**Supplementary**

**Materials and Methods**

**Sample collection and storage**

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll (Ficoll-Paque PLUS; GE Healthcare) density-gradient centrifugation and stored in liquid nitrogen until further use. Serum and plasma samples were separated from coagulated and sodium heparinized peripheral blood according to standard procedures, aliquoted and stored at -80 OC. For RNA studies, whole blood was collected in PAXgene Blood RNA Tubes (PreAnalytiX) and stored at -80 OC. Additional samples were shipped on dry ice by outside hospitals for patients 3, 4, 5, 8, and 9 and stored as above until further use.

**Whole-exome sequencing**

Genomic DNA was prepared from patients’ and family members’ peripheral blood (Maxwell). We performed whole exome sequencing (WES) and data analysis as previously described [1]. Novel candidate variants were filtered by ExAC, 1000 genomes, dbSNP, NHLBI GO Exome Sequencing Project, ClinSeq database, and an in-house database of over 600 exomes, and selected based on autosomal recessive inheritance. All variants identified by WES were confirmed by Sanger sequencing.

**Sanger sequencing**

Genomic DNA was prepared from patients’ and family members’ peripheral blood (Maxwell). The 8 coding exons of *TRNT1* were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was performed on a 3130xl Genetic Analyzer (Applied Biosystems) and data were analyzed using Sequencher (Gene Codes). Patient 9 was found to have *TRNT1* mutations in another institute.

**Deep sequencing of tRNA**

Total RNA was isolated from fibroblasts derived from patients (n=2) and healthy donors (n=3) using TRIzol Reagent (Invitrogen), per manufacturer’s instructions. RNA quality was assessed with the Agilent 2200 TapeStation Controller. Due to their secondary-structure, tRNAs are not amenable to standard small RNA cDNA library preparation methods. We performed HydroRNAseq, that was developed to allow for small RNA cDNA library preparation from tRNA fragments [2-4]. Briefly, tRNA was isolated from total RNA based on molecular weight separated by electrophoresis on a 12% denaturing polyacrylamide gel. 200 ng of isolated tRNA were hydrolyzed in 10 mM Na2CO3 and 10 mM NaHCO3 in a total volume of 15 μl, for 5 min at 90 ºC, followed by dephosphorylation with 1μl of calf intestinal phosphatase (NEB) in NEB buffer 3 (NEB), in a 50 µl reaction, at 37 ºC for 1 hr. RNA was extracted by phenol/chloroform/isoamyl alcohol, precipitated in ethanol and re-phosphorylated with 1 μl of T4 polynucleotide kinase (NEB) in a 20 µl reaction in 1x PNK buffer containing 1 mM ATP (NEB), at 37 ºC for 1 hr. Rephosphorylated RNA was converted into barcoded small RNA cDNA libraries as described previously [3, 4] and cDNA of 29-50 base-pair length was then sequenced on an Illumina HiSeq 2500 device in the NIAMS Genome Analysis Core Facility.

**tRNA Sequencing analysis**

A method has been developed to specifically quantify the mature tRNA expression levels at the very 3’ end. The method involves the genomic mapping reads first to hg19 with bowtie0.12.8 [5], then performing a second mapping for those reads that have failed in mapping number one, after removing the CCA from the 3’ ends of the failed reads. Mature tRNA expression values were calculated by overlapping mapped reads with tRNA coordinates downloaded from UCSC’s Genome Browser, using BESTools [6]. The expression values were normalized to the total mapped reads per million. Reads density profiles over the 3’ end of all the expressed tRNAs were generated using ngs.plot [7].

**RNA-Seq**

Total RNA was isolated from whole blood collected in PAXgene Blood RNA Tubes using PAXgene Blood RNA Kit (IVD) (PreAnalytiX), per manufacturer’s instructions. RNA quality was assessed with the Agilent 2200 TapeStation Controller. 65 ng of total RNA per sample were used for cDNA library preparation [TruSeq Stranded mRNA Library Preparation for NeoPrep (Illumina)]. RNA-Seq data were generated with an Illumina HiSeq 2500 system at NIAMS Genome Analysis Core Facility. Raw sequencing data were processed with CASAVA 1.8.2 to generate FastQ files. Reads of 50 bases were mapped to the human transcriptome and genome hg19 using TopHat 2.1.0 [8]. BAM alignments that map to hemoglobin genes (HBA1, HBA2, HBB, HBD, HBG1, HBE1, HBM, HBQ1 or HBZ) and intergenic alignments were removed using bedtools (https://www.ncbi.nlm.nih.gov/pubmed/25199790). Gene expression values (RPKM: Reads Per Kilobase exon per Million mapped reads) were calculated with Partek Genomics Suite 6.6. Ingenuity Pathway Analysis (QIAGEN) was used to determine the differentially regulated generation of canonical pathways and upstream analysis.

**Electron microscopy of bone marrow and skin biopsy tissues**

Processing for EM was carried out according to Ogilvy et al. [9] Briefly, cells were doubly fixed in glutaraldehyde and osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections (~90nm thick) were mounted on 200-mesh uncoated cupper grids, doubly stained with uranyl acetate and lead citrate, and examined and photographed with a JEOL JEM-1010 transmission electron microscope.

**Flow cytometry of bone marrow aspirate smear**

Bone marrow aspirates were analyzed by flow cytometry and B-cell maturation was assessed as previously described [10].

**TNF and IL-1β** **immunohistochemistry of the GI tract**

Immunohistochemistry for TNF and IL-1β was performed on formalin fixed, paraffin embedded clinical biopsy specimens. 5 μm sections were deparaffinized in xylene, rehydrated in graded alcohol. Antigen retrieval was performed with citrate buffer, pH9 in a pressure cooker for 20 minutes. A 2% casein block was applied for slides stained with IL-1β. The TNF antibody is a rabbit polyclonal antibody (Abca, AB6671) at 1:250 and the IL-1β antibody is a rabbit polyclonal antibody (Abcam, AB2105) at 1:250 for 60 minutes at room temperature. Dako Envision Dual Link and DAB were applied to detect antibody-antigen complex, slides were dehydrated in graded alcohols and xylene and coverslipped. Normal human lymph node was used as positive control for both antibodies.

