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A transcriptomic study of Hereditary Angioedema attacks

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Disclosure of potential conflict of interest

The rest of the authors declare that they have no relevant conflicts of interest.

Abstract

Background: Hereditary Angioedema (HAE) due to C1-inhibitor deficiency is a lifelong illness characterized by recurrent acute attacks of localized skin or mucosal edema. An activation of the kallikrein/bradykinin pathway at endothelial cell level has a relevant pathogenetic role in acute HAE attacks. Moreover, other pathways are involved, given the variable clinical expression of the disease in different patients.

Objective: To explore the involvement of other putative genes in the edema formation.

Methods: We performed a Peripheral Blood Mononuclear Cells microarray gene expression analysis on RNA isolated from HAE patients during acute attack and compared them with the transcriptome profile of the same patients in the remission phase.

Results: Gene expression analysis identified 23 genes significantly modulated during the acute attacks that are primarily involved in “natural killer cells signaling” and “leukocyte extravasation signaling” pathways. GSEA showed a significant activation of relevant biological processes such as the “response to external stimulus” and “protein processing” ($q < 0.05$) suggesting the involvement of PBMCs during HAE acute attacks. Up-regulation of two genes, Adrenomedullin (ADM) and Cellular Receptor for Urokinase Plasminogen Activator (uPAR) that occurs during the acute attack, was confirmed in PBMCs of 20 additional HAE patients by real-time PCR; finally, *in vitro* studies demonstrated the involvement of uPAR in the generation of bradykinin and endothelial leakage.

Conclusions:

Our study demonstrates the increase of ADM and uPAR in PBMC during the acute attack of HAE. Activation of these genes usually involved in the regulation of vascular tone and in inflammatory response might have a pathogenic role by amplifying bradykinin production and edema formation in patients with HAE.

Key messages

The focus of this study is to identify the genes modulated during the acute attack of HAE that might have a pathogenic relevance in this rare disease.

Capsule summary

During an acute attack of HAE the up-regulation of two genes, ADM and uPAR, occurs and the products of these genes might be involved in bradykinin formation and edema amplification.

Key words:

Hereditary angioedema, C1 inhibitor deficiency, peripheral blood mononuclear cells, genes, acute attacks, vascular permeability; plasmin

Abbreviations:

HAE: Hereditary angioedema

C1-INH: C1 inhibitor protein

C1INH: C1 inhibitor gene

BK: bradykinin

HAE-R : HAE patients collected during the remission phase

HAE-A : HAE patients collected during the acute attack

HS : healthy subjects

ADM : Adrenomedullin

uPAR : Cellular Receptor for Urokinase Plasminogen Activator

PBMCs : Peripheral Blood Mononuclear Cells

C1-IHN-HAE: Hereditary angioedema caused by C1-INH deficiency

FXII-HAE: Hereditary angioedema with normal C1-INH and with mutation in Factor XII gene

GSEA : gene set enrichment analysis

FDR : false discovery rate

IPA: Ingenuity Pathway Analysis software

RT-PCR: real-time PCR

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Introduction

Hereditary angioedema (HAE; OMIM #106100) is a potentially life-threatening rare disease with autosomal dominant transmission (1-2), caused by reduced antigenic levels (type I) or activity (type II) of C1 esterase inhibitor protein (C1-INH), due to a mutation in the C1 inhibitor gene (*C1INH*, *SERPING1*; OMIM #606860) (3,4). Other forms of HAE with normal C1-INH have been classified (5-7). Some of these patients carry mutation in Factor XII gene (FXII-HAE), in others the genetic defect is still unknown and inheritance is derived from the segregation of symptoms (8). In one family a mutation in angiopoietin 1 gene has been recently shown to segregate with angioedema symptoms (9). Severe episodes of edema localized at the sub-cutaneous and mucosal layers characterize the acute attacks of HAE that can be extremely debilitating, particularly if the airways are affected (10). The symptoms are caused by the local leakage of fluids from the capillaries as a result of uncontrolled activation of the plasma contact system and generation of vasoactive mediators (5, 11).

C1-INH is a serine protease inhibitor, which acts as a modulator of different pathways such as coagulation and fibrinolytic cascades, complement and contact-kinins systems (12-14). During the attacks of HAE, the uncontrolled activation of these pathways is enhanced as a result of endothelial cell activation (15) and generates factors that increase vascular permeability, provoking edema and inflammation (16). It is now well established that bradykinin is the principal mediator of symptoms of acute attacks of angioedema and that local trauma or emotional stress can represent triggering factors for an acute attack (17-20). However, the molecular events that culminate with the release of bradykinin and generation of tissue edema are not completely understood (21-22).

Different evidences (23-24) report that biomarkers for coagulation system activation (FXII) and for fibrinolytic system activation (plasmin) are functionally linked to bradykinin production (25) associated with endothelial cell activation (26).

Finally, data from the literature suggest that leukocytes might be involved in the pathogenesis of edema formation (27) and cytokines are actively released in HAE (28). Circulating and tissue infiltrating leukocytes can migrate at cutaneous and mucosal levels where they can produce C1-IHN and other Complement factors and might potentially contribute to edema formation (29).

In this study we analyzed the transcriptomic profile of patients during the acute attack of HAE in comparison with the same patients in the remission phase and also comparing HAE patients with healthy subjects in order to identify the genes that may have a pathogenetic relevance in HAE.

Methods

Extended version in supplementary material.

Patient recruitment

We studied 8 patients with C1-INH-HAE during the remission phase (HAE-R) (free of attacks for at least 1 month) and during the acute attack (HAE-A) involving oral submucosal tissues in 1 patient, subcutaneous tissue in 4 patients and the abdomen in 3 (Table 1A). Blood samples were collected in PAX-gene Blood RNA tubes (Becton Dickinson, Italia) within 5 hours from the beginning of symptoms. All patients provided written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Milan, “Luigi Sacco” Hospital.

Laboratory Measurements

The following measurements were performed in non-activated plasma: C1-INH activity was measured using a chromogenic assay (Technochrom C1-inhibitor; Techno-clone GmbH, Vienna, Austria), and C1-INH antigen by means of radial immunodiffusion (RID) (NOR-Partigen, Behring, Marburg, Germany) (Table 1B).

RNA extraction

Total RNA was isolated using PAX-gene Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) according to the manufacturer’s instructions. RNA purity and integrity was verified using lab-on-chip technology (Agilent 2100 Bioanalyzer, Palo Alto, CA, USA).

Gene expression profiling

Transcriptome data were generated using the HumanHT-12 v3 Expression BeadChip (Release 38, Illumina, San Diego, CA. USA) according to the manufacturer’s instructions. The Illumina arrays were then scanned with the HiScanSQ.

Microarray statistical analyses were performed by Genespring GX 11.0 software (Agilent Tech Inc., Santa Clara, CA, USA). Identification of genes differentially expressed between HAE-A and HAE-R patients was carried out with false discovery rate (FDR) method of Benjamini-Hochberg (30) and gene probe sets were filtered on the basis of the FDR, (adjusted-P value with multiple testing on 1000 permutations) and fold-change (FC) (31).

Moreover, gene set enrichment analysis (GSEA) was performed in pairwise comparisons of HAE-A versus HAE-R patients (30).

To assess biological relationships among differently regulated genes, we used the Ingenuity Pathway Analysis software (IPA, Ingenuity System, Redwood City, CA, USA; <http://www.ingenuity.com>).

The Illumina microarray data are MIAME (Minimum Information About a Microarray Experiment) compliant and the raw data have been deposited in the database The European Bioinformatics Institute (*EMBL-EBI*) and are accessible through Experiment ArrayExpress accession: E-MTAB-5897.

cDNA synthesis and quantitative real-time PCR analysis

Real-time PCR (RT-PCR) was performed on total RNA samples (n=20) using an ABI Prism 7900HT Sequence detection system (Applied Biosystems, Foster City, CA, USA). RNA was reverse transcribed into cDNA using a cDNA synthesis kit (QuantiTect Reverse Transcription Kit, Qiagen). RT-PCR amplification reactions were performed using the QuantiTect Primer Assay and the Quantifast SYBR Green PCRmix (Qiagen).

Cell culture

The Jurkat T-cell lines, purchased from ATCC, were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine, at 37°C and 5% CO₂ and 100 µg/ml penicillin and streptomycin (Sigma Aldrich, USA).

Primary Human Umbilical Vein Endothelial Cells (HUVEC) were isolated and cultured on bovine gelatin (Sigma Aldrich, USA) in human endothelial medium supplemented with 10% fetal bovine serum and 100 µg/ml penicillin and streptomycin (Invitrogen, USA) at 37°C and 5% CO₂.

Bradykinin ELISA

The measurement of Bradykinin in cell lysate and cell culture supernatant was assessed using a human Bradykinin enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp., USA), according to the manufacturer's instructions. The cells, seeded in 6-well plates at a density of 2×10^6 cells/well, were pre-incubated with 10 µg/ml and 20 µg/ml anti-uPAR antibody (Immunological Sciences, Italy), to neutralize the uPA-uPAR interaction, for 1 hour and then treated with 100 nM urokinase-type Plasminogen Activator (uPA) (Urokinasi Hospira, Italy), for 15 minutes. Cell lysates and their supernatants were subsequently collected. Bradykinin concentration was measured at 450 nm and calculated from a standard curve.

Endothelial leakage

The *in vitro* endothelial leakage assay was performed as previously described (33). Briefly, HUVEC were seeded on gelatin coated 24 well plate transwell (Costar, USA) and grown till confluence. The medium for the experiment was supplemented with 10% normal human serum and anti-uPAR antibody was added to endothelial cells at 10 µg/ml and at 20 µg/ml, for 15 minutes and then the plasminogen was activated with 100 nM uPA for 30 minutes. Then, the FITC-BSA leaked into the lower chamber was evaluated.

Statistical analysis and bioinformatics

For the remaining experimental analysis, we analyzed data with statistical software (GraphPad Prism; GraphPad, San Diego, CA, USA). All data were expressed as the mean $\pm$ SD of data obtained from at least three independent experiments. Statistical analysis was carried out using two-

tailed paired and unpaired Student's *t* test and one-way ANOVA and Tukey post-hoc tests. P-values lower than 0.05 were considered statistically significant.

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Results

Differences in gene expression during the acute attack of hereditary angioedema

In the first step of the study, in order to identify genes/pathways specifically modulated during acute attacks, we compared the whole-genome gene expression profiles of HAE patients' PBMC collected during the remission phase (HAE-R) with samples from the same patients during the acute attack (HAE-A). We identified 23 genes significantly modulated during the acute attack with a FDR <0.05 and a FC >1.5 (Table 2). These genes were all upregulated during the acute attacks and the Principal Component Analysis (PCA, Figure 1A) showed that these 23 genes well discriminated the acute attack and the remission phase. Then, to determine whether in HAE-A patients there was a coordinated expression or “enrichment” in a set of functionally related genes, we performed a gene set enrichment analysis (GSEA). This computational method allowed us to determine whether an a priori defined set of genes (involved in a specific pathway or biological process) shows statistically significant, concordant differences between PBMCs from HAE-A and HAE-R patients. We identified 10 gene sets, each identifying a specific biological process, significantly modulated ($q < 0.05$) during acute attacks (Table 3). Specifically, the sets of genes up-regulated during the acute attack were mostly associated with “response to external stimulus” and “protein processing” processes ($q < 0.05$). Among genes contributing to “response to external stimulus” process, we found uPAR, called also PLAUR, and ADM (supplemental Table 1). These two genes are factors involved in the regulation of vascular tone and could be involved during the acute HAE attack.

Pathway analysis

We performed a pathway analysis to get a global characterization of the biological functions over-represented by the sets of genes modulated during the acute attack. The analysis revealed that the genes identified during the acute attack were primarily involved in “natural killer cells signaling”

and “leukocyte extravasation signaling” canonical pathways, as indicated in Figure 1B. In particular, the top-ranked network included several genes encoding for regulators of these pathways (score 24.8 associated genes, $p < 0.01$, Figure 1C). Analysis of this network revealed a central role of genes important for the regulation of the vascular tone. Adrenomedullin (ADM) gene is coding for a pre-pro-hormone, which is cleaved to form a potent hypotensive and vasodilator agent. Dysferlin (DYSF) gene is coding for a key calcium ion sensor involved in the Ca^{2+} -triggered synaptic vesicle-plasma membrane fusions and in muscle contraction. Finally the Fc Fragment of IgG Receptor III (FCGR3) gene is coding for a receptor of the Fc region of immunoglobulin gamma that binds IgG complexes and mediates antibody-dependent cellular cytotoxicity and other antibody-dependent responses, such as phagocytosis.

Differences in gene expression between HAE patients and healthy subjects

The second step of the study was to perform a whole-genome gene expression analysis using microarray technology in order to identify genes specifically expressed or modulated in the disease. For this purpose, we compared the genomic profile of PBMCs from HAE-R, HAE-A and healthy subjects (HS).

Using ANOVA with Tukey HSD post hoc test we found 603 genes significantly modulated in HAE patients compared to HS (Supplemental Table 2). PCA showed that these genes could well distinguish the three analyzed sample classes (Figure 2A). Most of identified genes had more different levels of expression than HS whether during the attack or the remission phase (Figure 2B). Then, we performed a GSEA comparing HAE-R and HS to identify the biological processes that could be directly involved in the HAE pathogenesis, since HAE-R patients are in a physiological condition similar to that of HS. The analysis revealed that “cell matrix adhesion”, “cell substrate adhesion” and “g protein signaling coupled to camp nucleotide second messenger” gene sets were the most represented processes differentially regulated in the remission phase of HAE (Table 4).

