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Genetic manipulation of the *ApoF/Stat2* locus supports an important role for Type I Interferon signaling in atherosclerosis

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Abstract

Apolipoprotein F (ApoF) is a sialoglycoprotein that is a component of the HDL and LDL fractions of human serum. We sought to test the hypothesis that ApoF plays an important role in atherosclerosis in mice by modulating lipoprotein function. Atherosclerosis was assessed in male low density lipoprotein receptor knockout (*LDLR* KO) and *ApoF/LDLR* double knockout (DKO) mice fed a Western diet for 16 weeks. *ApoF/LDLR* DKO mice showed a 39% reduction in lesional area by en face analysis of aortas ($p < 0.05$), despite no significant differences in plasma lipid parameters. *ApoF* KO mice had reduced expression of Interferon alpha ($IFN\alpha$) responsive genes in liver and spleen, as well as impaired macrophage activation. Interferon alpha induced gene 27 like 2a (*Ifi27l2a*), Oligoadenylate synthetases 2 and 3 (*Oas2* and *Oas3*) were significantly reduced in the *ApoF* KO mice relative to wild type controls. These effects were attributable to hypomorphic expression of *Stat2* in the ApoF KO mice, a critical gene in the Type I IFN pathway that is situated just 425 base pairs downstream of ApoF. These studies implicate STAT2 as a potentially important player in atherosclerosis, and support the growing evidence that the Type I IFN pathway may contribute to this complex disease.

Keywords

ApoF; STAT2; Atherosclerosis; Type I IFN

Introduction

Apolipoprotein F (ApoF) is a component of the HDL and LDL fractions of human serum ¹ that occurs at concentrations of about 80 $\mu\text{g} / \text{mL}$ in human plasma ². Approximately 75% of the circulating ApoF is associated with small dense HDL3, while the remaining 25% resides on electronegative LDL ^{3,4}. We previously showed that Apo F overexpression reduces HDL cholesterol levels in mice, and results in HDL particles that are more efficient acceptors of macrophage cholesterol ⁴. Surprisingly, *ApoF* deficiency does not substantially alter plasma

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lipid or lipoprotein levels in mice⁵. In the current study, we considered the possibility that Apo F may reside on lipoproteins for a function other than simple lipid transport and may play an important role in atherosclerosis.

Signal Transducer and Activator of Transcription 2 (Stat2) is a transcription factor that plays a critical role in the Type I Interferon signaling. Type I interferons (IFN) consisting of IFN- α and IFN- β are secreted by hematopoietic and non-hematopoietic cell types in response to viral infection⁶. Binding of Type I IFN to the IFN α Receptor (IFNAR) induces cross-phosphorylation of tyrosine residues on Tyk2 and Jak1, which are physically associated with the cytoplasmic tails of the receptor⁷. This results in phosphorylation of Stat2, which then recruits and phosphorylates Stat1. The active pStat2/pStat1 complex then translocates to the nucleus and associates with (Interferon response factor) IRF-9 to form a trimolecular transcription factor complex also known as ISGF3⁸. ISGF3 binds to Interferon Stimulated Response Elements (ISRE) in the promoters of target genes to activate transcription. This activates an orchestrated program of genes that work together to mount the innate antiviral immune response by coordinately shutting down transcription, translation and replication of the virus. For example, 2'-5' Oligoadenylate Synthetases (OAS) are direct Stat2 target genes, and a key part of the antiviral response. OAS proteins catalyze the formation of 2'-5' oligoadenylate from ATP, which binds to and activates RNase L. Activated RNase L then degrades viral RNAs to prevent replication and dissemination of the virus⁹. Activation of the Type I IFN pathway is critical for the innate immune response to viral infection, and is also required for effective priming of the adaptive immune response¹⁰. While Stat1 readily partners with other Stat family members and participates in IFN gamma signaling, Stat2 is more specific to the Type I IFN pathway¹¹. As such, it offers a unique leverage point to investigate the role of Type I IFN signaling in atherosclerosis.