**Serum and plasma cytokine and chemokine profiling, and soluble CD14 (sCD14)**

The concentrations of 48 cytokines in serum and plasma were measured using the Bio-plex pro human cytokine 27-plex and 21-plex immunoassays (Bio-Rad) according to the manufacturer’s instructions. The serum and plasma concentrations of soluble TNF-R1 (sTNF-R1) and TNF-R2 (sTNF-R2) were measured using the Bio-plex pro human cytokine α-test immunoassays (Bio-Rad) according to the manufacturer’s instructions. The Bio-plex pro human cytokine standard group I and group II were used as standards for the assays. Each sample was assessed in duplicates or triplicates. Serum soluble CD14 (sCD14) was quantitated using human sCD14 Quantikine (R&D Systems, Minneapolis, MN) ELISA kits.

**NK cell frequency and phenotype**

Thawed PBMC were stained with ethidium monoazide (EMA), anti-CD19-PeCy5, anti-CD3-AlexaFluor700 (both from BD Biosciences), and with either anti-CD14-PECy7 (BD Biosciences) or anti-CD14-PeCy5 (AbD Serotec, Raleigh, NC) to exclude dead cells, T cells, B cells, and monocytes, respectively. NK cells were identified using anti-CD56-PeCy7 (BD Biosciences). In addition, FITC-conjugated antibodies against HLA-DR (BD Biosciences), PE-conjugated antibodies against TRAIL (BD Biosciences), NKG2A, and NKp44 (Beckman Coulter, Brea, CA), APC-conjugated antibodies against CCR5 (BD Biosciences), were used.

**NK cell degranulation and cytokine production**

Thawed PBMC were cultured overnight at 37°C, 5% CO2 in RPMI1640 with 10% FBS (Serum Source International), 1% penicillin/streptomycin, 2 mM L-glutamine and 10 mM HEPES (Mediatech). The next day, PBMCs were counted and cultured with or without K562 cells (ATCC, Manassas, VA) to assess NK cell degranulation as described [11] but without addition of cytokines. Cells were stained with anti-CD107-PE (BD Biosciences) in addition to EMA and the lineage-specific antibodies described above. For cytokine analysis, thawed PBMC were incubated with or without IL-12 (0.5 ng/ml; R&D Systems) and IL-15 (20 ng/ml; R&D Systems) for 14h, followed by addition of brefeldin A (BD Biosciences) for 4h as previously described [11]. Cells were then washed and stained with EMA and the lineage-specific antibodies described above. Cells were washed again, fixed and permeabilized with the Cytofix/Cytoperm Kit and stained with anti-IFNγ-PE. All samples were analyzed on an LSRII flow cytometer using FacsDiva Version 6.1.3 (BD Biosciences) and FlowJo Version 9.8.5 software (Tree Star, Ashland, OR).

**Derivation of patient-specific skin fibroblasts**

Healthy controls and TRNT1 patient-derived fibroblasts were generated from 3-4-mm skin biopsies under approved protocols.  After digested for 1h at 37C in digest solution with 0.1% Collagenase Type II (No.17101-015, Thermo Fisher Scientific) / 0.25 U/ml Dispase (No. 17105-0411, Thermo Fisher Scientific), the skin pieces were transferred for culture in Dulbecco’s modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum and antibiotics in 20% O2, 5% CO2 incubator. Once confluent, the fibroblasts then were passaged and routinely cultured in DMEM medium supplemented with 10% fetal bovine serum and antibiotics.

**Cell viability assay and ROS detection**

Cultured fibroblast cells from patients and healthy controls were harvested using 0.05% Trypsin–EDTA, washed with DMEM, and seeded in 96-well black plate with clear bottom at a density of 5000 cells/well in culture media (DMEM plus 10% FBS, 1% antibiotics), followed by incubation at 37 o C and 5% CO2 for 24 and 72-hour periods. At the indicated time points, cells were washed once with 1x PBS. For measurement of live cell numbers, cells were incubated at 37 o C and 5% CO2 with CyQUANT® Direct Cell Proliferation Assay reagents (ThermoFisher) in 1x PBS for 1 hour. For intracellular ROS detection cells were incubated with 10 μM 2’,7’- dichlorodihydrofluorescein diacetate (H2DCFDA, ThermoFisher) in 1x PBS for 1 hour. Fluorescence intensity at 480/535 nm from plate bottom was recorded afterwards.

**Autophagy and proteasome studies**

Fibroblasts derived from patients and healthy controls were detached from plates using 0.05% Trypsin–EDTA. Cells was washed once with PBS, resuspended in DMEM/10%FBS at 5x10^5 cells/ml, and then treated with 20 μg/ml TNF with or without 10 μM MG132 for 3h.  Cells were lysed and immunoprecipitation was conducted with antibodies against ubiquitin followed by blotting with antibodies against K48 ubiquitin. The LC3B levels were determined with antibody from Cell Signaling. Band density for LC3B-I, LC3B-II and actin was measured by Quantity One (Bio-Rad).

For supplementary figure 5**:** Fibroblasts from patients and healthy controls were harvested using 0.05% Trypsin–EDTA, washed with DMEM media, seeded in 6-well plates (1x106 cells/ well) in culture media (DMEM plus 10% FBS, 1% antibiotics), and incubated at 37 o C and 5% CO2 for 16 hours. The next day, the complete medium was removed and plates were washed once with 1xPBS. To induce serum starvation, DMEM without FBS was added to each well, with or without chloroquine (CQ) 100 μM. After incubation for 2 hours, or a time course of 30, 90 and 180 minutes, medium was removed, the plate was washed once with 1xPBS and then cells were lysed in the plate with lysis buffer plus protein inhibitors (Cell Signaling). Western blot was conducted using antibody against p62 (Cell Signaling) and LC3B (Cell Signaling). Cells cultured in serum containing media and without CQ served as negative controls. Band density for LC3B-II and actin was measured by Quantity One (Bio-Rad).

**Primary human monocyte differentiation and stimulation**

Monocytes were purified from PBMCs by negative selection (Monocyte Isolation Kit II; Miltenyi Biotec). Monocytes were suspended in monocytes attachment medium (PromoCell) and seeded at a density of 150,000/cm2 for 2 hours. Primary human monocytes were differentiated into M1 macrophages using 20 ng/mL human granulocyte-macrophage colony-stimulating factor (hGM-CSF, Sigma) for 7 days. hGM-CSF differentiated M1 macrophages were stimulated with or without 100 ng/mL LPS (Sigma) together with 20 ng/mL IFN-γ for 24 hours. Cell supernatants were analyzed by ELISA for IL-1β (R&D Systems).