A further pathway analysis of genes modulated in HAE-R compared to HS revealed among most significant biological processes primarily involved in “inflammatory response” and “immunological disease” canonical pathways, as indicated in Supplemental Figure 1.

In particular, 53 genes belonged to the category of “inflammatory response” (p-value = 2.73E-07), and 51 genes belonged to the category of “immunological disease” (p-value = 8.26E-09) (Table 5). Surprisingly, among genes involved in these biological functions we found again ADM and uPAR, that resulted significantly down-regulated in HAE-R patients compared to HS, while Granzyme and Perforin1 were up-regulated.

Moreover, also “Granzyme B signaling” and “VEGF signaling” pathways, have been found significantly modulated in HAE-R compared to HS (Supplemental Figure 2).

Differential expression of ADM and uPAR genes at transcriptional level

Two of the genes identified by microarray analysis were of great importance for the pathogenesis of the disease because they are involved not only in the regulation of vascular tone and in the inflammatory response but also in bradykinin-forming mechanisms controlled by C1-IHN. Therefore, in order to confirm the gene expression modulation of these two genes, we performed a quantitative RT-PCR for ADM and uPAR in an independent set of 20 HAE patients with the same clinical and demographic characteristics of those used for microarray experiments. We found that ADM and uPAR gene expression was significantly higher during the acute attack episode compared to the same subject in the remission phase (**p<0.02, *p<0.05 respectively, Figure 3 A-B). These results were in line with those obtained by the gene expression array.

Blocking the uPA-uPAR interaction alters bradykinin pathway

The mechanism of action of uPAR in the production of bradykinin was examined. We neutralized the uPA-uPAR interaction with a blocking anti-uPAR antibody both at 10 μ g/ml and at 20 μ g/ml, for 1 hour and then we activated the plasminogen with 100 nM uPA for 15 minutes. Then, we measured the T cell bradykinin production.

The treatment with anti-uPAR antibody attenuated the production of bradykinin, induced by urokinase plasminogen activator (uPA), in a dose-dependent manner, both in the cell lysate (p=0.0002 at 10 μ g/ml and p<0.0001 at 20 μ g/ml, Figure 3C) and in the supernatant (p=0.01 at 10 μ g/ml and p=0.001 at 20 μ g/ml, Figure 3D). Anti uPAR-treated cells showed a significantly reduction of bradykinin production also in the control medium without uPA stimulation (supplemental Figure 3 A-B).

Blocking the uPA-uPAR interaction alters endothelial leakage

The role of uPAR in the endothelial leakage was evaluated by in vitro assay as previously described (32). Anti-uPAR antibody was added to the endothelial cells at 10 μ g/ml and at 20 μ g/ml, for 15 minutes and then we activated the plasminogen with 100 nM uPA for 30 minutes. Then, the FITC-BSA leaked into the lower chamber was evaluated. The treatment with anti-uPAR antibody inhibited the endothelial leakage induced by uPA (Figure 3 E).

Discussion

The first finding emerging from our analysis conducted on PBMCs isolated from HAE patients is that the genes identified during the acute attacks were primarily involved in “natural killer cells signaling” and “leukocyte extravasation signaling” pathways. Our bioinformatics analysis demonstrated for the first time that the expression of two genes, ADM and uPAR, was up-regulated during acute attacks when comparing HAE-A vs HAE-R and was down-regulated in HAE-R vs HS. These genes codify for molecules with vasodilator effects and are involved not only in the regulation of vascular tone and in the inflammatory response, but also in the bradykinin-forming mechanisms, the principal mediator of HAE attacks.

Our results shed more light on the role for adaptive immunity in the development of HAE that remained controversial over time. Before us, only Lopez-Lera and colleagues analyzed the whole-genome RNA expression of PBMCs in three HAE type-I families according to the presence of mutation and the clinical symptoms (27). They could not find differentially regulated genes in PBMCs of HAE patients. However, the design of their study was very different and based on comparisons between patients with mutation versus patients without mutation within the analyzed families. Moreover, recent lines of evidence demonstrate that neutrophil and mast cell activation is functionally linked to bradykinin production through the contact system activation on endothelial cells (23). In this regard, the results reported by the last evidences and by our study, support the hypothesis that the adaptive immunity function may affect the pathogenesis of HAE.

Adrenomedullin (ADM) is a 52-amino-acid vasoactive peptide with vasodilator activity mediated by the cyclic adenosine monophosphate (cAMP), NO and renal prostaglandin systems (33). ADM acts via a G protein–coupled 7-transmembrane domain receptor, which associates the calcitonin receptor-like receptor (CRLR). It modulates proteins, namely receptor activity-modifying proteins (RAMP) 2 and RAMP 3 (34).

ADM could act as an endogenous immunomodulatory factor, with predominant anti-inflammatory effects (35). It is produced in many types of tissue, but the endothelium (36) and the monocytes (37)

are the principal source, in particular in response to proinflammatory cytokines (38). ADM reduces endothelial hyper-permeability and is an apoptosis survival factor (39).

Our microarray data confirmed the study recently published by Kajadacsi and colleagues (40). They demonstrated that during attacks of hereditary angioedema due to C1-IHN, the excess of bradykinin that enhanced the endothelial cells permeability and the activation of endothelial cells, was associated with elevated levels of several vasoactive peptides, such as ADM, AVP and ET-1. In particular, they suggested that the cooperation of these vasoactive peptides might be necessary to counterbalance the vasodilatory activity of bradykinin and to limit the attacks. Recently, Xie and colleagues (37), confirmed these data in acute Systemic Capillary Leak Syndrome (SCLS) characterized by abrupt and transient episodes of hypotensive shock and edema due to plasma leakage into peripheral tissues.

Finally, it is important to note that Complement Factor H is a serum-binding protein for ADM. (41) The interaction between Complement Factor H (also known as adrenomedullin binding protein 1, AMBP-1) and ADM, has been shown to have possible therapeutic application in various inflammatory diseases (42) and emerged also by our transcriptomic data (Figure 1C). There is plenty of evidence on the reciprocal effects of binding on Complement factor H and ADM activity (43).

Initially, the complement activation was proposed in the literature as the principal pathway involved in the pathogenesis of C1-IHN-HAE, but it was later discredited (22, 44-46). As known, the coagulation, fibrinolysis, and plasma kallikrein cascades are interrelated as shown in Figure 3F and these cascades are controlled by C1-IHN (47). In the last years several studies focused on the role of fibrinolysis as contributor to angioedema (25,48) and our data are in accordance with these evidences.

Cellular Receptor for Urokinase Plasminogen Activator (PLAUR or uPAR) is a glycosylphosphatidylinositol (GPI)-anchored protein (49) and is able to bind both the pro- and active forms of Urokinase Plasminogen Activator (uPA) (50). Once activated, the primary function

of uPA is the conversion of plasminogen to plasmin, a broad-spectrum enzyme capable of widespread extracellular matrix degradation and activation of several pro-MMPs. Several lines of evidence indicate that, in addition to fibrinolysis, the uPA–uPAR system also modulate numerous steps of the inflammatory cascade and influence the development of immune responses (51).

The inflammation is an adaptive response to damage of vascularized tissues (52). There is a lot of evidence to suggest that the plasminogen activation system is implicated in the regulation of all phases of the inflammatory response, but it plays a crucial role in leucocyte recruitment to the site of the inflammation (53-54).

uPAR is expressed constitutively in various immunologically active cells, included monocytes, macrophages and activated T-cells and also in endothelial cells (55-56). Is well known that uPAR interacts with components of the bradykinin-forming cascade, but uPAR actually binds HK best when it has been cleaved (57) and is an important binding protein for Factor XII (58).

We demonstrated that when we neutralized the uPAR expressed on T cells, the production of bradykinin was reduced in a dose-dependent manner. These new data demonstrate the involvement of uPAR in the production of bradykinin, that is the pivotal mediator of edema formation, showing a plausible additive mechanism of action: the uPAR overexpression in HAE-A patients, promotes the plasminogen activation by uPA with the consequent formation of plasmin finally leading to increased levels of bradykinin and endothelial leakage. This mechanism is in addition to other pathways, such as that of the contact system in which kallikrein leads to bradykinin production (Figure 3F). Intriguingly, in endothelium when uPAR is expressed, plasminogen is activated also in the absence of fibrin (59). Moreover, recently, Joseph et al demonstrated that pro-inflammatory mediators (IL-1 and TNF- α), and estrogen stimulate release of urokinase from endothelial cells, which can convert plasminogen to plasmin and represents a possible source for plasmin generation (60).

These findings are in agreement with previous observations that, in patients deficient in C1-INH the role of plasmin, the key enzyme of the fibrinolytic cascade, appears more prominent (14, 22, 24).

We are aware of the limitations of our study. Although we have performed a detailed gene expression analysis both comparing HAE-A versus HAE-R patients and HAE patients versus HS, we have not investigated ADM and uPAR serum levels because our approved clinical protocol did not consider the serum collection.

In conclusion, our study suggests, for the first time, that during the acute attack of HAE two genes involved in the regulation of vascular tone, ADM and uPAR, are up-regulated in PBMC and the products of these genes could provide a further mechanism involved in the bradykinin production and edema formation.

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Figure legends

Figure 1. Differences in gene expression during the acute attack of C1-IHN-HAE and functional analysis of the top selected genes identified by microarray during the acute attack.

A. The PCA shows that gene expression profile is different between HAE-A and HAE-R patients. **B.** The most representative canonical pathways dysregulated during the acute attack are mainly involved in “natural killer cells signaling” and “leukocyte extravasation signaling”. Graph shows category scores; “threshold” indicates the minimum significance level [scored as $-\log(p\text{-value})$ from Fisher’s exact test, set here to 1.25]. “Ratio” (differential yellow line and markers) refers to the number of molecules from the dataset that map to the pathway listed divided by the total number of molecules that map to the canonical pathway from within the IPA knowledgebase. **C.** The network has been algorithmically constructed using IPA-software based on the functional and biological connectivity of genes. Genes are graphically symbolized as nodes and the biological relationships between genes as edges. Red nodes represent genes containing identified variants; empty nodes are biologically linked to the studied genes based on evidence in the literature. The analysis of this top-ranked network (score 24.8, $p < 0.01$) reveals the presence of ADM, gene important for the regulation of the vascular tone. The red line evidences the interaction between Complement Factor H and ADM.

Figure 2. Differences in gene expression between HAE patients and healthy subjects

The PCA (**A**) shows that gene expression profile is different among the three analyzed sample classes. The profile plot (**B**) represents the gene expression patterns at $FC \geq 1.5$ relative at 90 of 603 genes up- and down-regulated between HAE patients and healthy subjects. Up-regulated genes are shown in red, and down-regulated ones in blue.

Figure 3. Validation of gene expression levels and functional studies. The amount of ADM (**A**) and uPAR (**B**) in an independent set of 20 HAE patients has been evaluated by real-time PCR.

ADM- and uPAR-normalized gene expression levels are significantly higher during the acute attack of the disease compared with HAE-R (** $p < 0.02$, * $p < 0.05$ respectively, p-values obtained by paired t-test). The histograms represent the mean $\pm$ SD. The treatment with anti-uPAR antibody reduces the production of bradykinin, in a dose-dependent manner, in the Jurkat cell lysate (**C**, $p = 0.0002$ at 10 $\mu\text{g/ml}$ and $p < 0.0001$ at 20 $\mu\text{g/ml}$ compared to uPA) and in the supernatant (**D**, $p = 0.01$ at 10 $\mu\text{g/ml}$ and $p = 0.001$ at 20 $\mu\text{g/ml}$ compared to uPA). **E**. The treatment with anti-uPAR antibody inhibits the endothelial leakage induced by uPA ($p < 0.05$ compared to uPA). **F**. Schematic overview of interactions between the contact, complement and plasminogen activation system with a central role of uPAR in the production of bradykinin.

Table 1. A. Demographics characteristics of HAE patients included in the study, gene defects and type of symptoms during attacks. B. Measurements of complement parameter markers in 8 healthy control subjects (HS) and 8 patients with C1-inhibitor deficiency during the acute attack (HAE-A) and during the remission phase (HAE-R).

A.

Patient code	Sex	Age	Site of attack	SERPING1 transcript Mutation
BT	F	48	oral submucosal tissues	NM_000062.2: c.1481G>T
DG	F	62	abdomen	NM_000062.2:c.1029+84G>A
MG	F	68	subcutaneous tissue	NM_000062.2:c.1475T>A
PS	F	48	abdomen	NM_000062.2:c.1269_1270insA
FF	F	78	abdomen	NM_000062.2:c.1269_1270insA
CM	M	18	subcutaneous tissue	NM_000062.2:c.508T>C
LM	M	16	subcutaneous tissue	large deletion
AC	F	39	subcutaneous tissue	NM_000062.2:c.-99dup

B.

Laboratory tests	Healthy subjects (HS, n=8)	HAE during remission phase (HAE-R, n=8)	HAE during acute attack (HAE-A, n=8)
C1 IHN function (%)	75.9	10.8	4.1
C 1 IHN antigen (%)	103	26.9	21.0

Table 2

The most significant genes (probes) (FDR<0.05 and FC >1.5) differentially regulated during the HAE-Acute attack.