We report that ApoF-deficient mice are protected from western diet induced atherosclerosis on an *LDLR* KO background. This was accompanied by substantially reduced expression of Type I IFN responsive genes and impaired macrophage activation. These effects were attributable to reduced expression of the neighboring *Stat2* gene; and support an important role for the Type I interferon pathway in atherogenesis.

Methods

Animals

ApoF KO mice generated on a C57BL6 background⁵ were crossed with *Ldlr* KO mice to generate *ApoF/Ldlr* DKO mice. Mice were housed in an AAALAC accredited animal facility in polycarbonate cages with filter tops and maintained on a 6 am to 6 pm light cycle, with free access to food and water. Mice were fed a chow diet (Purina LabDiet 50100) from weaning until 10–12 weeks of age, and then switched to a western (0.21 % cholesterol 1 % corn oil (w/w), Research Diets D12079B) for 16 weeks until sacrifice. Aortas were harvested by dissection, fixed in formalin and stained with Oil Red O¹². Total lesional area was measured under 0.4X magnification using Image Pro software. Fasting plasma lipids were analyzed on a Roche Cobas-Mira autoanalyzer using commercially available reagents (Sigma). Intraperitoneal (IP) administration of poly(I:C) (Sigma-Aldrich) was performed by injecting wild type and *ApoF* KO mice with a 200 μ l of 0.9% saline or 100 μ g of poly(I:C) in saline. Plasma was taken at 6 hours post injection for IFN α measurements, and animals were sacrificed at 24 hours to examine innate immune cell activation status and gene expression in the liver and spleen. Adeno-associated viral (AAV) vectors containing an empty expression cassette (null) or encoding the mouse Apo F protein were generated by the Penn Vector Core and have been described⁴. Mice were injected with AAV vectors at a dose of 5×10^{11} genome copies (GC) via IP injection and examined 14 days later. Animal

procedures were performed with the approval of the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

Microarray

Total liver RNA was used for microarray analysis on the Agilent 4 X 44 Whole Mouse Genome Array. Microarray and data analysis were performed by the Penn Functional Genomics Core. Differential expression was determined using a False Discovery Rate of 10% and a minimum fold change of 1.5. *All expression data will be deposited into GEO upon publication of this manuscript.*

Real time RT-PCR

Approximately 20–50 mg of tissue were rapidly flash frozen in liquid nitrogen. Total RNA was isolated using the Trizol method according to the manufacturer's instructions (Invitrogen). One microgram of total RNA was reverse transcribed using random hexameric primers with the Superscript III RT (Invitrogen) according to the manufacturer's instructions. Real time RT-PCR was performed on five microliters of a 1:50 dilution of the original cDNA sample in a final volume of 12 μ L in an ABI 7900 384-well PCR machine. TaqMan primer/probe sets from Applied Biosystems were used to detect mouse *ApoF* and *Stat2*, while the remaining genes were detected using SYBR green detection (list of primers in supplemental table 1). The relative quantity of each mRNA was calculated using the delta delta CT method with β -Actin as the housekeeping gene.

Flow cytometry

Spleens were minced with a razor blade and digested with collagenase (0.5mg/ml) and DNase (20ug/ml) for 30 mins at 37°C before being passed through a 100 micron cell strainer to obtain a single cell suspension. Red blood cells were then lysed using ACK lysing buffer (Gibco). Cells were stained with Live/dead fixable stain in PBS (Invitrogen) before surface antibodies were added. To examine antigen presenting cells (APCs), a dump gate of anti-CD3, anti-CD5 and anti-CD19 were used to gate out T and B cells. Macrophages were identified by the high expression of F4/80 and CD11b. Cell surface expression of CD80 was used as a marker of macrophage activation. Samples were collected using the LSR II flow cytometer (BD) and analyzed using Flowjo.