**Gene knockdown assay**

siRNA targeting *TRNT1* and scrambled siRNA were purchased from Invitrogen. The three siRNAs for *TRNT1* knockdown were 5’-GCACUUUAUUUGACUACUUTT-3’ (s27415, si1), 5’-CAGUAUCUAUUCACAAUGATT-3’ (s27414, si2), and 5’-GGAUUUGGGUGGAACUGAATT-3’ (s27413, si3). For siRNA gene knockdown experiments, 50 – 250 pmol siRNA were electroporated into THP1 cells (2.5 X 106 cells per well) using a Neon transfection system (Invitrogen) in Tip 100 μl tips at 1400 mV/30 ms/1 pulse and replated in 12-well plates. After transfection, THP1 cells were differentiated into macrophages with 50 nM PMA for 48 h and further cultured for 5 days. Seven days after transfection, siRNA-transfected cells were incubated with RPMI containing 1 µg ml-1 LPS for 3 h with or without MCC950 (200 nM), then supernatants were analyzed by ELISA for IL-1β (R&D Systems).

**Zebrafish husbandry and ethics statement**

All zebrafish experiments were performed in compliance with NIH guidelines for animal handling and research under NHGRI Animal Care and Use Committee (ACUC) approved protocols G-05-5 and G-01-3 assigned to RS and SB, respectively. Wild-type zebrafish strain TAB-5 was used for all experiments. Zebrafish husbandry, embryo staging and microinjections were performed as described previously [12].

**Generation of *trnt1* knockout zebrafish by CRISPR/Cas9**

The single guide RNAs (sgRNAs) to target the *trnt1* gene (Genbank accession number NM\_001002159) were designed using the CRISPR design tool at <http://crispr.mit.edu>. An sgRNA with a target site in exon 6 (5’-CTCAGGCGAGAGGATCTGGG-3’) was selected. Synthesis of target oligonucleotides (IDT), preparation of mRNA, injections, evaluation of activity by CRISPR-STAT and founder screening were carried out as described previously [13]. Primers used for fluorescent PCR were: M13F-tailed forward primer (5’-TGTAAAACGACGGCCAGTAGATTCTATGGACGAGTGGC-3’), 6-FAM-labeled M13F primer (5’-TGTAAAACGACGGCCAGT) and PIG-tailedreverse primer (5’-GTGTCTTACTAGAACCTATGCTTCACC -3’). Two frame-shifted mutations, a 10bp deletion and a 13bp deletion, were selected for phenotype analysis. The same primers were used for subsequent genotyping experiments to identify the heterozygous adults from progeny of selected founders, to analyze survival of mutant fish and to perform genotype-phenotype correlations for all experiments.

**Analysis of knockout fish for morphological phenotypes during embryonic development and survival to adulthood**

The embryos generated from pairwise in-crosses of *trnt1* heterozygotes were observed daily for morphological phenotypes from 1 day post fertilization (dpf) to 6 dpf under a Leica MZ16F stereomicroscope. Images were taken using a Zeiss AxioCam HRc camera. For survival analysis, 48 embryos were collected at various time points from 2 dpf to 9 dpf and genotyped by fluorescent PCR as described above. To analyze the defective jaw, we performed Alcian blue staining using Wiki protocols from ZFIN website (https://wiki.zfin.org/display/prot/Clearing+And+Staining+For+Larval+Fish+Cartilage+And+Bone). Fixed embryos were embedded and sectioned for histology (Histoserv) and evaluated using a Zeiss Axioplan2 inverted microscope and a Zeiss AxioCam 105 color camera.

**Analysis of neuromasts and hair cells**

At 5 dpf, 24 normal-looking and 20 mutant embryos based on the morphological phenotype analysis were stained in a cell strainer (BD Falcon, Cat# 08-771-1) with 2 µM Yopro-1 (Molecular Probe, Cat# Y3603) for 30 minutes. The number of neuromasts in the posterior lateral line and hair cells in the P1-P4 neuromasts were counted as described [14] using an inverted Zeiss Axiophot fluorescent microscope. All embryos were then genotyped to demonstrate a correlation of observed phenotypes to their genotype for the *trnt1* mutation.

**Statistical analysis**

Non-parametric one-sample Wilcoxon signed rank test was performed to assess the statistical significance of non-symmetric distribution of down-regulated mature tRNAs over up-regulated ones (mean log2[ratio] against 0), using R3.2. Differences in IL-1β concentration in THP1 transfected cells and experiments in zebrafish embryos were assessed by unpaired Student’s *t*test. ROS production units (ROS/cell) were calculated based on average cell numbers present in each well.ROS/cell units and live cell numbers**/**well were transformed to log10scale and analyzed for differences between patients and controls with unpaired Student’s *t*test with Welch's correction. The non-parametric Mann-Whitney test was used to test for differences in the frequency of bone marrow B cell populations and in NK cell assays.

Statistics and plots were generated with GraphPad Prism 6.0h (GraphPad Software, La Jolla, CA) software package, except if stated otherwise. Two-tailed p-values <.05 were considered statistically significant, except if stated otherwise.

**Figure legends**

**Supplementary figure 1.** Impaired B-cell maturation in the bone marrow of SIFD patients. **A.** Flow cytometric analysis of CD19 positive lymphocytes in the bone marrow of a healthy pediatric control in comparison to a patient with SIFD. The control shows the normal B-cell maturation pattern of CD10-positive B-cell precursors (hematogones) in the red box which are negative for CD20 in early stages and gradually express CD20 during maturation. In the final stage of B-cell maturation highlighted in the purple box, CD10 is downregulated and CD20 is positive in mature B-cells. In the patient with SIFD, CD10-positive B-cell precursors are present, however, there are markedly reduced mature B-cells, suggestive of impaired B-cell maturation.

**B.** Cumulative results of CD10-positive B-cell precursors and mature B cells detected in the bone marrow from SIFD patients (n=4) and healthy pediatric controls (total n=6). Each symbol represents an individual patient or healthy control. Median with IRQ range is shown. Statistics: Mann-Whitney test, two-tailed p-value <0.05 was considered significant, exact p-value is shown.