Symbol	Entrez Gene Name	Entrez Gene ID	Fold Change
FCGR3B	Fc Fragment Of IgG, Low Affinity IIIb, Receptor (CD16b)	2215	1.5255
CEACAM1	carcinoembryonic antigen related cell adhesion molecule 1	634	1.5577
NCF1C	neutrophil cytosolic factor 1C pseudogene	654817	1.5198
HSH2D	hematopoietic SH2 domain containing	84941	1.5723
IL1RN	interleukin 1 receptor antagonist	3557	1.6564
ISG20	interferon stimulated exonuclease gene 20	3669	1.5261
HIST2H2AA4	Histone Cluster 2, H2aa4	723790	1.5875
TNFSF13B	TNF superfamily member 13b	10673	1.5530
IL1RN	interleukin 1 receptor antagonist	3557	1.7110
ADM	Adrenomedullin	133	1.5872
HIST2H2AA3	Histone Cluster 2, H2aa3	8337	1.6390
LRG1	leucine rich alpha-2-glycoprotein 1	116844	1.6178
HIST2H2AC	histone cluster 2 H2A family member c	8338	1.5242
PADI4	peptidyl arginine deiminase 4	23569	1.5031
NCF1	neutrophil cytosolic factor 1	653361	1.5944
LPAR2	lysophosphatidic acid receptor 2	9170	1.5020
MMP9	matrix metalloproteinase 9	4318	1.6025

DYSF	Dysferlin	8291	1.5576
SLC16A3	Solute Carrier Family 16 (Monocarboxylate Transporter), Member 3	9123	1.5833
LILRA5	Leukocyte Immunoglobulin-Like Receptor, Subfamily A (With TM Domain), Member 5	353514	1.6076
LILRA5	Leukocyte Immunoglobulin-Like Receptor, Subfamily A (With TM Domain), Member 5	353514	1.5303
FFAR2	Free Fatty Acid Receptor 2	2867	1.6071
TNFAIP6	TNF alpha induced protein 6	7130	1.5243

Table 3

The most represented processes ($q < 0.05$) identified by the GSEA during the acute attack of HAE. The “response to external stimulus” gene set built-in genes important for the structure or regulation of extracellular matrix (uPAR) and for the regulation of the vascular tone (ADM).

Gene Set	Total Genes	Genes found	p-value [HAE-A] vs [HAE-R]	q-value [HAE-A] vs [HAE-R]	ES [HAE-A] vs [HAE-R]	NES [HAE-A] vs [HAE-R]
REGULATION_OF_SECRETION	40	15	0	0.039	0.619	1.836
NEGATIVE_REGULATION_OF_CELLULAR_BIOSYNTHETIC_PROCESS	28	15	0	0.047	0.683	1.872
PROTEIN_SECRETION	32	17	0	0.017	0.671	2.009
EPIDERMIS_DEVELOPMENT	70	15	0.018	0.037	0.665	1.808
PROTEIN_PROCESSING	48	23	0	0.035	0.649	1.821
RESPONSE_TO_EXTERNAL_STIMULUS	306	137	0	0.026	0.519	1.913
PROTEIN_AMINO_ACID_PHOSPHORYLATION	267	132	0	0.041	0.526	1.825
NEGATIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS	29	16	0	0.031	0.650	1.817
ECTODERM_DEVELOPMENT	79	17	0.018	0.035	0.664	1.870
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	140	58	0	0.100	0.591	1.924

Table 4

The most represented processes ($q < 0.05$) identified by the gene set enrichment analysis comparing HAE-R patients and healthy subjects (HS).

Gene Set	Total Genes	Genes found	p-value [HAE R] vs [HS]	q-value [HAE R] vs [HS]	ES [HAE R] vs [HS]	NES [HAE R] vs [HS]
CELL_SUBSTRATE_ADHESION	38	16	0	0.013	-0.697	-1.941
G_PROTEIN_SIGNALING_COUPLED_TO_CAMP_NUCLEOTIDE_SECOND_MESSENGER	64	20	0	0.018	-0.551	-1.833
CELL_MATRIX_ADHESION	37	15	0	0.007	-0.71	-1.931

Table 5

The most representative canonical pathways dysregulated in HAE-R patients compared to HS, identified by Anova and Tukey tests. The genes ADM and uPAR were built-in both in the “inflammatory response” and “immunological disease” categories.

Categories	Diseases or Functions Annotation	p-Value	Activation z-score	Molecules	Molecules
Inflammatory Response, Organismal Injury and Abnormalities	inflammation of organ	2,73E-07	0,879	ADM ,AHNAK,ALOX12,BCL2L1, BCL6,BMX,C5AR2,CA4,CCR6, PNE1,CR1,CXCL1,CXCL16,F5, FCGR2B,FGR,FUT7,FYN,GNB1L, GP1BA, GZMB ,HCK,IFNAR1, IFNGR2,IMPDH1,IRF5,MAPK3, MGAM,NCF4,NFATC3,NFKBID, NLRP12,NLRX1,NOTCH1,OSM, PGLYRP1, PLAUR ,PREX1, PRF1 , PRKCD,SELL,SEMA4A,SMAD7,	53

				SMPD1,STX2,TBCE,TGM3,TLR5, TNFRSF1B,TNFRSF9,TUBB1, UTRN,ZFP36	
Immunological Disease	systemic autoimmune syndrome	8,26E-09	0,296	ACSL1, ADM ,ALOX12,ALPL, BCL2L1,BCL6,CASP8,CCR6,CD3E, CDA,CR1,CXCL1,CXCL16,DEPDC5 ,DNM1L,FCGR2B,GNLY,GP1BA, GZMB ,HCAR3,HCK,IFNAR1, IFNGR2,IMPDH1,IRF5,KCNJ15, LILRB3,LYZ,MAP4K4,MAPK3, MGAM,MSRA,NCF2,NCF4,NCR3, OSM,P2RY13,PADI2,PADI4, PGLYRP1, PLAUR ,PRF1,SELL, SIRPB1,SP1,TLR5,TNFRSF1B, TNFRSF9,UCP2,VPS37C,ZFP36	51