Immunohistochemistry

Paraffin embedded formalin fixed heart sections were prepared by the Penn Cardiovascular Institute Histology Core. Sections of the aortic sinus were subjected to staining with hematoxylin and eosin, Masson's Trichrome, or Rat anti-Mac 3 IgG (BD Pharmingen 553322), Goat anti Sm22 alpha IgG (Abcam ab10135), and Mouse anti-Stat2 IgG (Millipore 07-140) antibodies. Mac3 and Sm22 were visualized using a horseradish peroxidase conjugated secondary antibody (ImPRESS TSA system), and Stat2 was imaged by immunofluorescence. Analysis of positively stained areas was performed using ImageJ software.

Statistics

All data are reported as the mean $\pm$ standard deviation. Comparisons between groups were performed using a two-tailed student's t-test. One way ANOVA was used for experiments involving 3 or more groups, and Tukey's posttest was then used to test for significant differences between individual groups. In all cases, significance is assigned at $p < 0.05$.

Results

Targeted deletion of *ApoF* results in hypomorphic expression of *Stat2* and *ApoN*

We previously generated mice with a targeted deletion of the *ApoF* gene⁵ in which the *ApoF* coding sequence was replaced with a Beta galactosidase reporter cassette. Surprisingly, deletion of the *ApoF* gene had no discernible effect on circulating lipids or lipoproteins⁵. The murine *ApoF* gene is situated 12.1 kb downstream of *ApoN* (an uncharacterized apolipoprotein), and only 425 upstream of *Stat2*, a critical mediator of Type I IFN signaling. During the course of these studies we found that the *ApoF* KO mice also exhibited significantly reduced expression of *Stat2* and *ApoN* in the liver: *Stat2*: -66%, $p < 0.05$; *ApoN*: -71%, $p < 0.05$ (Figure 1, Supplemental Figure 1). The *ApoF* KO mice were generated isogenically using C57BL/6 derived ES cells, meaning that these expression differences could not be due to passenger gene effects from the more commonly used 129 derived ES cells. Rather, it seemed more likely that the manipulation of the *ApoF* gene itself directly resulted in silencing of the neighboring *ApoN* and *Stat2* genes, possibly through deletion of important *cis*-acting regulatory elements (Supplemental Figure 2).

Microarray analysis reveals alterations in the Type I IFN signaling pathway

Apo F is expressed exclusively by the liver^{1, 5} where it is secreted into the circulation and then attaches to lipoproteins. Since there was no discernible lipid phenotype in the *ApoF* KO mice, we performed a microarray analysis on livers from these animals to gain insight into the function of *Apo F*. Interestingly, only a very short list of transcripts showed statistically significant differences in regards to *ApoF* genotype based on a False Discovery Rate (FDR) threshold of 5% (Table 1). Surprisingly, the list was enriched in interferon alpha responsive genes (blue), including Interferon Induced transcript 27 like 2a (*Ifi27l2a*), and a number of 2'-5' oligoadenylate synthetases (*Oas3*, *Oas2*, *Oasl2*, and *Oasl1a*) which were significantly lower in the *ApoF* KO mice.