**Supplementary figure 2.** NK cell phenotype and function

 **A.** Frequency of total NK cells (CD56+CD16+) in PBMCs from SIFD patients (n=3) and age-matched healthy controls (n=3). Gate: lymphocytic. **B.** Frequency of the surface activating receptors CCR5, NKp44, HLA-DR and NKG2A in total NK cells. Each symbol represents an individual patient or control. **C.** Frequency and expression of cytotoxic surface receptor TRAIL in total NK cells, and CD107, a marker of cytotoxicity, in the cytotoxic NK cell subset (CD56dim NK). **D.** Frequency and expression of IFNγ+ CD56bright NK cells (cytokine producing NK cell subset), after overnight stimulation with IL-12 and IL-15 as described in material and methods. Each symbol represents an individual patient or control. Statistics: Mann-Whitney, two-tailed p-value <0.05 was considered significant, exact p-value is shown. **CCR5:** C-C chemokine receptor type 5; **HLADR:** Human Leukocyte Antigen - antigen D Related; **NKG2A:** Killer Cell Lectin Like Receptor C1; inhibitory receptor. **TRAIL:** Tumor necrosis factor (TNF)-related apoptosis-inducing ligand.

**Supplementary figure 3. ROS overproduction**

Decreased cell viability and increased spontaneous ROS production in patients’ fibroblasts.  Fibroblasts from patients (n=3) and healthy controls (n=2) were cultured on 10% DMEM for a total of 24 and 72 hours. (A) Numbers of live cells were measured by absorption of nucleic acid stain in live cells (CyQUANT® Direct Cell Proliferation Assay) at the indicated time points. (B) Intracellular ROS levels were measured by H2DCFDA fluorescent dye at the same time points. ROS units were normalized based on viable cell numbers (ROS/cell) for each patient and control. Values in the graphs are log10 transformed data for viable cell numbers and ROS/cell. Each symbol in the graph represents an individual patient or control. One representative experiment out of three is shown. Statistical analysis: Unpaired t test with Welch's correction, two-tailed p-value <0.05 was considered statistically significant.

**Supplementary figure 4. IL-1β production**

**A.** Cultured monocyte-derived macrophages from patient 4, a healthy volunteer (C1), and her healthy, non-carrier brother (C2) were stimulated with LPS and IFN-γ for a total of 24 hours. IL-1β was measured in cell supernatants by ELISA immunoassay. Shown here are the relative IL-1β levels divided by the IL-1β in unstimulated C1 cells. **B.** 3 days post transfection of human THP-1-cells with three different si-TRNT1 or a scrambled siRNA control (Ctrl), IL-1β was measured by ELISA immunoassay. Graph shows cumulative data for each group obtained from four independent experiments presented as mean (SD). Statistics: unpaired t test, two-tailed p-value <0.05 was considered significant. **C.** 7 days post transfection of human THP-1-cells with si-TRNT1 or scrambled siRNA control, cultured cells were stimulated with LPS for 3 hours with or without the NLRP3 specific inflammasome inhibitor MCC950, and IL-1β levels was measured in cultured supernatants by ELISA immunoassay. The graph shows cumulative data for each group obtained from three independent experiments and presented as mean (SD). Statistics: unpaired t test, two-tailed p-value <0.05 was considered significant

**Supplementary figure 5.** Defective degradation of the ubiquitin-scaffold protein, p62, in mutant cells

**A.** Cultured fibroblasts from two patients and one healthy control were detached from plates and after overnight rest, were incubated for 2 hours on serum free media (serum starvation, ss), to induce autophagy, with or without the lysosomal inhibitor chloroquine (CQ) 100 nM to block the degradation of the autolysosomes’ contents. Cells were lysed and proteins p62 and LC3B were detected by immunoblotting using antibodies against p62 and LC3B. Band densities for LC3B-II and actin were measured by Quantity One (Bio-Rad) and the LC3B-II to actin ratio was calculated as an estimate of autophagosome formation. **B.** Time course of p62 degradation in fibroblasts from one patient and healthy control. Cultured fibroblasts from patient 2 and a healthy control were detached from plates and after overnight rest, were incubated on serum free media (serum starvation, ss) only or with the addition of CQ 100 nM for the indicated time points. P62 and LC3B were detected in cell lysates as in **A**. LC3-II/actin ratio was calculated as above.

**Supplementary figure 6. Changes in gene expression after treatment with TNF-inhibitor**

RNA-Seq was performed on whole blood cells from patient 4 before and after the initiation of etanercept, and 3 pediatric healthy controls. **A.** Heat map depicting 234 genes that were differentially expressed in the patient before treatment compared to the average value of healthy controls, and their expression changed by at least two-fold following treatment. **B.** Heat map panel shows a selected set from the 234 DEGs that are regulated by TNF, generated byIPA pathway analysis software. Heat maps are shown log2-normalized expression values (RPKM)

**Supplementary figure 7.** Viability and phenotype of *trnt1* knockout zebrafish embryos

**A**. **Survival of *trnt1*-/- fish.** The histogram shows the percentage of live fish with homozygous WT, heterozygous, and homozygous null genotypes for both alleles [knockout mutant alleles del10 (Δ10) and del13 (Δ13)] at the indicated days post fertilization (dpf).Null fish die between 6 to 9 dpf and no null adults are recovered from heterozygous in-crosses. **B-E.** Defective morphological and histological phenotypes of *trnt1*-/- embryos at 5 dpf. WT embryos are shown in the left panel and mutant embryos are shown in the right panel. **B**. *trnt1-/-* embryos with abnormal jaw (black arrow), pericardial edema (red arrow) and uninflated swim bladder (blue arrow). **C-D**. Abnormal pharyngeal arches shown by histology analysis (**C**, black arrow) and cartilage staining (**D**) **E**. Abnormal intestinal development. Mutant embryos show lack of epithelial folds in the bulb of intestine. Black arrows mark the indicated structures. Both alleles had similar phenotypes, phenotype for del10 allele is shown here.

**Supplementary figure 8.** Reduced neuromast hair cells in *trnt1* mutant embryos

**A.** Fluorescent images of WT (left) and mutant (right) embryos at 5 dpf stained with Yopro-1, showing the location of neuromasts. Both embryos are shown with their head to the left. **B.** Magnified images of hair cells in an individual neuromast in the WT and mutant embryos**. C-D**. Quantification of neuromast numbers **(C)** and hair cell numbers **(D)** in embryos from het in-crosses. Both graphs show the average and the standard error of the mean. The reduction in the number of neuromasts and hair cells is significant in the mutant (t-test, p <0.001). Both alleles had similar phenotypes, phenotype for del10 allele is shown here.

