GENE EXPRESSION PROFILING IN CHRONIC SPONTANEOUS URTICARIA

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BACKGROUND

Phenotypic and genotypic profiling of Chronic Spontaneous Urticaria (CSU) an IgE-associated disease still represents an unmet need[1]. Some of the mechanisms involved in mast cell activation and the role of the released mast cell mediators in CSU are well known. However the knowledge about the genetic susceptibility and genes expressed in the wheal and the non lesional skin of CSU patients remain limited.

AIM OF THE STUDY

The primary purpose of this study was to identify and characterize the gene expression profile in peripheral blood, lesional (wheal) and non lesional skin in a series of patients suffering from severe CSU.

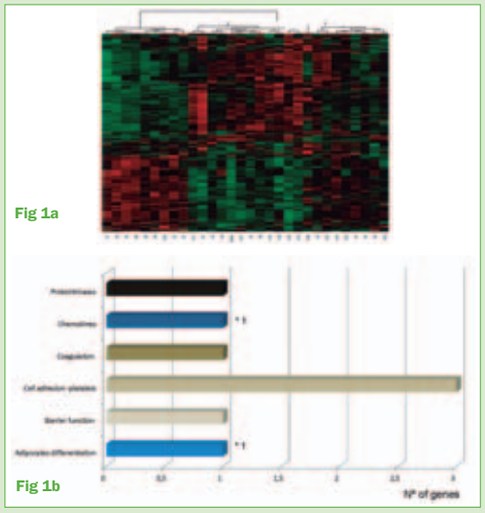
POPULATION STUDIED

Twenty patients with severe CSU and 10 healthy volunteers were included in the study. Healthy subjects showed a 1.1 ratio female: male and a mean age of 27,5 years. Patients suffering CSU were 70% female and 30% male and showed a mean age of 51.55 years. Mean duration of the disease was 5.2 years and the mean Urticaria Activity Score (UAS) in consultation was 4,8 (0-6) and the mean UAS7 was 33,6 (0-42).[2] Clinical features, therapeutic response and pathology of this sample of patients were analyzed according with the obtained genomic data.