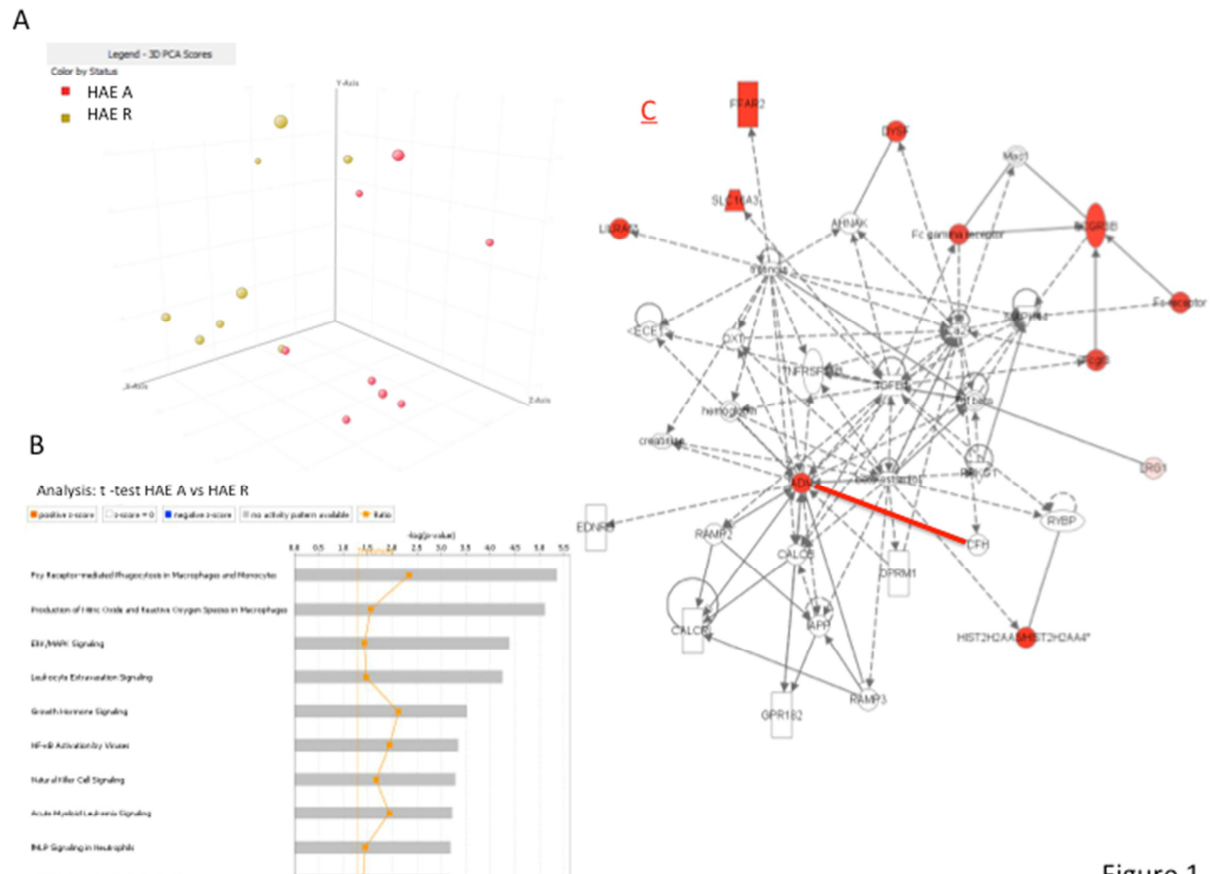


Figure 1

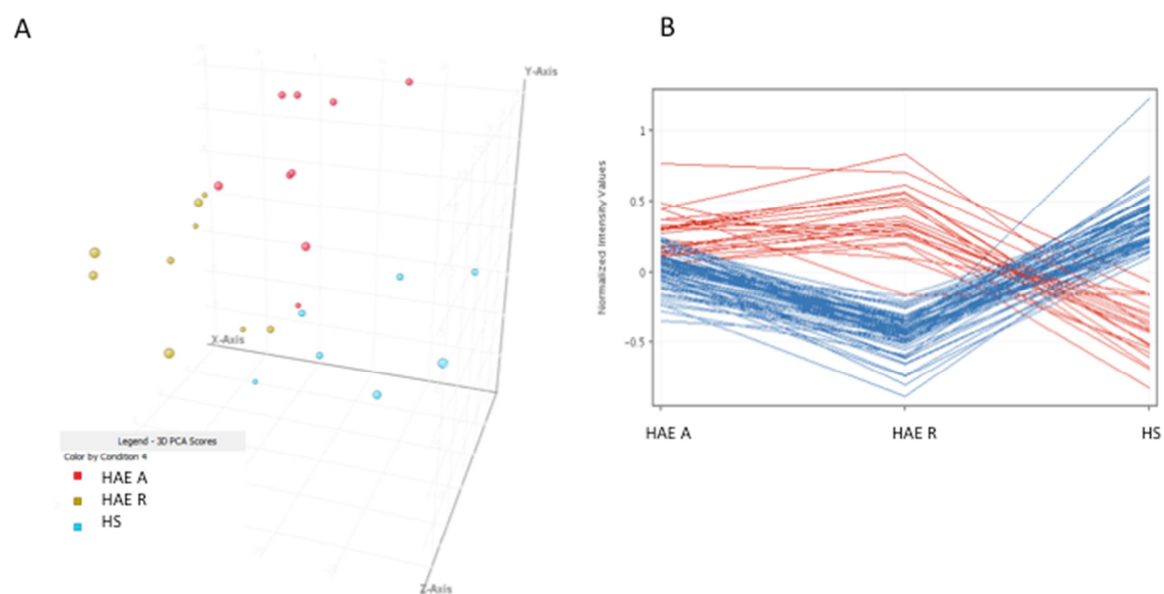


Figure 2

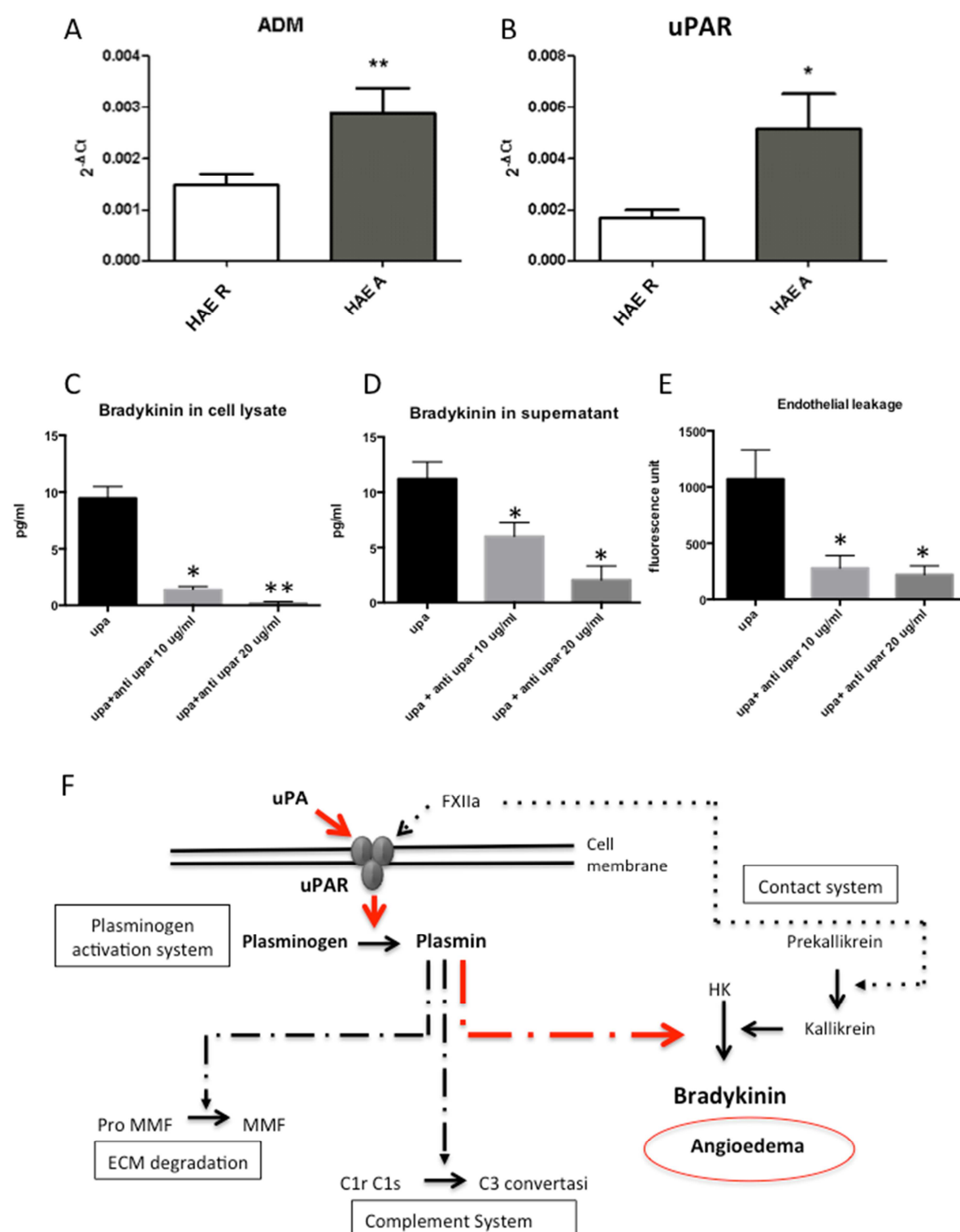
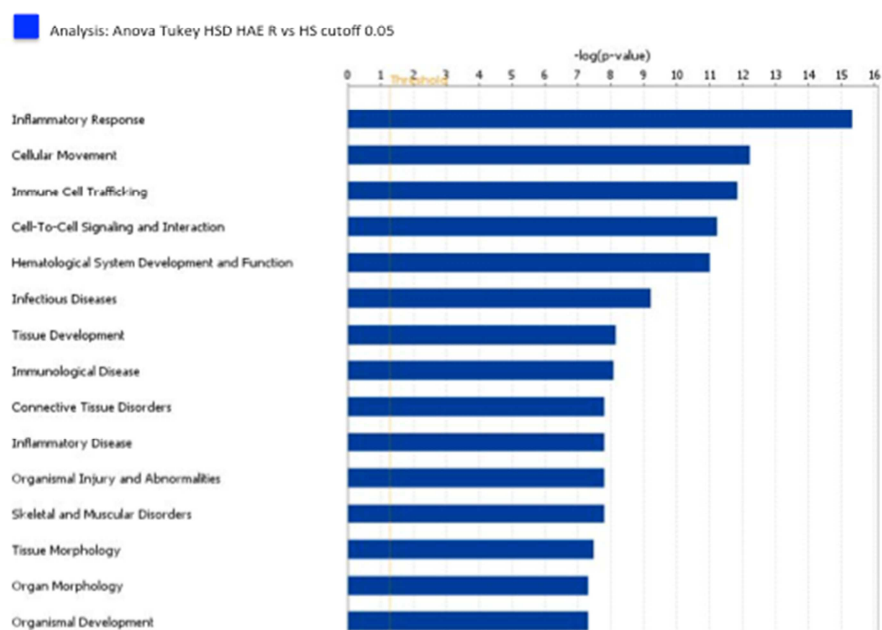
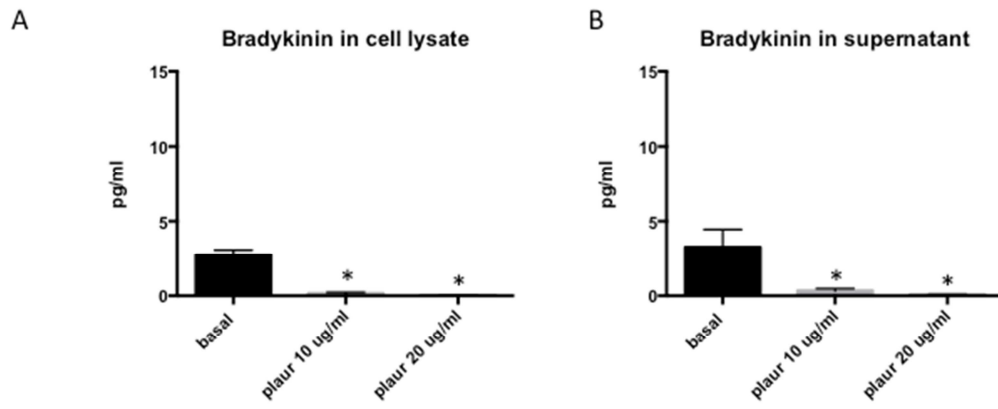


Figure 3



Supplemental Figure 1



Supplemental Figure 3

Supplemental Table 1

The most significant genes (FC >1.5) included in the response to external stimuli gene set, identified by the GSEA during the acute attack of HAE.

ProbeID	Symbol	Entrez_Gene_ID	Definition
3930343	ALOX5AP	241	arachidonate 5-lipoxygenase-activating protein (ALOX5AP)
5080367	CKLF	51192	chemokine-like factor (CKLF)
1030463	PROK2	60675	prokineticin 2 (PROK2)
1030270	FPR1	2357	formyl peptide receptor 1 (FPR1)
4670750	TNFRSF1A	7132	tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A)
6960630	CD97	976	CD97 molecule (CD97)
20446	CEBPB	1051	CCAAT/enhancer binding protein (C/EBP), beta (CEBPB)
3990703	IL10	3586	interleukin 10 (IL10)
110685	SYK	6850	spleen tyrosine kinase (SYK)
5670465	ADM	133	adrenomedullin (ADM)
5670719	IL10RB	3588	interleukin 10 receptor, beta (IL10RB)
6250615	PGLYRP1	8993	peptidoglycan recognition protein 1 (PGLYRP1)
6520180	IL18RAP	8807	interleukin 18 receptor accessory protein (IL18RAP)
6220671	PLAUR	5329	plasminogen activator, urokinase receptor (PLAUR)
2140242	TNFAIP6	7130	tumor necrosis factor, alpha-induced protein 6 (TNFAIP6)

Supplemental Table 2

The most significant genes differentially regulated in HAE compared to HS, identified by Anova with Tukey HSD post hoc test.

Symbol	Entrez Gene ID	Entrez Gene Name	FC HAE A vs HS	FC HAE R vs HS
FCGR2B	2213	PREDICTED: Fc fragment of IgG, low affinity IIb, receptor (CD32)	-1.0850	-1.1365
ARID3A	1820	AT rich interactive domain 3A (BRIGHT-like)	1.0401	-1.2279
UBN1	29855	ubinuclein 1	1.0096	-1.4148
MGLL	11343	monoglyceride lipase	1.1461	1.0964
KCNJ15	3772	potassium inwardly-rectifying channel, subfamily J, member 15	-1.0702	-1.2911
TUBB1	81027	tubulin, beta 1	1.6711	1.6685
SULT1A1	6817	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	-1.2613	-1.3965
ALOX5AP	241	arachidonate 5-lipoxygenase-activating protein	1.0567	-1.3066
LOC729580	729580	PREDICTED: hypothetical LOC729580	1.0827	1.1051
CD69	969	CD69 molecule	1.0290	1.2100
ALPL	249	alkaline phosphatase, liver/bone/kidney	-1.6717	-2.4399
KLC1	3831	kinesin light chain 1	-1.0570	-1.0814
TBC1D24	57465	TBC1 domain family, member 24	1.0132	-1.1668
FLJ77644	728772	PREDICTED: similar to transmembrane protein 106A, transcript variant 3	1.0955	-1.0114
LILRA5	353514	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	1.1685	-1.1681
SMC2	10592	structural maintenance of chromosomes 2	1.0287	1.1107
SLC25A44	9673	solute carrier family 25, member 44	-1.0934	-1.4758
KIAA1949	170954	KIAA1949	-1.0224	-1.3549
RXRA	6256	retinoid X receptor, alpha	1.0156	-1.2679
MOBK12A	126308	MOBK1, Mps One Binder kinase activator-like 2A	-1.4378	-1.7528
TSHZ3	57616	teashirt zinc finger homeobox 3	-1.0768	-1.2084
SMAD7	4092	SMAD family member 7	-1.0035	1.0872
CYTH3	9265	cytohesin 3	1.1092	1.0289
LOC729660	729660	PREDICTED: misc_RNA (LOC729660)	-1.3524	-1.4311
RBM23	55147	RNA binding motif protein 23	-1.0192	-1.3458
HAUS8	93323	HAUS augmin-like complex, subunit 8	-1.0834	-1.0547
ZNF83	55769	zinc finger protein 83	-1.0590	1.1295
SEMA4B	10509	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4B	-1.0718	-1.4289
EFHD2	79180	EF-hand domain family, member D2	1.0829	-1.1926
TRIM8	81603	tripartite motif-containing 8	-1.0544	-1.3995
SULT1A4	445329	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 4	-1.5594	-1.7406
PDLIM7	9260	PDZ and LIM domain 7	1.0597	-1.2310
BST1	683	bone marrow stromal cell antigen 1	-1.1865	-1.2706
TUBGCP4	27229	tubulin, gamma complex associated protein 4	1.1200	1.1271
DOK2	9046	docking protein 2, 56kDa	1.1955	1.2283
PSG3	5671	pregnancy specific beta-1-glycoprotein 3	-1.0430	-1.4443
GNLY	10578	granulysin	1.7589	1.9861
CARS	833	cysteinyI-tRNA synthetase	-1.1183	-1.1165

RPGR	6103	retinitis pigmentosa GTPase regulator	-1.0555	-1.0166
WDR33	55339	WD repeat domain 33	1.0173	1.0856
STAT3	6774	signal transducer and activator of transcription 3 (acute-phase response factor)	1.1233	-1.1494
STAT3	6774	signal transducer and activator of transcription 3 (acute-phase response factor)	1.0725	-1.2223
SNORA33	594839	small nucleolar RNA, H/ACA box 33	1.0624	1.0725
TBC1D2	55357	TBC1 domain family, member 2	-1.0997	-1.2352
LOC100132885	100132885	PREDICTED: misc_RNA (LOC100132885)	-1.2266	-1.2813
COPS8	10920	COP9 constitutive photomorphogenic homolog subunit 8 (Arabidopsis)	-1.0875	1.0392
		K-EST0218892 L18POOL1n1 cDNA clone L18POOL1n1-30-G08 5	1.0673	-1.0022
DCAF16	54876	DDB1 and CUL4 associated factor 16	1.1478	1.1713
MIR1978	100302173	microRNA 1978	1.5839	1.4861
APIP	51074	APAF1 interacting protein	1.0349	1.0993
GPR109B	8843	G protein-coupled receptor 109B	-1.2265	-1.4402
PROK2	60675	prokineticin 2	-1.4579	-2.2962
TOP1	7150	topoisomerase (DNA) I	1.0763	1.0901
PIM3	415116	PREDICTED: pim-3 oncogene	-1.2105	-1.4518
FYN	2534	FYN oncogene related to SRC, FGR, YES	1.1699	1.2573
ABHD2	11057	abhydrolase domain containing 2	-1.1141	-1.2163
ABHD2	11057	abhydrolase domain containing 2	-1.0701	-1.1248
OTUD6B	51633	OTU domain containing 6B	-1.0416	1.0253
FUT7	2529	fucosyltransferase 7 (alpha (1,3) fucosyltransferase)	-1.0927	-1.2621
		PREDICTED: hypothetical LOC388227	-1.0489	-1.0771
		cDNA FLJ26784 fis, clone PRS04220	1.1290	1.1560
TREML2	79865	triggering receptor expressed on myeloid cells-like 2	1.0339	-1.2392
GRB14	2888	growth factor receptor-bound protein 14	-1.0028	-1.0820
CR1	1378	complement component (3b/4b) receptor 1 (Knops blood group)	-1.1901	-1.2758
DCAKD	79877	dephospho-CoA kinase domain containing	1.1712	1.2621
ISL2	64843	ISL LIM homeobox 2	-1.1012	-1.1444
PHEX	5251	phosphate regulating endopeptidase homolog, X-linked (hypophosphatemia, vitamin D resistant rickets)	-1.0552	-1.0045
PPAP2C	8612	phosphatidic acid phosphatase type 2C	-1.2828	-1.3724
RASL11A	387496	RAS-like, family 11, member A	-1.0822	-1.1013
TAF1L	138474	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 210 kDa-like	-1.0909	1.0127
C21orf57	54059	chromosome 21 open reading frame 57	1.1985	1.2717
PFKFB4	5210	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	1.0106	-1.4665
SCARNA3	677679	small Cajal body-specific RNA 3	1.2362	1.0504
STX2	2054	syntaxin 2	-1.1162	-1.1853
SIRPB1	10326	signal-regulatory protein beta 1	-1.1202	-1.3031
AIF1L	83543	allograft inflammatory factor 1-like	1.1315	1.0969
OPRL1	4987	opiate receptor-like 1	-1.2272	-1.5766
GNLY	10578	granulysin	1.3916	1.6201
GNLY	10578	granulysin	1.8514	2.0271
FOLR3	2352	folate receptor 3 (gamma)	-2.2055	-3.1798
LOC730286	730286	PREDICTED: misc_RNA (LOC730286)	-1.4064	-1.9013