**Table 1. *TRNT1* mutations identified in 9 SIFD patients.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleotide Alteration** | **cDNA** **Alteration1** | **Amino Acid** **Alteration** | **EXAC Frequency2** | **1061 Arabian Controls** | **SIFT and** **PolyPhen2** | **Patient ID** | **Inheritance** |
| chr3:3179090C>T | c.295C>T | p.Arg99Trp | 0.00002477 | / | Damaging | 4 & 5 | Compound heterozygous |
| chr3:3179124C>T | c.329C>T | p.Thr110Ile | 0 | / | Damaging | 6 & 7 (siblings) | Compound heterozygous |
| chr3:3182234A>G (rs148398677) | c.383A>G | p.Asp128Gly | 0.00009064 | / | Damaging | 6 & 7 (siblings) | Compound heterozygous |
| chr3:3186274A>T (rs146717589) | c.488A>T | p.Asp163Val | 0.00005792 | / | Damaging | 3, 4, & 5 | Compound heterozygous |
| chr3:3188149A>G | c.644A>G | p.His215Arg | 0 | 0 | Damaging | 1 & 2 (siblings) | Homozygous |
| chr3:3188173T>C | c.668T>C | p.Ile223Thr | 0.00001649 | / | Damaging | 3 & 9 | Compound heterozygous |
| chr3:3189779A>G | c.1246A>G | p.Lys416Glu | 0.00004969 | / | Damaging | 8 | Compoundheterozygous |
| chr3:3189778\_3189779insA | c.1245\_1246insA | p.Ser418Lys*fs*\*9 | 0.0004386 | / | / | 8 & 9 | Compound heterozygous |

1. cDNA positions are according to the reference NM\_182916.2.

2. The ExAC database includes 61,486 exomes.

**Supplementary table 2: Clinical characterization of NHGRI Cohort of SIFD Patients (Patients 1-5)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Patient 1+ | Patient 2 + | Patient 3  | Patient 4 | Patient 5  |
| Ancestry | Saudi Arabian | Saudi Arabian | Mixed European | Mixed European | Mixed European |
| Consanguinity | Yes | Yes | No | No | No |
| *TRNT1* mutations | H215R/H215R | H215R/H215R | D163V/I223T | R99W/ D163V | R99W/ D163V |
| Fever onset  | 1-month-old | 2-year-old  | Soon after birth  | 3-week-old | 6-week-old |
| Frequency and duration of febrile episodes | Initially every 4 weeks, increased to weekly, lasting for 5-7 days | Every 2-4 weeks, lasting for 5-7 days | Every 2-3 weeks, lasting for 5-7 days | Initially every 3 weeks, increased to every 7-10 days, lasting for 3 to 5 days | Every 4-6 weeks, lasting from 2-3 days to 10-14 days |
| Inflammatory markers | Elevated during febrile episodes | Elevated during febrile episodes | Elevated during and between febrile episodes | Elevated during and between febrile episodes | Elevated during and between febrile episodes |
| Hematologic  | Chronic hypochromic, microcytic anemia.Elevated ferritin.Acute anemia during fevers | SA. Elevated ferritin.Acute on chronic anemia and blood transfusions with fevers | SA. Acute on chronic anemia, blood transfusions with fevers.Hemophagocytosis on bone marrow aspirate during flares, high ferritin and elevated LFTs (incomplete criteria for secondary HLH) | SA. High ferritin.Acute on chronic anemia and blood transfusions with fevers. Evidence of hemophagocytes on bone marrow aspirate smear | Chronic hypochromic, microcytic anemia, non transfusion dependent (rare sideroblasts in bone marrow biopsy)Elevated ferritin in the past, now normal |
| Immunologic | Leukocytosis with left shift and lymphopenia during fever | Leukocytosis or -penia, during fever.Toxic granulosis independently of fevers.Low-normal NK cell number.Fluctuating monocytes | Absent B cells.Agammaglobulinemia.Low-normal NK cell number | Very low B cell numberAgammaglobulinemia. Fluctuating neutropenia, monocytopenia.Toxic granulosis independently of fevers | Very low B cell number.Agammaglobulinemia. Very low NK cell number |
| Neurologic | Vertigo, febrile seizures during febrile episodes | Dizziness/ vertigo, opsoclonus during febrile episodesMild developmental delay | Severe growth retardation, hypotonia, developmental delay. Seizures; suspected CNS MAS. Abnormal brain MRI (end stage cerebral damage; hemorrhage, volume loss and leukomalacia) | Developmental delay and speech delay. Mildly wide based gait, intermittent opsoclonus and nystagmus (worse with fevers).Normal brain imaging | Developmental delay, improved.Mild deficits (mild ataxia and proximal muscle weakness, mild balance difficulties. Absent reflexes at the ankles) |
| Gastrointestinal | Oral ulcers, vomiting, abdominal pain, diarrhea with fevers.Hepatosplenomegaly | Sore throat, oral ulcers, vomiting, abdominal pain, diarrhea with fevers.Splenomegaly | Pseudoobstruction with ileostomy at birth. Necrotizing enterocolitis 6-week-old. Feeding intolerance/TPN dependence. Elevated liver enzymes during fevers. Hepatosplenomegaly | Oral mucosa and tongue ulcerations, diarrhea with fevers; transient pancreatic insufficiency, feeding intolerance, chronic constipation. GI biopsy: acute focal colitis.Hepatosplenomegaly, elevated liver enzymes. | History of oral ulcers and tongue swelling with fevers, feeding intolerance, prolonged diarrhea. GI biopsy: acute and chronic inflammation in the mucosa of the stomach, terminal ileus, colon. Hepatosplenomegaly (resolved) |
| Opthalmologic | Reportedly normal | Normal  | Optic nerve atrophy  | Early retinal degeneration  | Retinitis pigmentosa, retinal degeneration, and optic nerve atrophy. Hyperopia, bilateral cataracts. Pseudophakia status post cataract surgery. Myopic astigmatism. Legally blind |
| Hearing | Reportedly normal | Normal | Not officially tested | Bilateral sensorineural hearing loss; cochlear implants. Normal inner ear MRI.  | Bilateral sensorineural hearing loss; cochlear implants. |
| Musculoskeletal | Knee arthritis started at 2 years of age, partial response to steroids and colchicine | Normal | Normal  | Elevated muscle enzymes; normal MRI of the thighs.  | Arthritis of medium and large joints. Diffuse, tender inflammatory, soft tissue masses. Subcutaneous nodules with overlying skin erythema (scalp, external ear canal, limbs, chest, back). Muscle biopsy: Acute myositis/fasciitis; inflammatory infiltrates by lymphocytes and macrophages. |
| Mucocutaneous/Skin | Oral ulcers  | Normal | Normal | Cellulitis of both feet and face; ulcerated tongue; subcutaneous, tender, erythematous nodules during febrile episodes. | Erythematous rash and purpura. Cellulitis after minor trauma, tongue swelling.  |
| Pulmonary | Reportedly normal | Upper respiratory symptoms (nasal congestion, rhinorrhea), wheezing. History of stridor in infancy  | None | Nasal congestion, rhinorrhea, sinusitis. | History of recurrent croup, stridor and epiglottitis in infancy in association with fevers  |
| Infections | Typically, not identified | Typically, not identified.Avian Influenza A Virus infection  | Majority of microbial cultures negative. History of *Acinetobacter ursingii* sepsis, *Escherichia coli* acute pyelonephritis, *Staphyloccocus aureus* sepsis while on immunosuppressants  | History of prolonged course of Enteroviral gastrointestinal infection, *Clostridium difficile* colitis; bacterial urinary tract infections; RSV pneumonia. Majority of microbial cultures were negative.  | Few bacterial UTIs. Majority of microbial cultures were negative. |
| Other | Homozygous for V377I mutation in the *MVK* gene | Mild facial syndromic featuresShort stature; received growth hormone replacement therapy | Preterm delivery and perinatal complications.History of thrombosis of femoral central vein catheter.  | Short stature, mild dysmorphic features (sparse hair, deep seated eyes, long thin tapering fingers) | Mild dysmorphic features (plagiocephaly, sparse hair, deep seated eyes, long thin tapering figures). Bilateral small kidneys on abdominal ultrasound but normal kidney function. |
| Treatment | Acute: hemodynamic support often in ICU, blood transfusions, systemic corticosteroids, empiric antibiotics.Chronic: colchicine. | Acute: blood transfusions, systemic corticosteroids, empiric antibiotics.Chronic: Low dose daily oral steroids and colchicicine.Etanercept SC (onset 8 yo) | Acute: hemodynamic support, systemic corticosteroids, empiric antibiotics.Cyclosporin A.IgG replacement therapy Unrelated HLA matched donor PBSCT  | Acute: hemodynamic support, blood transfusions, empiric antibiotics. Chronic: IgG replacement Trial of Anakinra SC Etanercept SC (onset 4 yo) | Acute: hemodynamic support, empiric antibiotics, steroids. IgG replacement.Etanercept, switched toInfliximab and azathioprine due to development of inflammatory colitis |