Impaired Type I IFN response and macrophage activation in the *ApoF* KO mice

Real time RT-PCR confirmed significantly lower mRNA expression of *Ifi27l3a*, *Oas2*, and *Oas3* in both the livers and the spleens of the *ApoF* KO mice (Figure 2 A,B). We next challenged these animals with polyinosinic:polycytidilic acid (poly I:C) to assess whether the Type I interferon (IFN) pathway is functionally intact in the *ApoF* KO mice. Poly I:C is a Toll Like Receptor 3 (TLR3) agonist that mimics the double stranded RNA ligands present during a viral infection, resulting in secretion of IFN α/β . Poly(I:C) challenge produced a robust upregulation of *Ifi27l2a* and *Oas2* mRNA levels in the spleen at 24 hours post-injection which was substantially blunted in the *ApoF* KO mice (Figure 2 C,D). Interestingly, plasma levels of IFN alpha and cell surface expression of Interferon alpha receptor (IFNAR) both increased after Poly I:C treatment, but did not differ between the WT and *ApoF* KO mice (Figure 2 E,F). Poly I:C treatment resulted in a robust increase in macrophage CD80 and CD86 expression, early activation co-stimulatory molecules, which was dramatically blunted in the *ApoF* KO mice (Figure 2 G,H). This led us to suspect that these differences may actually be due to a cell intrinsic effect in immune cells. In line with this, we observed an 85% decrease in *Stat2* mRNA expression in isolated bone marrow macrophages (BMM) (Figure 2I). This is consistent with the fact that *Ifi27l2a*, *Oas*, and *Mx1* are well established direct transcriptional targets of *Stat2*. We therefore surmised that the decrease in expression of these genes and impaired immune cell activation could be due to reduced *Stat2* expression rather than a signaling event caused by loss of *Apo F*.

***ApoF* Deficient mice are resistant to Western Diet induced atherosclerosis**

We sought to test the hypothesis that Apo F may affect lipoprotein function and hence susceptibility to atherosclerosis. To accomplish this, male *Ldlr* KO and *ApoF/Ldlr* DKO mice were fed a Western Diet for 16 weeks for assessment of atherosclerosis by *en face* analysis and oil red O staining. We observed a significant 39% reduction in the total lesion area in the *ApoF/Ldlr* DKO mice relative to controls, $p < 0.05$ (Figure 3 A). Aortic roots contained complex lesions composed of necrotic core, cholesterol crystals, macrophage foam cells, and smooth muscle cells (Supplemental Figure 3). Smooth muscle cell (Sm22) and collagen (blue Trichrome) content did not significantly differ by genotype (Supplemental Figures 4,5). We did however observe a 38% reduction in macrophage positive area in the *ApoF/Ldlr* DKO mice ($p < 0.05$) (Supplemental Figure 6,7). Immunostaining for *Stat2* produced robust signal on the aortic valves, with less organized positive staining dispersed throughout the lesions (Supplemental Figure 8). Perhaps most intriguing, the *ApoF/Ldlr* DKO mice did not exhibit any changes in plasma lipid parameters that could explain the decreased lesion burden observed by *en face* analysis (Figure 3 B).

Reconstitution of Apo F does not restore expression of *Stat2* and Type I IFN responsive genes

We performed an experiment to test whether the loss of Apo F could be responsible for the immune phenotype and altered Type I IFN signaling in these animals. We used an AAV8 based vector driven by the liver specific thyroxine binding globulin (TBG) promoter to overexpress Apo F only in the livers of the *ApoF* knockout mice. In parallel, a control group of *ApoF* KO mice was given an empty vector (Null). AAV-mediated delivery of Apo F resulted in a 24-fold increase in liver *ApoF* mRNA levels relative to wild type mice (Figure 4A). In contrast, liver *Stat2* mRNA levels in these animals were unaffected by ApoF overexpression (Figure 4B). Apo F overexpression did not rescue the lower expression of IFN α -responsive genes in the liver (Figure 4 C,D) or in the spleen (Figure 4 E,F), indicating that these gene expression differences are Apo F-independent.

Discussion

The overall goal of this study was to determine whether physiological levels of Apo F expression have an impact on atherosclerosis. We found that the *ApoF* KO mice are protected from Western Diet induced atherosclerosis on an *Ldlr* deficient background- an effect that could not be explained by changes in plasma lipids or lipoproteins. We discovered that the *ApoF* KO mice have expression of IFN α responsive genes, a diminished response to poly(I:C) challenge, and impaired macrophage activation. These effects are most likely due to hypomorphic expression of the neighboring *Stat2* gene.