ODF2L	57489	outer dense fiber of sperm tails 2-like	-1.042954	1.0269
FPR1	2357	formyl peptide receptor 1	-1.1604	-1.7086
UBAP2	55833	ubiquitin associated protein 2	1.0949	1.1035
LIMK2	3985	LIM domain kinase 2	1.0138	-1.134
C15orf63	25764	chromosome 15 open reading frame 63	1.0956	1.0728
TMEM38B	55151	transmembrane protein 38B	1.0136	1.0297
TTC38	55020	tetratricopeptide repeat domain 38	1.3882	1.4599
TMEM129	92305	transmembrane protein 129	-1.0552	-1.0848
PSRC1	84722	proline/serine-rich coiled-coil 1	-1.0594	-1.1037
LOC285908	285908	hypothetical protein LOC285908	-1.2209	-1.2728
IRF5	3663	interferon regulatory factor 5	-1.2733	-1.3882
NCF4	4689	neutrophil cytosolic factor 4, 40kDa	-1.0040	-1.4514
NCF4	4689	neutrophil cytosolic factor 4, 40kDa	-1.2420	-1.8273
LOC651816	651816	PREDICTED: similar to Ubiquitin-conjugating enzyme E2S (Ubiquitin-conjugating enzyme E2-24 kDa) (Ubiquitin-protein ligase) (Ubiquitin carrier protein) (E2-EPF5)	1.1752	1.0567
C19orf38	255809	chromosome 19 open reading frame 38	-1.0953	-1.1746
TNFRSF1A	7132	tumor necrosis factor receptor superfamily, member 1A	1.0759	-1.2237
AVEN	57099	apoptosis, caspase activation inhibitor	1.0145	1.0995
GPR109A	338442	G protein-coupled receptor 109A	-1.1960	-1.3678
PSMD12	5718	PREDICTED: proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	1.3002	1.0955
SH2D3C	10044	SH2 domain containing 3C (SH2D3C)	-1.0830	-1.3340
MPP5	64398	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)	-1.0272	1.0429
LOC100128326	100128326	PREDICTED: misc_RNA (LOC100128326)	-1.5690	-2.0849
KISS1R	84634	KISS1 receptor	-1.1913	-1.1727
HNRNPL	3191	heterogeneous nuclear ribonucleoprotein L	1.4004	1.3333
UBE2C	11065	ubiquitin-conjugating enzyme E2C	1.1062	1.0198
PCNX	22990	pecanex homolog	-1.0588	-1.3585
LOC730746	730746	PREDICTED: similar to Heterogeneous nuclear ribonucleoprotein A1 (Helix-destabilizing protein) (Single-strand RNA-binding protein) (hnRNP core protein A1) (HDP)	-1.0280	-1.0293
VPS37C	55048	vacuolar protein sorting 37 homolog C	-1.0705	-1.3410
RARA	5914	retinoic acid receptor, alpha	1.3248	-1.0231
GPAM	57678	glycerol-3-phosphate acyltransferase, mitochondrial, nuclear gene encoding mitochondrial protein.	-1.0525	1.0048
TYMS	7298	thymidylate synthetase	1.1615	1.0955
NCF4	4689	neutrophil cytosolic factor 4, 40kDa	-1.0250	-1.3456
LOC648370	648370	PREDICTED: hypothetical protein LOC648370	1.0505	1.0804
LOC729852	729852	PREDICTED: misc_RNA (LOC729852)	-1.0141	1.0348
ZCRB1	85437	zinc finger CCHC-type and RNA binding motif 1	-1.0495	-1.0483
FRAT2	23401	frequently rearranged in advanced T-cell lymphomas 2	-1.1042	-1.4857
LOC642947	642947	2 hypothetical protein LOC642947	1.2957	1.0701
NFKBIB	4793	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta	1.1632	1.0990

PRF1	5551	perforin 1 (pore forming protein)	1.7736	2.2484
BCL2L1	598	BCL2-like 1 (BCL2L1), nuclear gene encoding mitochondrial protein	1.3895	1.7159
RBM43	375287	RNA binding motif protein 43	1.1833	1.1589
HERC4	26091	hect domain and RLD 4	-1.0579	-1.0735
TGFBR3	7049	transforming growth factor, beta receptor III	1.3750	1.4809
ESRRAP2	144832	estrogen-related receptor alpha pseudogene 2 on chromosome 13.	-1.1258	-1.2282
PRMT5	10419	protein arginine methyltransferase 5	-1.0440	-1.1611
SLC43A3	29015	solute carrier family 43, member 3	1.1043	1.0472
SEPX1	51734	selenoprotein X, 1	1.0864	-1.3003
GAB2	9846	GRB2-associated binding protein 2	1.0496	-1.3718
PIP4K2B	8396	phosphatidylinositol-5-phosphate 4-kinase, type II, beta	1.0444	1.1107
LOC728741	728741	PREDICTED: hypothetical LOC728741	1.0879	1.1637
SLA2	84174	Src-like-adaptor 2	1.2010	1.1937
AGPAT2	10555	1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	-1.0150	-1.2410
MSRA	4482	methionine sulfoxide reductase A	-1.1083	-1.3057
ACP2	53	acid phosphatase 2, lysosomal	1.2762	1.1638
PSMD5	5711	proteasome (prosome, macropain) 26S subunit, non-ATPase, 5	-1.126	-1.0740
EIF2AK3	9451	eukaryotic translation initiation factor 2-alpha kinase 3	1.0120	1.0485
UTRN	7402	utrophin	1.1604	1.1304
SLC2A14	144195	solute carrier family 2 (facilitated glucose transporter), member 14	-1.0901	-1.1448
NLRX1	79671	NLR family member X1	-1.2312	-1.3672
ARHGEF19	128272	Rho guanine nucleotide exchange factor (GEF) 19	-1.0950	-1.1362
CD14	929	CD14 molecule	-1.0198	-1.3402
PDSS1	23590	prenyl (decaprenyl) diphosphate synthase, subunit 1	-1.0061	1.0414
PTGDS	5730	prostaglandin D2 synthase 21kDa	1.2804	1.4703
		cDNA FLJ44441 fis, clone UTERU2020242	1.2022	1.2367
TNFRSF9	3604	tumor necrosis factor receptor superfamily, member 9	-1.0892	-1.1048
HRASLS3	11145	HRAS-like suppressor 3	1.3182	1.3195
CEACAM1	634	carcinoembryonic antigen-related cell adhesion molecule 1	1.1526	-1.2060
CEACAM1	634	carcinoembryonic antigen-related cell adhesion molecule 1	1.2035	-1.3174
KCTD15	79047	potassium channel tetramerisation domain containing 15	-1.1100	-1.1208
DDAH2	23564	dimethylarginine dimethylaminohydrolase 2	-1.1256	-1.2548
C13orf18	80183	chromosome 13 open reading frame 18	-1.2190	-1.4936
IRF5	3663	interferon regulatory factor 5	-1.6279	-1.8597
TNRC6A	27327	trinucleotide repeat containing 6A	1.2181	1.2347
MTMR12	54545	myotubularin related protein 12	-1.1194	-1.0760
NHSL2	340527	NHS-like 2	1.0128	-1.1691
LILRA2	11027	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	-1.0449	-1.5674
LOC143666	143666	PREDICTED: hypothetical protein LOC143666	-1.0688	-1.0992

CCL4L2	388372	chemokine (C-C motif) ligand 4-like 2	1.3521	1.4545
CCR6	1235	chemokine (C-C motif) receptor 6	1.2436	1.1731
		RC2-CT0522-150900-012-g04 CT0522 cDNA, mRNA sequence	1.1733	1.1210
PABPC5	140886	poly(A) binding protein, cytoplasmic 5	1.0070	-1.1041
MAPK3	5595	mitogen-activated protein kinase 3	-1.1454	-1.5170
CXCL16	58191	chemokine (C-X-C motif) ligand 16	-1.1295	-1.5139
H2AFJ	55766	H2A histone family, member J	-1.1550	-1.6046
LOC392713	392713	PREDICTED: similar to PMS1 protein homolog 2 (DNA mismatch repair protein PMS2)	1.1353	1.1032
NAT1	9	PREDICTED: N-acetyltransferase 1 (arylamine N-acetyltransferase)	-1.0822	-1.0121
HIST1H2BK	85236	histone cluster 1, H2bk	1.2164	-1.1427
HIST1H2BK	85236	histone cluster 1, H2bk	1.2069	-1.1402
LOC100132041	100132041	PREDICTED: hypothetical protein LOC100132041	-1.1834	-1.2556
AES	166	amino-terminal enhancer of split	1.3381	1.4107
TNRC18	27320	PREDICTED: trinucleotide repeat containing 18	-1.0209	-1.1223
CXCL1	2919	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	-1.0905	-1.1967
PITPNA	5306	phosphatidylinositol transfer protein, alpha	-1.0129	-1.1276
PPDPF	79144	pancreatic progenitor cell differentiation and proliferation factor homolog	1.2571	1.1892
ZNF438	220929	zinc finger protein 438	-1.0262	-1.2454
BRI3	25798	brain protein I3	-1.0620	-1.4453
ORM1	5004	orosomucoid 1	-2.0090	-2.1537
ITGAM	3684	integrin, alpha M (complement component 3 receptor 3 subunit)	-1.0108	-1.3800
LOC730744	730744	PREDICTED: similar to macrophage erythroblast attacher (LOC730744)	-1.2089	-1.6264
FASTK	10922	Fas-activated serine/threonine kinase	1.1505	1.1413
		7p17e02.x1 NCI_CGAP_Br22 cDNA clone IMAGE:3646155 3, mRNA sequence	1.1287	1.1313
TTYH2	94015	tweety homolog 2	-1.1273	-1.0982
CLDN9	9080	claudin 9	-1.0611	-1.2105
LCK	3932	lymphocyte-specific protein tyrosine kinase	1.1324	1.1038
C10orf54	64115	chromosome 10 open reading frame 54	1.0839	-1.2717
INPP5D	3635	inositol polyphosphate-5-phosphatase, 145kDa	1.1172	-1.2980
BCL6	604	B-cell CLL/lymphoma 6 (zinc finger protein 51)	-1.1797	-1.7830
POLR2J4	84820	polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa pseudogene	1.1241	1.0389
NKG7	4818	natural killer cell group 7 sequence	1.4741	1.6933
CEBPB	1051	CCAAT/enhancer binding protein (C/EBP), beta	-1.0177	-1.4942
PRO0628	29053	hypothetical LOC29053 (PRO0628), non-coding RNA.	1.2563	1.1234
TP53I11	9537	tumor protein p53 inducible protein 11	-1.0733	-1.1991
NAT6	24142	N-acetyltransferase 6 (GCN5-related)	1.12091	1.1011
ABI2	10152	PREDICTED: abl interactor 2	1.20173	1.0875
C11orf31	280636	chromosome 11 open reading frame 31	1.08032	1.0729
MANSC1	54682	MANSC domain containing 1	-1.2029	-1.3316

SERTAD3	29946	SERTA domain containing 3	1.0889	-1.1323
NLGN2	57555	neuroligin 2	-1.0571	-1.0506
TOB2	10766	transducer of ERBB2, 2	-1.0826	-1.0198
PARM1	25849	prostate androgen-regulated mucin-like protein 1	-1.0781	-1.0952
		DA086964 BRACE2 cDNA clone BRACE2041741 5	1.1159	1.0773
CAV2	858	caveolin 2	-1.0895	-1.1731
LOC649553	649553	PREDICTED: misc_RNA (LOC649553)	1.1820	1.1586
MED23	9439	mediator complex subunit 23	-1.0111	1.0709
ZFP36	7538	zinc finger protein 36, C3H type, homolog	-1.1466	-1.6339
SMPD1	6609	sphingomyelin phosphodiesterase 1, acid lysosomal	1.1144	1.1239
IL8RBP	3580	PREDICTED: interleukin 8 receptor, beta pseudogene	1.1982	1.1101
DOPEY1	23033	dopey family member 1	-1.0181	1.0316
SV2A	9900	synaptic vesicle glycoprotein 2A	-1.098	-1.1403
LPPR2	64748	lipid phosphate phosphatase-related protein type 2	1.0773	-1.228
SFTPD	6441	surfactant protein D	1.0308	-1.0573
		primary neuroblastoma cDNA, clone:Nbla10527, full insert sequence	1.2554	1.2928
		17000600107276 GRN_PREHEP cDNA 5, mRNA sequence	1.0663	1.1136
SNORD4A	26773	small nucleolar RNA, C/D box 4A	1.0883	1.1331
ABR	29	active BCR-related gene	1.0658	-1.0033
ABR	29	active BCR-related gene	-1.0883	-1.4179
CASP8	841	caspase 8, apoptosis-related cysteine peptidase	1.1923	1.2062
RN5S9	100169760	RNA, 5S ribosomal 9	1.7733	1.7049
KLRD1	3824	killer cell lectin-like receptor subfamily D, member 1	1.4373	1.6374
CEACAM3	1084	carcinoembryonic antigen-related cell adhesion molecule 3	-1.2251	-1.7678
NRGN	4900	neurogranin (protein kinase C substrate, RC3)	1.7010	1.5508
RNF122	79845	ring finger protein 122	1.1664	1.1016
LOC730385	730385	PREDICTED: hypothetical LOC730385	-1.3457	-1.5239
HPCAL1	3241	hippocalcin-like 1	-1.1680	-1.3469
CISD2	493856	CDGSH iron sulfur domain 2	-1.0028	1.0792
LRRC25	126364	leucine rich repeat containing 25	-1.4270	-1.7341
CTTN	2017	cortactin	1.3205	1.2979
DEF8	54849	differentially expressed in FDCP 8 homolog	-1.015	-1.4100
ZNF654	55279	zinc finger protein 654	1.1626	1.1787
SULT1A2	6799	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2	-1.2477	-1.4106
IL1B	3553	interleukin 1, beta	-1.1247	-1.5028
FBXL20	84961	F-box and leucine-rich repeat protein 20	-1.0359	-1.1769
CTDSPL	10217	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	1.2474	1.2199
SERPINB9	5272	serpin peptidase inhibitor, clade B (ovalbumin), member 9	1.1653	1.1800
RNF214	257160	ring finger protein 214	-1.1079	-1.1039
GUCY1A3	2982	guanylate cyclase 1, soluble, alpha 3	1.1636	1.08059
CHMP2A	27243	chromatin modifying protein 2A	-1.0334	-1.3445