+: siblings. SA: Sideroblastic anemia, IVIG: Intravenous immunoglobin, URIs: upper respiratory infections; HLH: hemophagocytic lymphohistiocytosis; LFTs: liver function tests

**Supplementary table 3: Clinical characterization of the NHGRI Cohort of SIFD Patients (Patients 6-9)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Patient 6 + | Patient 7 + | Patient 8  | Patient 9  |
| Ancestry | Mixed European | Mixed European | Mixed European | Mixed European |
| *TRNT1* mutations | T110I/ D128G | T110I/ D128G | K416E/S418K*fs*\*9 | I223T/S418K*fs*\*9 |
| Consanguinity | No | No | No | No |
| Fever onset  | 6-year-old  | 4-weeks-old | 2-month-old | 3-week-old |
| Frequency and duration of febrile episodes | Decreased after age 7 with initiation of IVIG | Every 10- 20 days, lasting 2-5 days, more frequent during the last year of life. | Every 1-4 weeks, lasting 3-7 days, more frequent during first year of life | Every 2-3 weeks |
| Inflammatory markers | NA/ND | NA/ND | Elevated during febrile episodes | NA/ND |
| Hematologic | SA. Initially diagnosed with hereditary pyropoikilocytosis.Elevated ferritin, iron overload. | SA; underwent splenectomy for transfusion dependent anemia at the age of 3. Initially diagnosed with hereditary pyropoikilocytosis.  | SA, no transfusions needed  | SA, transfusion dependent. |
| Immunologic | Hypogammaglobulinemia, diagnosed with CVID | Combined B and T cell immunodeficiency.Hypogammaglobulinemia, diagnosed with CVID. | Initially low total B cell count, normalized at age 2 years HypogammaglobulinemiaNormal NK cells | Combine B and T cell immunodeficiency. |
| Neurologic | Mild developmental delay, resolved.Attention deficit disorder. | Mild developmental delay.History of central catheter-related venous thrombosis of superior vena cava complicated by pseudotumor cerebri.  | Developmental delay | Developmental delay |
| Gastrointestinal | Mild symptoms with fevers (nausea, vomiting, diarrhea). Hepatosplenomegaly | Mild symptoms with fevers (nausea, vomiting, diarrhea) Nodular regenerative hyperplasia and hemosiderosis seen in liver autopsy postmortem. | Failure to thrive. Mild symptoms with fever (diarrhea) | Protein losing enteropathy. Feeding intolerance/ TPN dependence. Pancreatic insufficiency.Hypoglycemia |
| Opthalmologic/Hearing | Retinitis pigmentosa; decreased night time vision.Hyperopic astigmatism | Retinitis pigmentosa. Decreased night time vision | Bilateral sensorineural hearing loss; cochlear implants. Bilateral cataracts. | Retinitis pigmentosa.Bilateral sensorineural hearing loss; hearing aids |
| MusculoskeletalMucocutaneous/Skin | Normal | Asymmetric erythema and swelling of digits of the hands and feet, diffuse swelling of the dorsum of the feet, erythema nodosum on lower extremities. Skin biopsy: septal panniculitis, superficial and deep lymphocytic dermatitis | Recurrent swelling of digits of the hands and knee. | Normal |
| Pulmonary | Asthma, history of abnormal PFT  | Asthma | Recurrent cough | NA/ND |
| Infections | Recurrent otitis media, URIs, sinusitis, pneumonia  | Recurrent otitis media, pneumonia  | Recurrent rhinitis and cough without proven infectious agent. Rotavirus gastroenteritis | CMV viremia, CMV pneumonitis.Pseudomonas necrotizing perianal colitis. |
| Other |  | Undetermined hypercoagulable syndrome, DVT on anticoagulation |  |  |
| Treatment | Blood transfusions during childhood.IgG replacement (6.5 years old) | Blood transfusions, splenectomy IgG replacement (3.5 years old)Oral steroids, NSAIDS.Died at the age of 9; autopsy: *St. aureus* septic shock. | IgG replacementAnakinra for 3 months, discontinued due to lack of effect on fever episodes. | Blood transfusions IgG replacementSteroidsEtanercept (started 21 months old) |