The reduced expression of *Ifi2712a*, *Oas2*, *Oas3* and other genes involved in the innate antiviral response agrees with studies in other *Stat2* deficient mouse models. A *Stat2* hypomorphic mouse designated “P117” harbors a splice site mutation resulting in only trace amounts of full length *Stat2* protein expression¹³. Primary mouse embryonic fibroblasts (PMEFs) from these animals are completely incapable of activating *Oas* and *Pkr* expression in response to IFN α . This is accompanied by impaired dendritic cell activation¹³. Mice with a targeted deletion of *Stat2* have been reported and studied extensively for their response to viral infection^{10, 14, 15}. PMEFs from the *Stat2* deficient mice do not activate the *Oas* and *Mx* genes in response to IFN α stimulation. Importantly, *Oas* and *Mx* expression are induced less than 50% in the *Stat2* heterozygous mice¹⁰. These defects in IFN α signaling render the P117 mice as well as the *Stat2* KO mice particularly susceptible to viral infections. Taken together, the 70–85% reduction in *Stat2* mRNA in the *ApoF* KO mice is adequate to explain the reduced expression of IFN α -responsive genes and the defective macrophage activation.

The lower levels of *ApoN* and *Stat2* are most likely a direct result of the targeting of the *ApoF* gene. It is worth noting that *Stat2* resides only 425 bp downstream of the *ApoF* gene. Despite our best efforts to generate these mice on a pure C57BL6 background to avoid strain differences and passenger genes, unintended regulatory effects of the insertion may have been unavoidable. ChIP Sequencing data on the human genome indicates the presence of acetylated histones within the second exon of *ApoF* that extend through the 3'UTR and well into the 425 bp gap between *APOF* and *STAT2*¹⁶, suggesting that the *APOF* gene is contiguous with the *STAT2* promoter (Supplemental Figure 2). It is plausible that important *cis*-acting regulatory elements contained within the *ApoF* coding sequence, intron or 3'UTR were removed by our targeting vector. It is also conceivable that the silencing of *ApoN* and *Stat2* is due to negative effects of the beta galactosidase reporter cassette or the neomycin resistance (Neo) gene. There are other examples of local silencing of neighboring genes at a targeted locus by insertion of foreign DNA sequences such as Neo^{17, 18}.

Recent data from other mouse models support a causal relationship between Type I IFN signaling and atherosclerosis. *Ldlr* KO mice fed a western diet have increased atherosclerosis with low dose IFN α treatment. In this study, treatment with IFN α increases atherosclerotic plaque area ($\uparrow 64\%$, $p < 0.008$) relative to saline injected control mice¹⁹. Likewise, IFN β administration promoted atherosclerosis in both a collar-induced model in *ApoE* KO mice, as well as in western diet fed *Ldlr* KO mice²⁰. In contrast to the report with IFN α stimulation, treatment with IFN β did not alter plasma cholesterol levels. Bone marrow transplant experiments showed that *Ldlr* KO mice with a myeloid-specific deletion of the *Ifnar* gene were in fact protected from atherosclerosis in the absence of exogenously supplied IFN β . Interestingly, the control *Ldlr* KO mice in this study expressed the *Stat2* target genes *Oas1*, *Oas2*, *Mx1* and *Irf9* in aortic arches, supporting a role for constitutive activation of this pathway- even in the absence of viral infection. Plasmacytoid dendritic cells (pDCs) are the most potent producers of Interferon alpha in the body, producing as much as 1000-fold more than other immune cell populations²¹. Recent work has demonstrated that pDCs exist within atherosclerotic lesions, and that antibody mediated depletion of these cells offers protection from atherosclerosis²². Accordingly, these authors also found that specific stimulation of pDCs with CpG oligonucleotides led to an increase in plaque burden in *ApoE* KO mice fed a high fat diet²². It is tempting to speculate that pDCs present within the lesion may be the source of the endogenous IFN alpha, secreted in response to either viral infection or necrotic cell death during lesion formation. Collectively, these data support a proatherogenic role for IFN α/β in atherogenesis, both of which require obligate signaling through *Stat2*.

