactions.

There are 24 connected components with the biggest one contains 134 nodes (Fig. 1).

In a network, high-degree nodes have more communication and interactions with other nodes; therefore, they play more important roles in the network. Figure 1 shows that the high-degree nodes in the network form a densely linked area. This area includes several gene families associated with immune function, such as the interferon regulatory factor family (genes IRF1~IRF9), interferon induced protein family (genes IFIT1, IFIT3, IFI16, etc.), and STATs family (genes STAT1, STAT2). The biological functions of these gene families are in line with the well-documented anti-inflammatory and anti-cancer actions of PPAR γ .

3.3. Validation of the differentially expressed genes

Finally to ensure that our data interpretation was accurate we performed real-time qRT-PCR analysis to confirm the differential expression of several genes previously shown by RNA-Seq data to be significantly up- or downregulated in PPAR γ KO cells. The rationale for the

selection of these genes was: 1) they are significantly up- or downregulated, 2) they are potential candidate genes previously studied in our laboratory, and 3) they are known target genes transcriptionally regulated by PPAR γ . As shown in Fig. 2, the mRNA levels of Aldh1a1 and Ankrd1 were 32 times higher in PPAR γ KO cells than that in WT cells, and levels of Xaf1, Ifi202b, Ncoa7, Hsd11b1, Adipoq, Plac8, and Lpl were all dramatically decreased in the PPAR γ KO cells compared with those in WT cells. The gene expression patterns revealed by qRT-PCR analysis (Fig. 2) was similar to that seen with the RNA-Seq data, validating our results. The fold changes (Log2) of the genes revealed by RNA-Seq are shown in parentheses under the corresponding genes in Fig. 2.

4. Conclusions

Taken together our results suggest that, in addition to controlling adipocyte differentiation and energy metabolism, PPAR γ also plays key roles in the development of the cardiovascular system, the control of blood pressure and in inflammation and immune functions.

4. Discussion

PPAR γ , a member of the nuclear receptor superfamily, is among the most extensively studied transcription factors due to its importance in human health. Since most biological processes are interlaced and PPAR γ can crosstalk with other factors and signaling pathways (Chu et al., 2014; Leonardini et al., 2009; Takada et al., 2012), it is critically important to streamline the relevant information collected from individual studies conducted in different settings (tissues or cell types) to a single source, e.g. NURSA (Nuclear Receptor Signaling Atlas, <https://www.nursa.org/nursa>) so that investigators in different areas can utilize this information to design their studies more effectively and interpret their data accurately (Becnel et al., 2017).

In the present study, we report our bioinformatics data analysis, of PPAR γ effects on global gene expression. The analysis was performed in a blinded fashion using data generated from deep sequencing of RNA samples isolated from multipotent mesenchymal stromal cells (MSCs) from either PPAR γ knockout or wild type mice. The original purpose of this RNA-Seq study was to identify novel genes whose expression is influenced by PPAR γ and which are involved in the regulation of MSC osteo- and adipogenic lineage commitment. Unexpectedly, the bioinformatics analysis revealed novel biological functions for PPAR γ in addition to its known role in cell lineage commitment. PPAR γ is a master regulator of adipogenesis and MSCs are a common progenitor for marrow adipocytes and osteoblasts. Therefore, it is generally thought that blockade of the marrow adipocyte pathway by targeting PPAR γ would force MSCs into the osteoblastic pathway and therefore increase bone mass. Potentially making this strategy an ideal anti-osteoporosis therapy. PPAR γ is known to be an important transcription factor that controls a broad variety of biological processes, including insulin sensitivity, energy metabolism, cardiovascular and blood pressure, and inflammation and immune functions. The results of this bioinformatics data analysis confirm and extend the known functions of the PPAR γ gene (Tables 1). Novel PPAR γ functions include: a role for PPAR γ in the regulation of cardiac function and blood

pressure control via the renin-angiotensin system (Finck, 2007; Huang et al., 2012; Márquez-Salom and Diez, 2013; Yu et al., 2015; Yu et al., 2012), a role in inflammation and immunity (Table 2), and a role in the development of inflammatory disorders such as diabetes (Murphy and Holder, 2000), Alzheimer's disease (Heneka et al., 2011), inflammatory bowel disease (Annese et al., 2012), Cushing's disease (Ambrosi et al., 2004), and arthritis (Bongartz et al., 2005; Fahmi et al., 2003). Furthermore, analysis of the gene profiles influenced by the differentially expressed genes revealed the central role of PPAR γ in the development of diseases related to the energy metabolism and immunity (Tables 3 and 4) including diabetes, insulin resistance, glucose and lipid metabolism, as well as many other metabolic and inflammatory disorders (Akoum, 2014; Cipolletta et al., 2012; Clark, 2002; Daynes and Jones, 2002; Harmon et al., 2011; Kersten et al., 2000; Rosen and Spiegelman, 2001; Semple et al., 2006; Zhang and Young, 2002).