+: siblings. NA/ND: Not available/ No done. SA: Sideroblastic anemia, IVIG: Intravenous immunoglobin, URIs: upper respiratory infections, DVT: deep venous thrombosis, PFTs: pulmonary function tests

**Supplementary table 4: T and B cell clinical immunophenotyping and function (Patients 1-5)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | P1 + | P2 + | P3 | P4 | P5 |
| T cell numbers | NA/ND | Within normal range, intermittently decreased CD4:CD8 ratio  | Within normal range, intermittently decreased CD4:CD8 ratio | CD4, CD8 cells within normal range, normal ratio. Elevated DNT and NKT cells.  | CD4, CD8 cells within normal range, normal ratio. Elevated DNT cells. |
| Naïve/memory (CD45RA/CD45RO) | NA/ND  | Normal | Normal | Normal | Normal |
| TCR alpha/beta and gamma/delta CD4+, CD8+ | NA/ND | NA/ND | Normal | Normal | Normal |
| T cell activation markers(HLA-DR, CD25) | NA/ND | Normal | Increased in the blood, bone marrow and CSF during fever | Normal | Normal |
| B cell numbers | NA/ND | Low-normal range  | Absent | Very low | Very low to absent |
| Serum immunoglobulins | NA/ND | Normal | Panhypo-gammaglobulinemia | Panhypo-gammaglobulinemia | Panhypo-gammaglobulinemia  |
| Mitogen\* proliferationstudies | NA/ND | NA/ND | Normal | Normal | Normal |
| Recall \*\*or alloantigen proliferation studies | NA/ND | NA/ND | NA/ND | Normal response to Candida and tetanus | Normal response to Candida, negative to tetanus |
| Antibody production | NA/ND | Protective levels of anti-diphtheria toxoid, anti-tetanus toxoid, and anti-pneumococcus antibodies, positive anti-CMV serology. | NA/ND | NA/ND | Protective anti-diphtheria toxoid levels, borderline low anti-tetanus toxoid antibody levels |
| Other  |  |  |  |  | Anergy to intradermal PPD, Candida and tetanus |

**+**: siblings. NA/ND: Not available/ No done.

\* Mitogens: PHA: phytohaemagglutinin, ConA: Concanavalin A, PWM: pokeweed mitogen

\*\* Recall antigens: Tetanus, Candida.

**Supplementary table 5: T and B cell clinical immunophenotyping and function (Patients 6-9)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | P6 + | P7 + | P8 | P9 |
| T cell numbers  | Low at diagnosis, then normal | Intermittently low | Normal | Low, normal CD4/CD8 ratio |
| Naïve/memory CD4+, CD8+ (CD45RA/CD45RO) | Normal | Adult pattern  | Normal  | Decreased  |
| TCR alpha/beta and gamma/delta CD4+, CD8+ | Normal | Decreased alpha/beta,normal gamma/delta | Normal | NA/ND |
| T cell activation markers(HLA-DR, CD25) | NA/ND | NA/ND | Normal | NA/ND |
| B cell numbers | Intermittently low  | Intermittently low | Low at diagnosis, then normal | Low |
| Serum immunoglobulins | Panhypo-gammaglobulinemia | Panhypo-gammaglobulinemia | Low IgM, IgA, normal IgG  | Low |
| Mitogen\* proliferationstudies | Normal | Low to PHA and PWM | Normal | Fluctuating  |
| Recall \*\*or alloantigen proliferation studies | Normal response to candida tetanus, single donor and pooled unrelated allogeneic cells | Low response to Candida, tetanus, and pooled unrelated allogeneic cells | Normal | Absent responses to Candida, tetanus. Reduced response to alloantigens  |
| Antibody production | Undetectable anti-diptheria toxoid and anti-tetanus toxoid antibodies | Undetectable anti-diptheria toxoid, anti-tetanus toxoid, and anti-pneumococcus antibodies | Undetectable anti-pneumococcus antibodies | NA/ND |

**+**: siblings. NA/ND: No done/ no available.

\* Mitogens: PHA: phytohaemagglutinin, ConA: Concanavalin A, PWM: pokeweed mitogen

\*\* recall antigens: Tetanus, Candida.

**Supplementary table 6: Frequency and absolute numbers of double negative T cells (DNT), their subtypes and NK T cells by flow cytometry of peripheral blood performed at the NIH**

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\*For P4 two different time points are available and shown