The reduced macrophage content in the lesions of the *ApoF/Stat2* DKO mice (Supplemental Figures 4 and 7), combined with the 85% reduction in macrophage *Stat2* expression (Figure 2I), are consistent with an important role for monocyte/macrophage Type I Interferon signaling in atherogenesis. The impaired macrophage activation in these animals raises the possibility that *Stat2* may act at the level of monocyte infiltration or foam cell formation. The lack of a difference in *Stat2* immunostaining by genotype may be due to the advanced nature of the lesions (Supplemental Figure 8), which is not ideally suited for examining early processes such as monocyte infiltration and foam cell formation. However, it is also worth noting that other cell types of the vessel wall may also play a role (such as endothelial and smooth muscle cells), and this merits further investigation in appropriate model systems.

Genetic evidence linking *STAT2* to CAD or MI risk in humans has not yet been reported. Interestingly, patients with systemic lupus erythematosus (SLE) have markedly elevated Type I IFN levels and downstream signalling²³⁻²⁷, which may contribute to their increased CAD risk²⁸. Likewise, a growing body of literature also supports a relationship between viral infections (particularly cytomegalovirus) and atherosclerotic disease risk and

severity²⁹. It is tempting to speculate that elevated Type I IFN signaling through STAT2 could be an underlying mechanistic link between autoimmune diseases, viral infection, and atherosclerosis. Future studies using the *Stat2* knockout mice will allow us to cleanly and definitively assess the role of this protein in atherosclerosis and the molecular mechanisms involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *ApoF* KO mice are protected from western diet induced atherosclerosis on an *LDLR* KO background, via a lipid independent mechanism.
- Reduced atherosclerosis is accompanied by decreased expression of IFN alpha responsive genes and impaired immune cell activation.
- Deletion of the *ApoF* gene results in hypomorphic expression of *Stat2*, supporting a role for the Type I IFN response in atherogenesis.

Figure 1. Decreased expression of flanking genes at the mouse ApoF locus

The positions of *ApoN* and *Stat2* are shown relative to *ApoF* using a display from the *Ensembl* genome browser. Message levels of *ApoN* and *Stat2* in mouse liver were measured in wild type and *ApoF* KO mice by real time RT-PCR with β -Actin as the housekeeping gene.

Figure 2. Impaired activation of the Type I IFN pathway in ApoF KO mice

Total RNA was isolated from the liver and spleen of wild type and *ApoF* KO mice for validation by real time RT-PCR. Message levels of *Ifi2712a*, *Oas2*, and *Oas3* in A) liver and B) spleen normalized to β -Actin. Spleen RNA was isolated from wild type and *ApoF* KO mice that received an intraperitoneal injection of either saline or 100 μ g of poly(I:C). Message levels of C) *Ifi2712a* and D) *Oas2* were measured by real time RT-PCR. E) Plasma levels of IFN α at 6 hours after poly(I:C) injection were measured by ELISA. Cell surface expression of F) Interferon alpha receptor, G) CD80, and H) CD86 on splenic macrophages that were harvested twenty four hours after poly(I:C) administration. I) Message levels of *Stat2* in primary bone marrow derived macrophages from wild type and *ApoF* KO mice.

Figure 3. Decreased Atherosclerosis in ApoF Deficient Mice

Male *LDLR* KO (n = 10) or *ApoF/LDLR* DKO (n = 9) mice were fed a Western diet for 16 weeks for assessment of atherosclerosis development. A) En face lesional area in Oil Red O stained aortas from *LDLR* KO and *ApoF/LDLR* DKO mice (* $p < 0.05$). B) Fasted plasma lipid parameters after 12 weeks on Western Diet.