Our findings are consistent with known PPAR γ functions and our RNA-Seq data was also confirmed by qRT-PCR results suggesting that the strategies we employed for data analysis are valid (Fig. 2). It is worth mentioning that we selected and tested 9 genes for confirmation, and all of them (100%) showed patterns of differential expression identical to those seen with the RNA-Seq data. Among these 9 genes, Adipoq (also known as adiponectin) and Lpl are adipocyte differentiation marker genes and are transcriptionally activated by PPAR γ (Maeda et al., 2001; Schoonjans et al., 1996). Both Lpl and Adipoq play important roles in regulating triglycerides and insulin sensitivity (Ferreira et al., 2001; Kim et al., 2001; Lihn et al., 2005; Okubo et al., 2007). Xaf1 is a gene involved in regulation of angiogenesis (Qiao et al., 2008). Ifi202b, which is induced by interferon, is involved in inflammation and immunity (Rozzo et al., 2001). Hsd11b1, is an enzyme that converts the inactive glucocorticoid, cortisone, to the active form cortisol and thus plays an important role in metabolic syndrome, inflammation and immune function (Cooper and Stewart, 2009; Stimson and Walker, 2007). Aldh1a1 and Plac8 have been shown to play roles in metabolism as well as immunity (Jimenez-Preitner et al., 2011; Johnson et al., 2012; Thomas et al., 1982; Ziouzenkova et al., 2007). Ankrd1 is a key regulator of the cardiovascular system (Crocini et al., 2013; Moulik et al., 2009).

In summary, we aimed to discover potential genes that regulate BMSC osteo- and adipogenic lineage commitment in a PPAR γ deficient setting. The study revealed new genes whose expression is influenced by PPAR γ but whose function in this setting is yet to be elucidated.

Acknowledgments

This work was supported by grants from The National Institute on Aging (AG046248), National Institutes of Health (NIH).

Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Numbers R01AG046248.

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- RNA sequencing analysis was performed to examine the expression profile of genes in PPAR γ -deficient bone marrow mesenchymal stem/progenitor cells (BMSCs).
- Results showed that PPAR γ plays key roles in the development of the cardiovascular system, the control of blood pressure and in inflammation and immune functions, in addition to its pivotal roles in regulating adipocyte differentiation and energy metabolism.
- This study provides new information for the Nuclear Receptor Signaling Atlas by demonstrating a novel role of PPAR γ on the expression of genes and pathways by using a BMSC PPAR γ deficient mouse model.

Fig. 1. Gene association network significantly influenced by differentially expressed genes. Diamond nodes denote differentially expressed genes.

Fig. 2.

Real-time qRT-PCR analysis of representative differentially expressed genes. Numbers shown in parentheses indicate the fold changes (Log2) of the corresponding genes in RNA-Seq data. Numbers above the bar graph indicate the levels of mRNA relative to control, which is set as 1. The PCR reaction was performed in triplicate. Abbreviations: Xaf1, XIAP associated factor 1; Ifi202b, interferon activated gene 202B; Ncoa7, nuclear receptor coactivator 7; Hsd11b1, hydroxysteroid 11-beta dehydrogenase 1; Aldh1a1: aldehyde dehydrogenase family 1, subfamily A1; Ankrd1, ankyrin repeat domain 1; Plac8, placenta-specific 8; Lpl, lipoprotein lipase.

Table 1

The most specific biological processes the differential expressed genes are involved in.