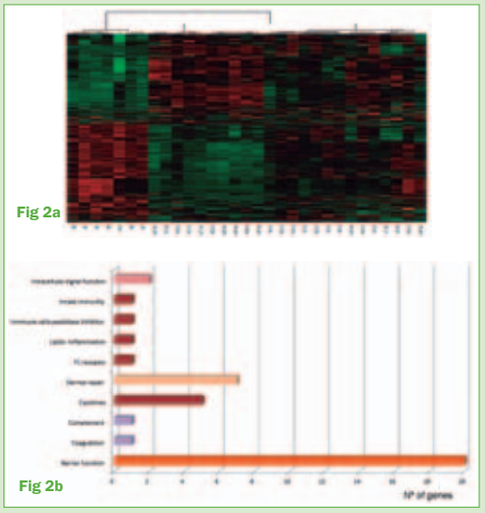
METHODOLOGY FOR THE SAMPLE ANALYSYS

- Gene expression (GE) was studied from peripheral blood, lesional (wheals) and non lesional skin in patients with CSU and from peripheral blood and normal skin samples from the control group. The following comparative analyses were performed;
  1. Peripheral blood results from CSU patients vs peripheral blood from healthy controls
  2. Non-lesional skin from CSU patients vs normal skin from healthy controls
  3. Lesional (wheal) vs non-lesional skin in CSU patients
- GE analysis using Agilent whole human genome oligomicroarrays 8x60v2 (one-color) and a bioinformatic study based in the raw data was performed. The GE comparison between CSU patients peripheral blood (n=20) and healthy controls (n=10) showed 278 transcripts differentially expressed (P adjusted value <0.05). We selected 7.729 transcripts from the paired comparison between patient wheal and patient non lesional skin (n=9). Finally, 1.213 transcripts were selected when non lesional skin from CSU patients (n=14 available) was compared with skin from healthy controls (n=6).
- Functional analysis was performed as a first step with Ingenuity Pathway Analysis (IPA) (http://www.ingenuity.com). All results lists were loaded and a core analysis performed.
- Based on technical recommendations (adjusted P value of <0.05 and a Log fold change of 1,2) and an accurate assessment of the genes up or down regulated according mast cell biology, wheal pathogenesis or urticaria knowledge, a number of genes were selected to be confirmed by quantitative real time PCR (Q-PCR). Thirteen genes from the peripheral blood comparison results, 144 from a total of 238 candidate genes selected from the wheal versus non lesional skin paired comparison results in CSU patients and 53 from a total of 57 genes from the non lesional skin results (CSU versus healthy skin), were processed by Q-PCR using Taqman Low Density Array (TLDA) platform. Up and downregulated genes were included.
- A TLDA 64 assay format was used, in which two samples per array were interrogated for the expression of 64 genes in triplicate (adjusted P value of <0.05 and a Log fold change of 1,2). This set of genes was studied not only in the same set of samples which were processed in the microarray analysis, but also in a set of independent sample. The percentage of validated genes by Q-PCR in these comparisons was a 62%, 72% and 75% respectively.

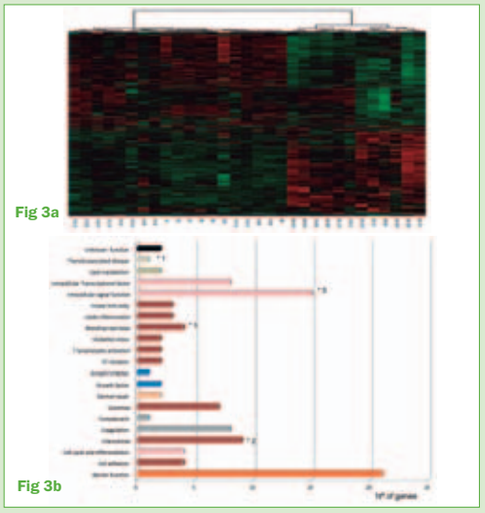
RESULTS



1. Peripheral blood in CSU patients vs peripheral blood from healthy controls (Fig 1a,b)
1.a. 278 transcripts were selected between the two conditions. Heat map showing all sample distribution according to these results. (B= healthy control; A=CSU patient)
1.b. Biological function of the 13 up and down expressed (*) genes validated by Q-PCR validation.



2. Non-lesional skin in CSU vs normal skin from healthy controls (Fig 2a,b)
2.a. 1213 transcripts were selected between the two conditions. Heat map showing all sample distribution according to these results. (B= healthy control;A-S = non lesional ; A-W = wheal)
2.b. Biological function of the 40 upregulated genes validated by Q-PCR.



3. Lesional skin (wheal) vs non-lesional skin in CSU patients
3.a. 7729 transcripts were selected between the two conditions. Heat map showing all sample distribution according to these results. (B= healthy control;A-S = non lesional; A-W = wheal)
3.b. Biological function of 103 upregulated and down expressed (*) genes validated by Q-PCR.

DISCUSSION - CONCLUSION

- The results obtained from this study are very robust and consistent according to the strict requirements of the methodology.
- Peripheral blood samples from patients suffering from severe CSU show several up regulated genes involved e.g. in platelet and endothelial adhesion or down-regulated involved e.g. in adipocyte differentiation.
- Non lesional skin from patients suffering from CSU shows a permanent inflammatory status where the most common up regulated genes are involved in the recovery of epidermal barrier, inflammation markers and dermal repair.
- The paired comparison between lesional (wheal) and non lesional skin in patients with CSU shows a broad spectrum of genes mainly involved in the inflammatory response that would need an individualized confirmation.
- A functional analysis of the genes over or under expressed according with the clinical characteristics, pathological findings, clinical course of the disease and response to the treatments was also performed.

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The authors would like to thank Novartis Pharma for the Research Grant that support part of this study.

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