Figure 4. AAV-mediated overexpression of ApoF in the ApoF knockout mice

ApoF KO mice were injected with 5×10^{11} genome copies of either an empty AAV vector (Null), or one encoding mouse ApoF (mApoF) under the control of a liver specific promoter. A) Expression of *ApoF* and *Stat2* in the livers of these mice. Expression of C) *Ifi2712a* and D) *Oas2* in the livers of these animals. Expression of E) *Ifi2712a* and F) *Oas2* in the spleen (*indicates significant differences at $p < 0.05$ by one way ANOVA followed by Tukey's post-test).

Table 1**Microarray results**

Genes are listed in order of lowest false discovery rate (FDR) and then greatest fold-change from top to bottom. Genes with a false discovery rate of less than 5% were considered statistically significant (above the bold line). Highlighted in blue are known IFN α target genes or genes with activities involved in innate immunity.

Gene Name	Description	FC	FDR	Adj P.
Apof	Apolipoprotein F	-	0	0.00000
Apon	Apolipoprotein N	-7.41	0	0.00740
Ifi2712a	IFN α -induced protein 27-like 2a	-5.46	0	0.01062
Gm4955		-4.53	0	0.01920
ENSMUST37976		-4.37	0	0.01482
Oas3	2'-5' oligoadenylate synthetase 3	-3.70	0	0.01062
Oas2	2'-5' oligoadenylate synthetase 2	-3.25	0	0.06874
Oasl2	2'-5' oligoadenylate synthetase-like 2	-2.92	0	0.00740
Oas1a	2'-5' oligoadenylate synthetase-like 2a	-2.85	0	0.00740
Oas1f	2'-5' oligoadenylate synthetase 1f	-2.51	0	0.03218
D030028A08Rik		-2.48	0	0.03218
Oas1a	2'-5' oligoadenylate synthetase-like 1a	-2.47	0	0.01987
Gm7998		-2.10	0	0.03218
Trim79	Tripartite Motif Protein 79	-1.92	0	0.05003
EG545385		-4.07	5.44	0.16180
LOC100044227		-3.18	5.44	0.19062
Erdr1	Erythroid differentiation regulator 1	-3.14	5.44	0.19062
Gm5431		-2.89	5.44	0.20147
Cd200r4	Cluster of Differentiation 200 receptor 4	-2.39	5.44	0.11829
Ifit3	IFN α -induced protein with tetratricopeptide repeats 3	-2.02	5.44	0.13465
Tex14	Testes expressed gene 14	-2.02	5.44	0.15613
Xaf1	XIAP-associated factor 1	-2.01	5.44	0.12067
Ly6a	lymphocyte antigen 6 complex, locus A	-1.94	5.44	0.16180
Slfn8	Schlafen 8	-1.91	5.44	0.16789
Ccl3	Chemokine (C-C motif) Cigand 3	-1.83	5.44	0.11810
Trim30	Tripartite Motif Protein 30	-1.78	5.44	0.12797
Csprs	Component of Sp100_rs	-1.71	5.44	0.12797
LOC100041903		-1.67	5.44	0.15613
Phf11	PHD Finger Protein 11	-1.67	5.44	0.13465
Mx1	Interferon-Induced GTP-binding Protein Mx1	-2.79	8.65	0.21366
Siglec1	Sialoadhesin (CD169)	-2.50	8.65	0.23638
LOC631406		-2.30	8.65	0.20973
Elov13	Elongation of very long chain fatty acids like 3	-2.03	8.65	0.19062
AA414992		-1.95	8.65	0.19557
Oas1l	2'-5' oligoadenylate synthetase-like 1	-1.93	8.65	0.20973

Gene Name	Description	FC	FDR	Adj P.
Gm9629		-1.86	8.65	0.19557
Gm684		-1.80	8.65	0.19062
ENSMUST100417		-1.75	8.65	0.20973