ARHGAP27	201176	Rho GTPase activating protein 27	1.0565	-1.2226
ACTN1	87	actinin, alpha 1	-1.2582	-1.5831
		MST131 mRNA, complete cds	-1.1174	-1.1404
MKNK1	8569	MAP kinase interacting serine/threonine kinase 1	-1.0595	-1.4290
FAM65A	79567	family with sequence similarity 65, member A	-1.3400	-1.5788
HSH2D	84941	hematopoietic SH2 domain containing	1.5175	-1.0029
SLC11A1	6556	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	-1.2678	-1.9033
FGFBP2	83888	fibroblast growth factor binding protein 2	1.7657	2.142
LOC100134571	100134571	PREDICTED: misc_RNA (LOC100134571)	1.1365	1.0794
TTC39B	158219	tetratricopeptide repeat domain 39B	-1.0528	1.0425
NTN3	4917	netrin 3	-1.2780	-1.3004
URB2	9816	URB2 ribosome biogenesis 2 homolog	1.1003	1.0760
PER3	8863	period homolog 3	-1.0216	-1.0999
PRR7	80758	proline rich 7 (synaptic)	1.1220	1.1219
NCRNA00085	147650	non-protein coding RNA 85	-1.1963	-1.2987
ZNF716	441234	PREDICTED: zinc finger protein 716	-1.0521	-1.1904
LOC653557	653557	PREDICTED: hypothetical LOC653557	1.0968	1.0851
FGR	2268	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	-1.0873	-1.4992
BATF3	55509	basic leucine zipper transcription factor, ATF-like 3	1.0315	-1.0679
DGAT2	84649	diacylglycerol O-acyltransferase homolog 2	-1.1443	-1.1821
FLOT2	2319	flotillin 2	-1.1362	-1.5409
KIAA1429	25962	KIAA1429	1.0251	1.0835
TLR5	7100	toll-like receptor 5	-1.3197	-1.6994
NFATC3	4775	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	1.0586	1.1369
CNN2	1265	calponin 2	-1.1110	-1.5361
FLJ45445	399844	FLJ45445 protein	-1.2598	-1.4054
DNAJB12	54788	DnaJ (Hsp40) homolog, subfamily B, member 12	-1.1938	-1.2691
C20orf194	25943	chromosome 20 open reading frame 194	-1.0819	-1.0291
ZSCAN21	7589	zinc finger and SCAN domain containing 21	-1.0866	-1.1288
TBC1D3I	643947	PREDICTED: TBC1 domain family, member 3I	-1.1801	-1.2856
ANKRD40	91369	ankyrin repeat domain 40	1.1152	1.1005
SP1	6667	Sp1 transcription factor	-1.0991	-1.3093
CR1	1378	complement component (3b/4b) receptor 1 (Knops blood group)	-1.1066	-1.1810
SH3BP2	6452	SH3-domain binding protein 2	1.0739	-1.0132
UCP2	7351	uncoupling protein 2 (mitochondrial, proton carrier), nuclear gene encoding mitochondrial protein	1.6137	1.7158
ITPRIP	85450	inositol 1,4,5-triphosphate receptor interacting protein	-1.0255	-1.3825
GJC1	10052	gap junction protein, gamma 1, 45kDa	1.3801	1.1534
P2RY13	53829	purinergic receptor P2Y, G-protein coupled, 13	-1.3405	-1.8268
DNAJC5	80331	DnaJ (Hsp40) homolog, subfamily C, member 5	1.0462	-1.1241
EXTL3	2137	exostoses (multiple)-like 3	-1.0988	-1.4403
HSPA7	3311	heat shock 70kDa protein 7	-1.1843	-1.5631
LRRC37A4	55073	leucine rich repeat containing 37, member A4	-1.0376	-1.0721

		(pseudogene), non-coding RNA.		
LOC729003	729003	PREDICTED: hypothetical protein LOC729003	1.0804	1.1006
C19orf59	199675	chromosome 19 open reading frame 59	-1.2776	-1.9209
IMPDH1	3614	IMP (inosine monophosphate) dehydrogenase 1	-1.2182	-1.6298
LOC730107	730107	PREDICTED: similar to Glycine cleavage system H protein, mitochondrial	1.0597	1.0662
DKFZp761E19 8	91056	DKFZp761E198 protein	1.1757	-1.0325
RGL4	266747	ral guanine nucleotide dissociation stimulator- like 4	-1.0927	-1.4154
		wf10a02.x1 Soares_NFL_T_GBC_S1 cDNA clone IMAGE:2350154 3	-1.0582	-1.1304
		clone 25194 mRNA sequence	1.2015	1.1616
ACSL1	2180	acyl-CoA synthetase long-chain family member 1	-1.1584	-1.8014
C19orf39	126074	chromosome 19 open reading frame 39	1.2224	1.1109
C2orf18	54978	chromosome 2 open reading frame 18	1.1404	1.0957
VEZT	55591	vezatin, adherens junctions transmembrane protein	-1.0050	1.1093
LOC100130914	100130914	PREDICTED: misc_RNA (LOC100130914)	-1.5265	-1.9200
RPL37	6167	ribosomal protein L37	1.0030	1.0565
SLC2A3	6515	solute carrier family 2 (facilitated glucose transporter), member 3	-1.2159	-1.6590
RAB43	339122	RAB43, member RAS oncogene family	1.1313	1.0034
NCF2	4688	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2	-1.0177	-1.4183
LOC648490	648490	PREDICTED: hypothetical protein LOC648490	-1.0826	-1.0643
TSEN34	79042	tRNA splicing endonuclease 34 homolog	-1.0345	-1.3810
TSEN34	79042	tRNA splicing endonuclease 34 homolog	-1.0776	-1.4641
SEMA4A	64218	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A.	-1.0868	-1.4223
VCL	7414	Vinculin	1.3806	1.3115
LOC441253	441253	PREDICTED: nuclear envelope pore membrane LOC441253	-1.0592	1.0025
LOC641972	641972	PREDICTED: similar to single-stranded DNA- binding protein isoform a (LOC641972)	1.1142	1.1948
BCR	613	breakpoint cluster region	1.0716	1.0594
HUS1	3364	HUS1 checkpoint homolog	1.0374	-1.0136
MEX3C	51320	mex-3 homolog C	1.0191	1.1407
LPCAT3	10162	lysophosphatidylcholine acyltransferase 3	-1.1126	-1.2598
C20orf107	388799	chromosome 20 open reading frame 107	-1.0637	-1.0836
FARSLB	10056	phenylalanine-tRNA synthetase-like, beta subunit	1.2331	1.0228
LFNG	3955	LFNG O-fucosylpeptide 3-beta-N- acetylglucosaminyltransferase	1.0452	1.1016
GGCT	79017	gamma-glutamyl cyclotransferase	1.0784	1.0432
FCRL2	79368	Fc receptor-like 2	-1.0696	-1.0644
CAPN12	147968	calpain 12	1.1086	1.1146
HNRNPA3	220988	heterogeneous nuclear ribonucleoprotein A3	1.1224	1.0786
LOC440926	440926	H3 histone, family 3A pseudogene	1.1347	-1.0492

FERMT3	83706	fermitin family homolog 3	1.2520	1.0447
LOC645693	645693	PREDICTED: misc_RNA (LOC645693	1.0104	1.0619
REPS2	9185	RALBP1 associated Eps domain containing 2	1.0996	-1.1176
HMGA1	3159	high mobility group AT-hook 1	1.2479	1.2147
UHRF1BP1	54887	UHRF1 binding protein 1	-1.0818	-1.0444
REL	5966	v-rel reticuloendotheliosis viral oncogene homolog	1.0932	-1.0116
HIPK2	28996	homeodomain interacting protein kinase 2	1.3517	1.2348
LOC728070	728070	PREDICTED: similar to chromosome 16 open reading frame 54	1.0991	1.0404
NCR3	259197	natural cytotoxicity triggering receptor 3	1.1105	1.2081
HSPA6	3310	heat shock 70kDa protein 6	-1.0162	-1.5857
LOC100008589	100008589	28S ribosomal RNA, non-coding RNA.	1.8002	2.3497
CSDE1	7812	cold shock domain containing E1, RNA-binding	-1.1011	-1.0949
RPA4	29935	replication protein A4, 34kDa	-1.0995	-1.1181
LRRC6	23639	leucine rich repeat containing 6	-1.2218	-1.3061
MAP3K3	4215	mitogen-activated protein kinase kinase kinase 3	1.0429	-1.2061
RXRG	6258	retinoid X receptor, gamma	-1.1059	-1.0978
PCBP2	5094	poly(rC) binding protein 2	1.2278	1.1991
TRAF7	84231	TNF receptor-associated factor 7	1.1574	1.0990
SSBP3	23648	single stranded DNA binding protein 3	1.1557	1.3233
ANPEP	290	alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	-1.0804	-1.3970
PTBP2	58155	polypyrimidine tract binding protein 2	-1.0513	1.0602
		xf55f09.x1 NCI_CGAP_Gas4 cDNA clone IMAGE:2621993 3	-1.0003	1.0218
KLRC3	3823	killer cell lectin-like receptor subfamily C, member 3	1.0647	1.2281
MSH5	4439	mutS homolog 5	-1.0777	-1.0849
LIMK2	3985	LIM domain kinase 2	1.0786	-1.0907
HEMK1	51409	HemK methyltransferase family member 1	-1.0328	-1.1130
PPBP	5473	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7	1.6811	1.6050
ZBTB25	7597	zinc finger and BTB domain containing 25	-1.0209	1.0644
OPLAH	26873	5-oxoprolinase (ATP-hydrolysing)	-1.1151	-1.3194
		AGENCOURT_8120841 Lupski_dorsal_root_ganglion cDNA clone IMAGE:6178568 5	1.0642	1.0587
LOC729486	729486	PREDICTED: similar to Interleukin-9 receptor precursor (IL-9R) (CD129 antigen)	-1.1585	-1.2176
ZNF124	7678	zinc finger protein 124	-1.0494	1.0112
C8orf38	137682	chromosome 8 open reading frame 38	-1.0630	1.0080
ERBB2	2064	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog	1.1280	1.1432
		603089645F1 NIH_MGC_120 cDNA clone IMAGE:5228627 5	-1.1095	-1.2191
MEIS1	4211	Meis homeobox 1	1.0756	1.0697
CALR	811	Calreticulin	1.3051	1.2779
TBCE	6905	tubulin folding cofactor E	1.0707	1.0736
NACC2	138151	NACC family member 2, BEN and BTB	-1.0871	-1.2783

		(POZ) domain containing		
FAM53C	51307	family with sequence similarity 53, member C	-1.3198	-1.8640
TCIRG1	10312	T-cell, immune regulator 1, ATPase, H ⁺ transporting, lysosomal V0 subunit A3	1.2785	1.1858
TGM3	7053	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)	-1.3617	-1.7067
HIST2H2AA4	723790	histone cluster 2, H2aa4	-1.1126	-1.7825
		cDNA FLJ33932 fis, clone CTONG2017798	-1.0878	-1.0220
MGC40489	146880	PREDICTED: hypothetical protein MGC40489	-1.0568	-1.0812
SLC25A4	291	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4, nuclear gene encoding mitochondrial protein	-1.0264	1.0595
GNG11	2791	guanine nucleotide binding protein (G protein), gamma 11	1.4629	1.4770
AGPAT6	137964	1-acylglycerol-3-phosphate O-acyltransferase 6 (lysophosphatidic acid acyltransferase, zeta)	1.0565	1.08137
FAM173B	134145	family with sequence similarity 173, member B	-1.0502	1.0220
PLD2	5338	phospholipase D2	-1.1152	-1.1547
PIK3R1	5295	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	1.4323	1.4014
LOC100128737	100128737	PREDICTED: hypothetical protein LOC100128737	-1.0853	-1.1282
IQSEC1	9922	IQ motif and Sec7 domain 1	-1.1419	-1.38597
ROPN1L	83853	ropporin 1-like	-1.1338	-1.4786
PGS1	9489	phosphatidylglycerophosphate synthase 1	-1.1317	-1.4974
PRSS23	11098	protease, serine, 23	1.1376	1.2150
NARF	26502	nuclear prelamin A recognition factor	-1.1560	-1.4566
TPD52L2	7165	tumor protein D52-like 2	-1.0550	-1.2415
ALPK1	80216	alpha-kinase 1	-1.0938	-1.5022
IL1RN	3557	interleukin 1 receptor antagonist	1.2259	-1.3739
LOC100131850	100131850	PREDICTED: hypothetical protein LOC100131850	1.0848	-1.0770
SLC39A4	55630	solute carrier family 39 (zinc transporter), member 4	1.0917	1.0576
TMCO6	55374	transmembrane and coiled-coil domains 6	-1.0714	-1.1320
KIAA0319L	79932	KIAA0319-like	1.1427	-1.0965
TIAM2	26230	T-cell lymphoma invasion and metastasis 2 (TIAM2), transcript variant 2, mRNA.	-1.0876	-1.2212
TAF1B	9014	TATA box binding protein (TBP)-associated factor, RNA polymerase I, B, 63kDa	1.0709	1.0895
		mRNA for KIAA0574 protein, partial cds	-1.0668	-1.0827
PFKFB3	5209	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	-1.1200	-1.2276
PFKFB3	5209	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	-1.1496	-1.7324
GGA1	26088	golgi associated, gamma adaptin ear containing, ARF binding protein 1	1.2035	1.1637
C9orf30	91283	chromosome 9 open reading frame 30	1.0917	1.1567
HIST1H3D	8351	histone cluster 1, H3d	-1.0074	-1.1423
C19orf6	91304	chromosome 19 open reading frame 6	1.1483	1.1377
CYP2S1	29785	cytochrome P450, family 2, subfamily S, polypeptide 1	-1.1046	-1.1158

