



Autoimmune disease-associated gene expression is reduced by BET-inhibition



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ABSTRACT

For many autoimmune diseases, the underlying mechanism is still unknown. In order to get more insight into the etiology of autoimmune diseases, we recently published a study where we performed epigenetic profiling and RNA sequencing on CD4⁺CD45RO⁺ T cells derived from the site of inflammation of Juvenile Idiopathic Arthritis (JIA) patients and compared this with healthy controls [1]. In this "Data in Brief", we focus on the analysis of our RNA sequencing data reported in this study, of which the raw and processed files can be found in GEO under GSE71595. We provide a detailed description of the downstream analysis, quality controls, and different analysis methods or techniques that validate the results obtained with RNA-sequencing.

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Specifications

Organism/cell line/tissue	<i>Homo sapiens</i> /CD4 ⁺ CD45RO ⁺ T cells/synovial fluid (SF) and peripheral blood (PB)
Sequencer or array type	Illumina NextSeq500 sequencer
Data format	Raw and analyzed
Experimental factors	Healthy control (HC)-derived cells obtained from PB vs. Juvenile Idiopathic Arthritis (JIA) patient-derived cells obtained from SF.
Experimental features	CD4 ⁺ CD45RO ⁺ cells were sorted from HC peripheral blood mononuclear cells (PBMC) and JIA synovial fluid mononuclear cells (SFMC), and cultured for 16 h in the presence of CD3/CD28 dynabeads and 300 nM JQ1(+) or JQ1(-). Total RNA was extracted, followed by next-generation sequencing to study global transcriptome differences between HC and JIA patients and to assess the effect of JQ1(+) on gene expression in JIA patient-derived T cells.
Consent	Informed consent was obtained from all patients either directly or from parents/guardians when the patients were younger than age 12 years.
Sample source location	University Medical Center Utrecht, Utrecht, The Netherlands.

1. Direct link to deposited data

The deposited data can be found at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71595>.

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2. Experimental design, materials and methods

2.1. Study population and sample collection

Four HC and three oligoarticular JIA patients were included who all had active disease at the time of sampling. SF was collected upon therapeutic joint aspiration and PB was drawn at the same moment. SFMCs and PBMCs were isolated using Ficoll Isopaque density gradient centrifugation and were frozen down in FCS containing 10% DMSO.

2.2. RNA sample preparation and sequencing

CD4⁺CD45RO⁺ cells (ranging between 600.000–1.200.000 cells per sample) were isolated from frozen HC PBMCs and JIA SFMCs using flow cytometry, activated with human T-activator CD3/CD28 dynabeads (1 cell: 3 beads) and cultured for 16 h in the presence of 300 nM JQ1(+) or JQ1(-). Subsequently, cells were treated for 4 h with phorbol 12-myristate 13-acetate (100 ng/ml) and ionomycin (1 µg/mL). Total RNA was extracted using the RNeasy kit (Qiagen), according to the manufacturer's instructions. The quality of the RNA was assessed based on the RNA profile generated by the bioanalyzer using the picochip. Removal of rRNA and globin-encoding mRNA, RNA fragmentation, cDNA generation, adapter ligation and PCR amplification was done using the TruSeq stranded total RNA with ribo-zero globin sample preparation kit (Illumina) and 75 bp single-end sequencing was performed using an Illumina NextSeq 500 platform through the Utrecht DNA sequencing facility.