GO Term	Level	Total genes	Mapped genes	p
regulation of blood volume by renin-angiotensin	11	14	3	0.000514
regulation of blood vessel size by renin-angiotensin	11	5	2	0.005401
regulation of systemic arterial blood pressure by renin-angiotensin	10	30	3	0.003824
renal control of peripheral vascular resistance involved in	9	5	2	0.005401
regulation of systemic arterial blood pressure				
regulation of systemic arterial blood pressure by hormone	9	42	3	0.008131
regulation of metanephric glomerulus development	9	7	2	0.00955
type I interferon-mediated signaling pathway	8	113	5	0.000453
renal system process involved in regulation of systemic arterial blood pressure	8	25	3	0.002435
negative regulation of DNA biosynthetic process	8	6	2	0.007227
ISG15-protein conjugation	7	7	3	8.13E-05
cytokine-mediated signaling pathway	7	406	8	0.000167
cellular response to type I interferon	7	114	5	0.000465
protein homooligomerization	7	315	6	0.003359

Table 2

The biological processes most significantly enriched with differentially expressed genes.

GO Term	Level	Total genes	Mapped genes	p
cellular response to cytokine stimulus	6	513	11	1.51E-06
negative regulation of multicellular organismal process	4	527	11	1.51E-06
innate immune response	4	915	13	2.91E-06
negative regulation of type I interferon production	6	39	5	5.31E-06
regulation of type I interferon production	5	86	6	5.93E-06
response to cytokine stimulus	4	651	11	7.96E-06
cellular response to organic cyclic compound	6	257	8	8.71E-06
cellular response to chemical stimulus	4	2379	18	2.77E-05
positive regulation of cellular process	4	4825	25	5.29E-05
cell surface receptor signaling pathway	5	3167	20	6.38E-05
ISG15-protein conjugation	7	7	3	8.13E-05

Table 3

The most specific biological processes the affected genes.

GO Term	Level	Total genes	Mapped genes	p
IMP biosynthetic process	15	27	7	1.52E-05
positive regulation of transcription from RNA polymerase II promoter	14	1112	36	0.000314
positive regulation of transcription, DNA-templated	13	1559	47	7.84E-05
positive regulation of interferon-beta biosynthetic process	12	10	4	0.000973
glycine biosynthetic process from serine	11	11	11	2.37E-18
glycine biosynthetic process	11	18	11	4.94E-14
positive regulation of nucleic acid-templated transcription	11	1559	47	7.84E-05
positive regulation of RIG-I signaling pathway	11	7	4	0.000226
arginine biosynthetic process	11	9	4	0.000641
Leydig cell differentiation	11	22	5	0.001378
purine nucleotide biosynthetic process	11	198	11	0.008948
L-serine biosynthetic process	10	15	8	3.21E-09
IMP metabolic process	10	28	7	1.99E-05
negative regulation of viral-induced cytoplasmic pattern recognition receptor signaling pathway	10	5	4	4.14E-05
negative regulation of RIG-I signaling pathway	10	5	4	4.14E-05
positive regulation of MDA-5 signaling pathway	10	6	4	0.000111
positive regulation of RNA biosynthetic process	10	1588	47	0.000118
T cell lineage commitment	10	26	5	0.002905
cardiac chamber morphogenesis	10	132	9	0.00846

Table 4

The biological processes most significantly enriched with the affected genes.

GO Term	Level	Total genes	Mapped genes	p
defense response to virus	6	181	49	3.13E-45
response to virus	4	290	57	9.50E-45
cellular response to type I interferon	5	114	31	4.27E-28
response to type I interferon	4	115	31	5.33E-28
type I interferon signaling pathway	7	113	30	6.65E-27
L-serine metabolic process	6	36	20	3.47E-25
cellular response to cytokine stimulus	4	513	49	1.59E-23
positive regulation of type I interferon production	5	57	22	1.65E-23
cytokine-mediated signaling pathway	6	406	43	2.68E-22
glycine metabolic process	6	56	20	1.63E-20
negative regulation of type I interferon production	4	40	17	8.90E-19
glycine biosynthetic process from serine	11	11	11	2.37E-18
serine family amino acid metabolic process	5	93	20	1.05E-15
positive regulation of interferon-beta production	5	30	13	3.03E-14
glycine biosynthetic process	11	18	11	4.94E-14
positive regulation of cytokine production	4	362	32	4.94E-14
regulation of innate immune response	4	301	29	1.37E-13
cell surface receptor signaling pathway	5	2561	88	5.32E-13
negative regulation of viral process	5	58	15	8.35E-13
interferon-gamma-mediated signaling pathway	7	127	18	8.22E-11