IFITM2	10581	interferon induced transmembrane protein 2 (1-8D)	1.0755	-1.1803
LASP1	3927	LIM and SH3 protein 1	-1.0438	-1.3192
ADM	133	adrenomedullin	-1.1471	-2.0211
GDPD3	79153	glycerophosphodiester phosphodiesterase domain containing 3	-1.0379	-1.2708
HIST2H2AA3	8337	histone cluster 2, H2aa3	-1.2374	-1.8447
HIST2H2AA3	8337	histone cluster 2, H2aa3	-1.0922	-1.8292
PADI2	11240	peptidyl arginine deiminase, type II	1.0109	-1.1029
ARMC2	84071	armadillo repeat containing 2	-1.0398	-1.0642
DNM1L	10059	dynammin 1-like	1.1624	1.2089
LOC642726	642726	PREDICTED: hypothetical protein LOC642725, transcript variant 1	1.0528	1.0642
		cDNA clone IMAGE:30332316	-1.2312	-1.1992
BANP	54971	BTG3 associated nuclear protein	-1.1756	-1.3725
Mar-03	115123	PREDICTED: membrane-associated ring finger (C3HC4) 3	-1.0766	-1.0789
TAF1D	79101	TATA box binding protein (TBP)-associated factor, RNA polymerase I, D, 41kDa	1.0198	1.0426
WDR51A	25886	WD repeat domain 51A	-1.0491	-1.1296
RGPD1	400966	RANBP2-like and GRIP domain containing 1	1.2697	1.1484
CDA	978	cytidine deaminase	-1.1115	-1.5716
N4BP2	55728	Nedd4 binding protein 2	1.1032	1.1624
LOC100131096	100131096	PREDICTED: hypothetical protein LOC100131096	-1.0287	-1.2880
PGD	5226	phosphogluconate dehydrogenase	-1.0907	-1.5008
AHNAK	79026	AHNAK nucleoprotein	1.1007	1.1369
PIK3R4	30849	phosphoinositide-3-kinase, regulatory subunit 4, p150	1.02184	1.0796
B3GNT8	374907	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	1.0472	-1.0891
XPO6	23214	exportin 6	-1.0241	-1.4786
CMTM4	146223	CKLF-like MARVEL transmembrane domain containing 4	-1.1286	-1.1308
STX4	6810	syntaxin 4	-1.1663	-1.2582
CTSB	1508	cathepsin B	1.4323	1.3194
SFRS12	140890	splicing factor, arginine/serine-rich 12	-1.0896	1.0128
KIAA0319L	79932	KIAA0319-like	1.0772	-1.2064
HCK	3055	hemopoietic cell kinase	-1.0895	-1.4273
RFX1	5989	regulatory factor X, 1 (influences HLA class II expression)	1.0947	-1.1480
NAT14	57106	N-acetyltransferase 14 (GCN5-related, putative)	-1.0684	-1.0966
EMR2	30817	egf-like module containing, mucin-like, hormone receptor-like 2	-1.4059	-1.7460
NINJ1	4814	ninjurin 1	1.0815	-1.3409
IL17RA	23765	interleukin 17 receptor A	1.1764	-1.1447
GZMB	3002	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	1.7769	2.1101
SNX27	81609	sorting nexin family member 27	-1.0762	-1.4195
SLC25A10	1468	solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10, nuclear gene encoding mitochondrial protein	-1.0665	-1.1018
CLUAP1	23059	clusterin associated protein 1	1.0829	1.0608

TACSTD2	4070	tumor-associated calcium signal transducer 2	1.2339	1.1566
CA4	762	carbonic anhydrase IV	-1.3823	-1.7724
PSME1	5720	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)	1.3058	1.1063
LOC652775	652775	PREDICTED: similar to Ig kappa chain V-V region L7 precursor	-1.0673	-1.1671
		BX105310 Soares_testis_NHT cDNA clone IMAGp998J212640	1.1185	1.08231
LILRB3	11025	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	-1.0017	-1.4152
ALDOB	229	aldolase B, fructose-bisphosphate	1.1401	1.0368
FCGR2A	2212	Fc fragment of IgG, low affinity IIa, receptor (CD32)	-1.0067	-1.4417
LOC100134407	100134407	PREDICTED: hypothetical protein LOC100134407	1.1096	1.0239
LOC401002	401002	PREDICTED: similar to single-stranded DNA-binding protein isoform a LOC401002	1.0396	1.1592
ZNF195	7748	zinc finger protein 195	-1.0287	1.0867
		cDNA: FLJ21199 fis, clone COL00235	-1.1155	-1.3051
PGLYRP1	8993	peptidoglycan recognition protein 1	-1.1369	-1.5666
DHRS13	147015	dehydrogenase/reductase (SDR family) member 13	-1.2246	-1.5772
CXorf12	8269	chromosome X open reading frame 12	-1.0623	-1.0625
WDR86	349136	WD repeat domain 86	-1.1565	-1.2043
MBD5	55777	methyl-CpG binding domain protein 5	-1.0843	-1.0673
CHMP6	79643	chromatin modifying protein 6	1.1561	1.1320
LRG1	116844	leucine-rich alpha-2-glycoprotein 1	-1.0815	-1.7307
C5orf62	85027	chromosome 5 open reading frame 62	-1.0381	-1.0860
REPS2	9185	RALBP1 associated Eps domain containing 2	-1.0063	-1.2805
RN7SL1	6029	RNA, 7SL, cytoplasmic 1	1.2219	1.1149
OSBPL2	9885	oxysterol binding protein-like 2	1.0359	-1.2925
HIST2H2AC	8338	histone cluster 2, H2ac	-1.1430	-1.7534
GNB1L	54584	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	-1.1385	-1.1908
BCL2L14	79370	BCL2-like 14 (apoptosis facilitator)	-1.0330	-1.0380
PDPK1	5170	3-phosphoinositide dependent protein kinase-1	1.22468	1.1779
LOC100130764	100130764	PREDICTED: p150-like LOC100130764	1.2259	1.0789
		AGENCOURT_6505119 NIH_MGC_125 cDNA clone IMAGE:5588241 5	1.0550	1.1116
LRPAP1	4043	low density lipoprotein receptor-related protein associated protein 1	-1.0980	-1.1903
C9orf80	58493	chromosome 9 open reading frame 80	1.1140	1.1760
NUSAP1	51203	nucleolar and spindle associated protein 1	1.0997	1.0433
GPR97	222487	G protein-coupled receptor 97	-1.1490	-1.5313
GPR97	222487	G protein-coupled receptor 97	-1.0187	-1.4418
GPR97	222487	G protein-coupled receptor 97	1.0093	-1.4187
PADI4	23569	peptidyl arginine deiminase, type IV	-1.1057	-1.6063
KIT	3815	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	-1.07186	-1.0167
LOC646990	646990	PREDICTED: hypothetical protein LOC646990	1.0969	1.0899
ADCK4	79934	aarF domain containing kinase 4	1.2297	1.1533
CLIC3	9022	chloride intracellular channel 3	1.3860	1.4457

PRKCD	5580	protein kinase C, delta	-1.0604	-1.5671
KIAA0652	9776	KIAA0652	1.1001	1.0073
DENND5A	23258	DENN/MADD domain containing 5A	-1.1057	-1.4687
TECPR2	9895	tectonin beta-propeller repeat containing 2	-1.1084	-1.1842
KCTD9	54793	potassium channel tetramerisation domain containing 9	-1.0235	1.0298
TMEM106C	79022	transmembrane protein 106C	1.0411	1.1346
TIMM23	10431	translocase of inner mitochondrial membrane 23 homolog, nuclear gene encoding mitochondrial protein	1.0112	1.0735
MED27	9442	mediator complex subunit 27	1.1846	1.1902
GPR77	27202	G protein-coupled receptor 77	-1.0806	-1.16518
GP1BA	2811	glycoprotein Ib (platelet), alpha polypeptide	1.2346	1.1129
THSD1P	374500	thrombospondin, type I, domain containing 1 pseudogene XR_001335	-1.1039	-1.06414
S1PR5	53637	sphingosine-1-phosphate receptor 5	1.4022	1.62804
BMX	660	BMX non-receptor tyrosine kinase	-1.0713	-1.1584
VEGFB	7423	vascular endothelial growth factor B	1.0194	1.0834
GLI4	2738	GLI family zinc finger 4	1.1040	1.0834
		mRNA; cDNA DKFZp547E107 (from clone DKFZp547E107)	-1.2029	-1.1608
LOC730387	730387	PREDICTED: misc_RNA (LOC730387)	-1.2994	-1.5417
DHX34	9704	DEAH (Asp-Glu-Ala-His) box polypeptide 34	-1.2370	-1.4563
LOC374443	374443	CLR pseudogene (LOC374443), non-coding RNA. XR_001315	-1.0081	1.0728
DEPDC5	9681	DEP domain containing 5	-1.0758	-1.0813
LPAR2	9170	lysophosphatidic acid receptor 2	-1.0380	-1.4937
LOC644745	644745	PREDICTED: misc_RNA (LOC644745)	1.2961	1.1152
TIMP2	7077	TIMP metalloproteinase inhibitor 2	1.1434	-1.1657
SFT2D3	84826	SFT2 domain containing 3	-1.1381	-1.0458
ETF1	2107	eukaryotic translation termination factor 1	1.0809	1.0861
SLC9A8	23315	solute carrier family 9 (sodium/hydrogen exchanger), member 8	-1.0469	-1.2693
ABHD12B	145447	abhydrolase domain containing 12B	-1.0776	-1.0854
NLRP12	91662	NLR family, pyrin domain containing 12	-1.1681	-1.3469
NLRP12	91662	NLR family, pyrin domain containing 12	-1.1539	-1.5289
TRK1	7206	tRNA lysine 1 on chromosome 17.	1.3089	1.3445
MRPL50	54534	mitochondrial ribosomal protein L50, nuclear gene encoding mitochondrial protein	1.0442	1.1619
CTDSP1	58190	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	1.2295	1.2846
LZTS2	84445	leucine zipper, putative tumor suppressor 2	1.0072	-1.0500
MAP4K4	9448	mitogen-activated protein kinase kinase kinase 4 (MAP4K4), transcript variant 3, mRNA.	-1.1392	-1.2441
		7o98h02.x1 NCI_CGAP_Ov18 cDNA clone IMAGE:3644403 3	1.2109	1.2178
XIAP	331	X-linked inhibitor of apoptosis	1.1055	1.0748
PIAS1	8554	protein inhibitor of activated STAT, 1	1.3414	1.0188
FLJ20628	55006	hypothetical protein FLJ20628	-1.0371	1.0212
GPR56	9289	G protein-coupled receptor 56	1.7228	1.9667
LAMP2	3920	lysosomal-associated membrane protein 2	1.0459	-1.3954
RAD17	5884	RAD17 homolog	-1.0641	1.0066

SELL	6402	selectin L	-1.0671	-1.4637
ZNF761	388561	zinc finger protein 761	-1.0372	1.0499
ZNF467	168544	zinc finger protein 467	-1.3046	-2.0131
DTWD1	56986	DTW domain containing 1	-1.0208	1.0984
PRRG4	79056	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	1.1621	1.0487
UBTD1	80019	ubiquitin domain containing 1	-1.0127	-1.2735
EMR3	84658	egf-like module containing, mucin-like, hormone receptor-like 3	-1.2127	-1.6297
DEFB123	245936	defensin, beta 123	-1.0597	-1.1075
CILP	8483	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	-1.0252	-1.0639
NFKBID	84807	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	-1.1151	-1.1626
GCLC	2729	glutamate-cysteine ligase, catalytic subunit	-1.0056	1.0873
CTBP1	1487	C-terminal binding protein 1	1.2319	1.2678
CD3E	916	CD3e molecule, epsilon (CD3-TCR complex)	1.5442	1.7140
SLC26A8	116369	solute carrier family 26, member 8	-1.0257	-1.3495
GPR137B	7107	G protein-coupled receptor 137B	1.1033	1.1213
FBS1	64319	fibrosin 1	1.1359	1.0101
KIAA0319L	79932	KIAA0319-like	1.4221	1.0326
PLEKHG1	57480	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	-1.0896	-1.0810
KLRD1	3824	killer cell lectin-like receptor subfamily D, member 1	1.3957	1.6184
CASK	8573	calcium/calmodulin-dependent serine protein kinase (MAGUK family)	-1.0877	1.0170
PRR13	54458	proline rich 13	1.0519	-1.2401
CABP5	56344	calcium binding protein 5	1.1114	1.1740
USP14	9097	ubiquitin specific peptidase 14 (tRNA-guanine transglycosylase)	1.1645	1.2070
DYSF	8291	dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	-1.0853	-1.6760
RENBP	5973	renin binding protein	-1.2546	-1.4591
PHF21A	51317	PHD finger protein 21A	-1.0841	-1.4272
SLC16A3	9123	solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	1.0760	-1.4550
TUBD1	51174	tubulin, delta 1	-1.0289	1.0015
EIF5A	1984	eukaryotic translation initiation factor 5A	1.3599	1.3593
LILRA3	11026	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3	-1.2170	-1.9661
C4orf27	54969	chromosome 4 open reading frame 27	1.0634	1.1322
IFNGR2	3460	interferon gamma receptor 2 (interferon gamma transducer 1)	-1.0479	-1.4883
MSH3	4437	mutS homolog 3	1.1842	1.1781
LOC654126	654126	PREDICTED: similar to leucine rich repeat containing 37B, transcript variant 2	1.0919	-1.0039
ZYX	7791	zyxin	1.0101	-1.3423
SNTB2	6645	syntrophin, beta 2 (dystrophin-associated protein A1, 59kDa, basic component 2)	1.1053	1.10139
RRP7B	91695	ribosomal RNA processing 7 homolog	1.2092	1.1498
LTC4S	4056	leukotriene C4 synthase	-1.0823	-1.0806
PREX1	57580	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	-1.1292	-1.4668