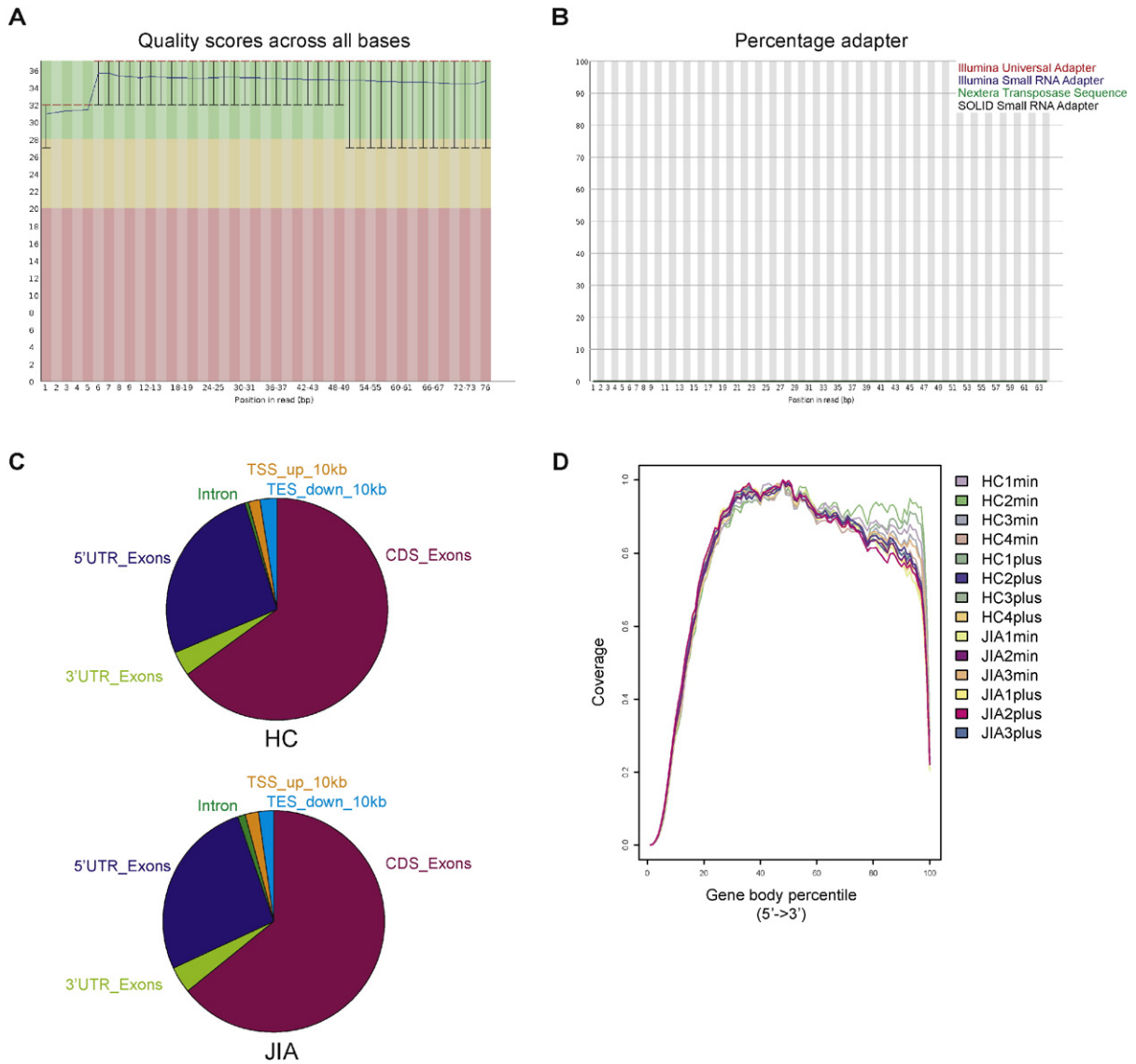


Fig. 1. Quality control steps in RNA-sequencing data analysis. (A) Read quality score across all bases, representative image of one sample is shown. (B) Percentage adapters in reads, representative image of one sample is shown. (C) Average percentage of read distribution over DNA regions from untreated 4 HC and 3 JIA samples (read distribution is also representative for JQ1(+) treated samples). (D) Read coverage of housekeeping genes for all samples.

2.3. Quality control

Read quality assessment, sample demultiplexing, and adapter trimming was performed by Utrecht DNA sequencing facility using BaseSpace (Illumina) software. Reads with quality score of $Q > 30$ were selected for downstream analysis. Before starting downstream analysis, FastQC (Babraham Bioinformatics) was used to confirm previous steps (Fig. 1A and B). As a human reference genome, hg19 was used, and reads were mapped to this reference sequence using Tophat v.2.0.9 [2] with Bowtie 2.1.0 [3] as index (tophat2 -p 8 -library-type fr-firststrand -g hg19.gtf). For each sample $\pm 91\%$ of the reads were successfully mapped to the genome, indicating sufficient coverage. RSeQC was used to calculate how mapped reads were distributed over DNA regions, such as coding sequences and UTRs, and to assess uniform read coverage of genes [4] (Fig. 1C and D).

2.4. Gene expression analysis and validation

Transcriptomes of each individual sample were generated using CuffLinks v2.2.1 with hg19 gene annotation as a guide [5,6]. Individual

transcripts belonging to the HC or JIA patient group were merged using Cuffmerge. The resulting transcriptome was used for differential gene expression analysis using Cuffdiff. Quartile normalization was performed (-library-norm quartile) to improve the detection of differential expression of less abundant transcripts. Reads mapping to rRNA and tRNA were masked (-M option) from the quantification. Cuffnorm was used to generate quartile normalized count and FPKM tables. Using an $FDR \leq 0.05$, 914 transcripts were identified that are significantly differentially expressed between HC and JIA patients. Among these transcripts, 637 were upregulated in JIA patients compared to HC, while 237 were downregulated in JIA patients. JQ1(+) and JQ1(-) treatments of JIA patient-derived memory/effector T (Tmem/eff) cells resulted in 633 differential transcripts, based on $FDR \leq 0.05$, of which 338 were downregulated upon JQ1(+) treatment and 295 upregulated.

Principle component analysis (PCA) on the complete transcriptome of HC and JIA patient-derived T cells, either with and without JQ1(+) treatment, demonstrated that four different groups can be distinguished, illustrating that HC and JIA cluster separately and that JQ1 affects gene expression in such way that treated samples cluster separately from untreated samples (Fig. 2A).

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