

Probe preparation and hybridization: 10 μ g of total cellular RNA was converted into double-stranded cDNA by using a SuperScript™ Choice system (Invitrogen, CA, USA). The biotinylated complementary RNA (cRNA) was synthesized using BioArray High Yield RNA Transcript Labeling Kit (Enzo Life Sciences, New York, NY, USA). Global gene expression was analyzed with microarray technology using the Affymetrix GeneChip 1.0 ST array (High Wycombe, UK), which provides probes for whole transcript coverage, and therefore a more complete picture of gene expression, for 28,869 well-annotated genes with 764,885 distinct probes from the human genome.

High-throughput real time PCR was performed to verify the candidate genes using TaqMan™ OpenArray® NTCyclerSystem. The PBMC cDNA samples analyzed on the OpenArray® system. Loading of samples, operation of instruments and RT-qPCR cycling were performed using standardized protocol for gene expression analysis provided by Life Technologies™. In short, 1.2 μ l of each cDNA sample was combined with 2.5 μ l of 2 \times TaqMan® OpenArray® Real-Time PCR Master Mix (Life Technologies™) and 1.3 μ l dH₂O and transferred to an OpenArray® 384-Well Sample Plate. Samples were loaded into the custom designed OpenArray® plates using QuantStudio™ 12 K flex AccuFill™ system. The OpenArray® plates were removed from the AccuFill™ system, transferred to an OpenArray® carrier, covered with immersion fluid (All reagents from QuantStudio™ 12 K flex OpenArray® accessories kit, Life Technologies™) and loaded into the QuantStudio™ 12 K flex instrument for RT-qPCR cycling. Samples were subjected to standard thermal cycling protocol provided by Life technologies™. Data was collected with Life Technologies™ Expression Suite analysis software v 1.0.3 using linear baseline correction method and the global auto Cq threshold method. Normalization of data was performed using the comparative Cq method [20]. The reference gene used in this study, ACTB (Actin, Beta) and TBP (TATA-binding protein) were selected based on internal control parameters included in software. A transcript was considered as undetected when unprocessed Cq value exceeded 35.

Western blotting

PBMCs were exposed to 100 μ M BeSO₄ for 0 min, 1h, 2h, 6h and 24h and harvested for assay. Subsequently, 2 - 3 \times 10⁶ cells were lysed using RIPA (Radioimmunoprecipitation) buffer containing protease inhibitors (Santa Cruz, CA) (21). 20 μ g proteins were subjected to 10% SDS-PAGE and transferred onto PVDF membranes (Amersham Biosciences, Les Ulis, France). The membranes were probed with anti-pJAK2, p-STAT1 and total JAK 2, STAT1 antibodies. Total Jak2 and STAT1 served as a loading control. The immunoblots were detected using the ECL plus detection kit (Pierce, Rockford, IL, USA) and quantified using the Quantity One Software (Bio-Rad Laboratories, CA)(21).

CBD and Sarcoidosis overlapping genes pathway analysis and network generation

IPA was carried out with $P < 0.002$ as the cutoff point. The genes were categorized according to the molecular functions using the software. The identified genes were also mapped to genetic networks in the IPA database and ranked by a score that denotes the probability that a collection of genes equal to or greater than the number in a network could be achieved by chance alone. The genetic network analysis focuses on the functional relationships that are present in the literature to create a network of genes with similar functions and recorded interactions. A network pathway is a graphical representation of the molecular relationships between genes or gene products. Genes or gene products are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). All edges are supported by at least one reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. The intensity of the node color indicates the degree of up- (red) or down-regulation (green). Nodes are displayed using various shapes that represent the functional class of the gene product (Ingenuity® Systems, www.ingenuity.com).