SPTLC1	10558	serine palmitoyltransferase, long chain base subunit 1	-1.0976	-1.0181
REM2	161253	RAS (RAD and GEM)-like GTP binding 2	-1.2119	-1.5243
LOC653450	653450	PREDICTED: hypothetical LOC653450	1.0599	-1.0381
SIGLEC9	27180	sialic acid binding Ig-like lectin 9	-1.0035	-1.1088
MXD3	83463	MAX dimerization protein 3	-1.0860	-1.2828
LILRA5	353514	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	1.1991	-1.3275
LILRA5	353514	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	1.0708	-1.3774
		BX090817 NCL_CGAP_Brn52 cDNA clone IMAGp998G155675 ; IMAGE:2290982	1.0632	1.1076
ALOX12	239	arachidonate 12-lipoxygenase	1.3198	1.2766
SAMD3	154075	sterile alpha motif domain containing 3	1.0568	1.2214
SLC10A3	8273	solute carrier family 10 (sodium/bile acid cotransporter family), member 3	1.1437	1.1090
TUFT1	7286	tuftelin 1	1.0388	-1.0764
CD1A	909	CD1a molecule	-1.1025	-1.0933
POLD3	10714	polymerase (DNA-directed), delta 3, accessory subunit	1.0211	-1.0612
DAB2	1601	disabled homolog 2, mitogen-responsive phosphoprotein	1.2182	1.1771
MGAM	8972	maltase-glucoamylase (alpha-glucosidase)	-1.2950	-1.4116
FAM131A	131408	family with sequence similarity 131, member A	-1.1552	-1.2869
TNFSF14	8740	tumor necrosis factor (ligand) superfamily, member 14	-1.0054	-1.1322
UBE2R2	54926	ubiquitin-conjugating enzyme E2R 2	-1.0840	-1.1239
UBOX5	22888	U-box domain containing 5	-1.0942	-1.0918
CUX1	1523	cut-like homeobox 1	-1.0070	-1.0826
TNFRSF1B	7133	tumor necrosis factor receptor superfamily, member 1B	-1.1012	-1.4989
C10orf78	119392	chromosome 10 open reading frame 78	-1.0044	1.1207
FICD	11153	FIC domain containing	1.2506	1.2043
IFNAR1	3454	interferon (alpha, beta and omega) receptor 1	-1.1182	-1.4856
NUP54	53371	nucleoporin 54kDa	-1.0463	-1.0018
LOC730284	730284	PREDICTED: misc_RNA (LOC730284	-1.4171	-1.7781
CXCR1	3577	chemokine (C-X-C motif) receptor 1	1.0717	-1.3991
CPNE1	8904	copine I	-1.3030	-1.4330
OSM	5008	oncostatin M	-1.0574	-1.2549
C5orf32	84418	chromosome 5 open reading frame 32	-1.2623	-1.5932
F5	2153	coagulation factor V (proaccelerin, labile factor)	-1.2057	-1.3882
PAFAH2	5051	platelet-activating factor acetylhydrolase 2, 40kDa	1.1198	1.2096
EEPD1	80820	endonuclease/exonuclease/phosphatase family domain containing 1	-1.0846	-1.1321
PLAUR	5329	plasminogen activator, urokinase receptor	-1.0198	-1.3410
NOTCH1	4851	Notch homolog 1, translocation-associated	-1.3468	-1.8587
SOCS3	9021	suppressor of cytokine signaling 3	1.0268	-1.2041
MCM8	84515	minichromosome maintenance complex component 8	1.0330	1.0873
MCM8	84515	minichromosome maintenance complex component 8	1.3971	-1.0567

CR2	1380	complement component (3d/Epstein Barr virus) receptor 2	-1.1087	-1.0516
		601067201F1 NIH_MGC_10 cDNA clone IMAGE:3453216 5	1.1687	1.0659
TRIM25	7706	tripartite motif-containing 25	1.1370	-1.3601
LOC644132	644132	PREDICTED: misc_RNA (LOC644132)	-1.0558	-1.1464
GPR56	9289	G protein-coupled receptor 56	1.8807	2.1690
LAMA5	3911	laminin, alpha 5	-1.2382	-1.2268
LOC100049716	100049716	PREDICTED: hypothetical protein LOC100049716	-1.0472	-1.0944
LOC646674	646674	PREDICTED: misc_RNA (LOC646674), miscRNA.	1.1089	1.2225
MRPL4	51073	mitochondrial ribosomal protein L4 (MRPL4), nuclear gene encoding mitochondrial protein	1.0937	1.0520
LSP1	4046	lymphocyte-specific protein 1	1.5601	1.1980
COG5	10466	component of oligomeric golgi complex 5	1.03248	1.1057
LYZ	4069	Lysozyme	-1.7441	-1.9607
TREM1	54210	triggering receptor expressed on myeloid cells 1	1.1972	-1.1888
UBE2E3	10477	PREDICTED: ubiquitin-conjugating enzyme E2E 3	-1.0793	-1.0458
BTN2A2	10385	butyrophilin, subfamily 2, member A2	-1.0850	-1.0918

Analysis: Tukey HSD HAE R vs HS cutoff 0.05

positive score score = 0 negative score if no activity pattern available Ratio



Supplemental
Figure 2

Methods

Patient recruitment

We studied 8 patients with C1-INH-HAE (2 men and 6 women, aged 46.8 ± 22.2 years [mean $\pm$ standard deviation (SD), range 78-16]) during the remission phase (HAE-R) (free of attacks for at least 1 month) and during the acute attack (HAE-A) involving oral submucosal tissues in 1 patient, subcutaneous tissue in 4 patients and the abdomen in 3 (Table 1A). The diagnosis of abdominal acute attack in the patients with known *C1-INH* deficiency was based on the presence of acute abdominal pain with or without vomiting and diarrhea, the absence of any other cause of abdominal pain and prompt reversal upon the infusion of C1-INH. The control group consisted of 8 healthy subjects (HS, 2 men and 6 women) with a mean age of 50.8 ± 11.7 years (range 60-24). Blood samples were collected in PAX-gene Blood RNA tubes (Becton Dickinson, Italia) and sodium citrate as anti-coagulant. Blood samples were collected from these patients within 5 hours from the beginning of symptoms. All patients provided written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Milan, “Luigi Sacco” Hospital.

Laboratory Measurements

The following measurements were performed in non-activated plasma: C1-INH activity was measured using a chromogenic assay (Technochrom C1-inhibitor; Techno-clone GmbH, Vienna, Austria), and C1-INH antigen by means of radial immunodiffusion (RID) (NOR-Partigen, Behring, Marburg, Germany) (Table 1B).

RNA extraction

For RNA isolation, 2.5 ml blood was drawn in PAX-gene Blood RNA tubes, incubated for two hours at room temperature and stored at -20°C . Tubes were thawed overnight at room temperature prior to RNA isolation. Total RNA was isolated using PAX-gene Blood RNA Kit (PreAnalytiX,

Hombrechtikon, Switzerland) according to the manufacturer's instructions. Total RNA concentration was measured using the Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and RNA purity and integrity was verified using lab-on-chip technology (Agilent 2100 Bioanalyzer, Palo Alto, CA, USA).

Transcriptome data were generated using the HumanHT-12 v3 Expression BeadChip (Release 38, Illumina, San Diego, CA, USA). In this process, 500 ng total RNA was used to synthesize biotin-labeled cRNA using the Illumina®TotalPrep™ RNA amplification kit (Applied biosystems/Ambion, USA). Quality of labelled cRNA was measured using the NanoDrop® ND-100 spectrophotometer and the Agilent 2100 Bioanalyzer. 750 ng biotinylated cRNA was used for hybridization to gene-specific probes on the Illumina microarrays. The Illumina arrays were then scanned with the HiScanSQ.

Microarray statistical analyses were performed by Genespring GX 11.0 software (Agilent Tech Inc., Santa Clara, CA, USA). Identification of genes differentially expressed between HAE-A and HAE-R patients was carried out with false discovery rate (FDR) method of Benjamini-Hochberg (1) and gene probe sets were filtered on the basis of the FDR, (adjusted-P value with multiple testing on 1000 permutations) and fold-change (FC) (2). Fold change filter was set to 1.5-fold in each comparison. Only genes that were significantly (adjusted-P value <0.05 and FC >1.5) modulated were considered for further analysis.

Moreover, gene set enrichment analysis (GSEA) was performed in pairwise comparisons of HAE-A versus HAE-R patients. GSEA analyzes gene expression data and determines whether a particular set of genes is over- or under-represented in the compared samples (1). C2 curated gene sets from the Broad Institute (<http://www.broad.mit.edu/index.html>), based on prior biological knowledge, were used for the analysis. Significance of differential expression, as determined by the enrichment analysis, was recalculated 1000 times. A corrected P-value was obtained from the analysis using the FDR q-value correction. On the basis of this correction, the cutoff for significance was established at a P-value <0.05.

To assess biological relationships among differently regulated genes, we used the Ingenuity Pathway Analysis software (IPA, Ingenuity System, Redwood City, CA, USA; <http://www.ingenuity.com>). The reference gene selection was performed by own software written in Java program language. IPA generates networks based on the connectivity of the genes and computes a score for each network according to the fit of the set of supplied focus genes. These scores indicate the likelihood of focus genes to belong to a network versus those obtained by chance. The canonical pathways generated by IPA are the most significant for the uploaded data set. Fischer's exact test with FDR option was used to calculate the significance of the canonical pathway.

The Illumina microarray data are MIAME (Minimum Information About a Microarray Experiment) compliant and the raw data have been deposited in the database The European Bioinformatics Institute (*EMBL-EBI*) and are accessible through Experiment ArrayExpress accession: E-MTAB-5897.

cDNA synthesis and quantitative real-time PCR analysis

Quantitative real-time PCR (RT-PCR) was performed on total RNA samples from the patients in the validation group (n=20) using an ABI Prism 7900HT Sequence detection system (Applied Biosystems, Foster City, CA, USA). RNA (500 µg) was reverse transcribed into cDNA using a cDNA synthesis kit (QuantiTect Reverse Transcription Kit, Qiagen) according to the manufacturer's instructions. RT-PCR amplification reactions were performed using the QuantiTect Primer Assay and the Quantifast SYBR Green PCRmix (Qiagen). Gene expression levels of one housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ADM and uPAR were determined. Expression levels of target genes were expressed relative to GAPDH.

Cell culture

The Jurkat T-cell lines, purchased from ATCC, were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine, at 37°C and 5% CO₂ and 100 µg/ml penicillin and streptomycin (Sigma Aldrich, USA).

Primary Human Umbilical Vein Endothelial Cells (HUVEC) were isolated and cultured on bovine gelatin (Sigma Aldrich, USA) in human endothelial medium supplemented with 10% fetal bovine serum and 100 µg/ml penicillin and streptomycin (Invitrogen, USA) at 37°C and 5% CO₂.

Bradykinin ELISA

The measurement of Bradykinin in cell lysate and cell culture supernatant was assessed using a human Bradykinin enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp.,USA), according to the manufacturer's instructions. The cells, seeded in 6-well plates at a density of 2×10^6 cells/well, were pre-incubated with 10µg/ml and 20µg/ml anti-uPAR antibody (Immunological Sciences, Italy), to neutralize the uPA-uPAR interaction, for 1 hour and then treated with 100 nM urokinase-type Plasminogen Activator (uPA) (Urokinasi Hospira, Italy), for 15 minutes. Cell lysates and their supernatants were subsequently collected. Bradykinin concentration was measured at 450 nm and calculated from a standard curve.

Endothelial leakage

The *in vitro* endothelial leakage assay was performed as previously described (3). Briefly, HUVEC were seeded on gelatin coated 24 well plate transwell (Costar, USA) and grown till confluence. The medium for the experiment was supplemented with 10% normal human serum and anti-uPAR antibody was added to endothelial cells at 10µg/ml and at 20µg/ml, for 15 minutes and then the plasminogen was activated with 100 nM uPA for 30 minutes. Then, the FITC-BSA leaked into the lower chamber was evaluated.

Statistical analysis and bioinformatics

For the remaining experimental analysis, we analyzed data with statistical software (GraphPad Prism; GraphPad, San Diego, CA, USA). All data were expressed as the mean $\pm$ SD of data obtained from at least three independent experiments. Statistical analysis was carried out using two-tailed paired and unpaired Student's t test and one-way ANOVA and Tukey post-hoc tests. P-values lower than 0.05 were considered statistically significant.

References

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Figure legends

Supplemental Figure 1. Functional analysis of the top selected genes identified comparing HAE-R patients and healthy subjects. The top 15 enriched biological functions of differentially expressed genes in HAE patients during the remission phase compared to healthy as determined by IPA software.

Supplemental Figure 2. Ingenuity Pathway Analysis revealed the top canonical pathways of the top 1% deregulated genes. Predominant canonical pathways dysregulated in HAE patients during the remission phase compared to healthy subjects were mainly involved in “Granzyme B” and “VEGF signaling”.

Supplemental Figure 3. Blocking the uPA-uPAR interaction alters the bradykinin pathway.

Anti uPAR-treated Jurkat cells show a significantly reduction of bradykinin production in the control medium without uPA stimulation both in the cell lysate (A) and in supernatant (B). * $p < 0.05$, p-values obtained by paired t-test.