**Supplementary table 7: Canonical Pathways that downregulated with treatment in patient 4 (generated by ingenuity pathway analysis).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Pathways** | **p-value** | **Gene ID** |   |
| Hepatic Fibrosis / Hepatic Stellate Cell Activation | 8.91E-07 | COL5A1,COL1A2,MYH10,COL1A1,COL5A2,CTGF,FN1,CD40,ACTA2,TGFB3,MMP2 |
| Agranulocyte Adhesion and Diapedesis | 3.55E-05 | MYH10,PODXL2,FN1,ACTA2,MMP14,MMP2,ACTG2,CXCL5,CLDN9 |
| Inhibition of Matrix Metalloproteases | 3.89E-04 | TIMP3,MMP14,THBS2,MMP2 |  |
| Autoimmune Thyroid Disease Signaling | 5.75E-04 | CD40,HLA-DRA,FCER1G,HLA-DQA1 |  |
| Osteoarthritis Pathway | 6.03E-04 | TIMP3,BGLAP,FN1,DDR2,SMO,HTRA1,TCF7L2,CASP5 |
| EIF2 Signaling | 7.59E-04 | ACTA2,RPL36A,RPL36AL,RPS23,RPL21,RPL26,ACTG2,RPS24 |
| Allograft Rejection Signaling | 9.55E-04 | CD40,HLA-DRA,FCER1G,HLA-DQA1 |  |
| Altered T Cell and B Cell Signaling in Rheumatoid Arthritis (RA) | 9.77E-04 | CD40,HLA-DRA,FCER1G,HLA-DQA1,TLR7 |
| Role of Osteoblasts, Osteoclasts and Chondrocytes in RA | 1.20E-03 | COL1A1,BGLAP,SFRP2,MMP14,SMO,CSF1R,TCF7L2,BMP1 |
| Crosstalk between Dendritic Cells and Natural Killer Cells | 1.35E-03 | CD40,ACTA2,HLA-DRA,TLR7,ACTG2 |  |
| Nur77 Signaling in T Lymphocytes | 1.38E-03 | HLA-DRA,FCER1G,NR4A1,HLA-DQA1 |
| Dendritic Cell Maturation | 1.55E-03 | COL1A2,COL1A1,CD40,DDR2,HLA-DRA,FCER1G,HLA-DQA1 |
| Tryptophan Degradation to 2-amino-3-carboxymuconate semialdehyde | 1.70E-03 | KMO,KYNU |  |
| Calcium-induced T Lymphocyte Apoptosis | 2.19E-03 | HLA-DRA,FCER1G,NR4A1,HLA-DQA1 |
| B Cell Development | 2.29E-03 | CD40,HLA-DRA,HLA-DQA1 |  |
| Leukocyte Extravasation Signaling | 2.88E-03 | TIMP3,ACTA2,MMP14,MMP2,ACTG2,CLDN9,ARHGAP8/PRR5-ARHGAP8 |
| Remodeling of Epithelial Adherens Junctions | 3.09E-03 | DNM1,TUBB3,ACTA2,ACTG2 |  |
| T Helper Cell Differentiation | 3.63E-03 | CD40,HLA-DRA,FCER1G,HLA-DQA1 |  |
| Caveolar-mediated Endocytosis Signaling | 4.07E-03 | ACTA2,CAV1,ACTG2,CAVIN1 |  |
| Calcium Signaling | 4.90E-03 | MYH10,ACTA2,TNNT3,TPM1,CHRNA10,SLC8A1 |
| RhoA Signaling | 5.25E-03 | ACTA2,NRP2,ACTG2,CDC42EP2,ARHGAP8/PRR5-ARHGAP8 |
| NAD biosynthesis II (from tryptophan) | 6.03E-03 | KMO,KYNU |  |
| Cdc42 Signaling | 6.61E-03 | CDC42BPA,HLA-DRA,FCER1G,HLA-DQA1,CDC42EP2 |
| Graft-versus-Host Disease Signaling | 7.59E-03 | HLA-DRA,FCER1G,HLA-DQA1 |  |
| Communication between Innate and Adaptive Immune Cells | 8.32E-03 | CD40,HLA-DRA,FCER1G,TLR7 |  |
| Factors Promoting Cardiogenesis in Vertebrates | 8.91E-03 | SMO,TGFB3,TCF7L2,BMP1 |  |
| Histamine Biosynthesis | 9.12E-03 | HDC |  |
| Human Embryonic Stem Cell Pluripotency | 9.33E-03 | SMO,TGFB3,TCF7L2,INHBA,BMP1 |  |
| Epithelial Adherens Junction Signaling | 1.02E-02 | MYH10,TUBB3,ACTA2,ACTG2,TCF7L2 |
| Virus Entry via Endocytic Pathways | 1.45E-02 | DNM1,ACTA2,CAV1,ACTG2 |  |
| OX40 Signaling Pathway | 1.45E-02 | HLA-DRA,FCER1G,HLA-DQA1 |  |
| Oxidative Phosphorylation | 1.51E-02 | NDUFS5,ATP5O,ATP5I,NDUFA1 |  |
| Glycine Degradation (Creatine Biosynthesis) | 1.82E-02 | GATM |  |
| Tight Junction Signaling | 1.82E-02 | MYH10,ACTA2,TGFB3,ACTG2,CLDN9 |
| Tryptophan Degradation III (Eukaryotic) | 1.86E-02 | KMO,KYNU |  |
| Gap Junction Signaling | 1.86E-02 | TUBB3,BGLAP,ACTA2,CAV1,ACTG2 |  |
| Tec Kinase Signaling | 1.91E-02 | ACTA2,GNAO1,FCER1A,FCER1G,ACTG2 |
| Wnt/β-catenin Signaling | 1.95E-02 | SFRP2,GNAO1,SMO,TGFB3,TCF7L2 |  |
| Sertoli Cell-Sertoli Cell Junction Signaling | 2.14E-02 | TUBB3,ACTA2,TGFB3,ACTG2,CLDN9 |
| Phagosome Formation | 2.19E-02 | FN1,FCER1A,FCER1G,TLR7 |  |
| iCOS-iCOSL Signaling in T Helper Cells | 2.29E-02 | CD40,HLA-DRA,FCER1G,HLA-DQA1 |  |
| Colorectal Cancer Metastasis Signaling | 2.45E-02 | MMP14,SMO,TLR7,TGFB3,MMP2,TCF7L2 |
| Basal Cell Carcinoma Signaling | 2.51E-02 | SMO,TCF7L2,BMP1 |  |
| Role of NFAT in Regulation of the Immune Response | 2.57E-02 | HLA-DRA,GNAO1,FCER1A,FCER1G,HLA-DQA1 |
| TREM1 Signaling | 2.63E-02 | CD40,TLR7,CASP5 |  |
| L-serine Degradation | 2.69E-02 | SDSL |  |
| ILK Signaling | 3.16E-02 | MYH10,FN1,ACTA2,FERMT2,ACTG2 |  |
| Heme Degradation | 3.63E-02 | HMOX1 |  |
| Catecholamine Biosynthesis | 3.63E-02 | DBH |  |
| Citrulline-Nitric Oxide Cycle | 4.47E-02 | ASS1 |  |
| Bladder Cancer Signaling | 4.57E-02 | THBS1,MMP14,MMP2 |  |
| Antigen Presentation Pathway | 4.68E-02 | HLA-DRA,HLA-DQA1 |   |

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