



Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.intl.elsevierhealth.com/journals/jods

Letter to the Editor

Dysregulated genes of extrinsic type of atopic dermatitis: 34K microarray and interactomic analyses

ARTICLE INFO

Keywords:

Atopic dermatitis

Extrinsic type

Interactomics

Microarray

Protein–protein interaction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease found in children and adults, and it is characterized by severe itching with typical skin lesions. The extrinsic type of AD (Ade) has a Th2 cell mediated high serum IgE level and this condition is associated with IgE-mediated sensitization; this is seen in 70–80% of AD patients. In this study, we focused on the Ade type by conducting a large-scale oligochip microarray (34K). We corroborated the microarray results with the protein–protein interaction (PPI) mapping to predict the genes related with Ade functioning as interaction hubs.

The Ade lesional skin (eczematous, lichenification and pruritic excoriation lesions) samples ($n = 30$) were collected from patients according to our previous reports [1]. Non-atopic normal control subjects ($n = 10$), who had neither a personal/family history nor signs of atopic diseases, had their samples selected from the skin that was removed via plastic surgery. The mean values for each group's total IgE level, the eosinophilia count, SCORAD score and the patients' age were 1739 U/mL, 625 μ L, 43.84 and 21.1 years old for the Ade group, respectively. The gender of all Ade samples was male. The total RNA preparation, labeling, hybridization, and analysis were conducted based on previous reports [2]. The GenePloer™ Human-34K chip (Digital Genomics, Seoul, Korea) was hybridized with a mixture of fluorescently labeled cDNAs from the control and Ade samples. As a result of SAM (more than a twofold intensity change with a 5% q value cutoff), 228 candidate genes were found in which 97 genes were up-regulated and 131 genes were down-regulated in Ade patients (Fig. 1a). The genes obtained from the microarray were classified into several groups according to the functions of the genes (supplementary Table 1). We performed RT-PCRs for making the qualitative compar-

isons of the expression levels (Fig. 1b). As a result, FcεRI, a marker of Ade disease, was detected to be significantly up-regulated. FABP7 and CPA3 were up-regulated, while ARSF, AREG, GJB2, GLTP, HAS3 and HIST4H4 were down-regulated in the Ade patients. These results matched to the results of the microarray data.

Next, we adapted bioinformatic tools such as PPI mapping, KEGG pathway and SNPs analyses to predict several important genes from the pools of candidate genes. The PPI prediction employs protein structural interactome MAP (PSIMAP) and protein experimental interactome MAP (PEIMAP) [3–5]. We used 205 candidate genes in supplementary Table 1 to conduct PPI prediction. We found that 104 genes had 458 interacting partners altogether (each gene had on average eight interacting partners). The hub genes for Ade (as shown in Fig. 2) that had high interaction scores: (1) ECM and cell adhesion-related genes (TNC, VCAM1, CXADR, CEACAM5 and COL12A1), (2) ATP/GTP binding genes (NTRK2), (3) immune response genes (FcεRI, CTSG and CD200R1), (4) proteolytic enzymes (KLK4, KLK6, KLK8, KLK14, TMPRSS11D and PLAT), and (5) structural genes (CDON and ACTG2). We also analyzed biochemical pathways for the up- and down-regulated genes, which were assigned to various annotations in KEGG pathways (supplementary Table 3). Physiochemical property predictions on the 205 candidate genes showed that some genes contained membrane domains. These were IFI27 (Interferon), MAL (T-cell differentiation protein), BENE (unknown), and GPR172B (unknown). We noted that BENE and GPR172B genes are not yet functionally annotated, and they may be important candidate genes (supplementary Table 4). Furthermore, we searched for SNPs in promoters, coding regions, and splice-sites in order to detect genetic variations on candidate genes. We selected the one-kilo base upstream region of each gene to be used for the search of SNPs using the promoter information given by TRANSPAC 8.4 and SNP@Promoter databases. For other non-synonymous SNPs for coding region and splice-sites, dbSNP version 126 was used. Of the hub genes, TNC and CEACAM5 had the greatest number of non-synonymous SNPs. Also, SNPs (rs12459365 and rs11671058) in the KLK6 gene are known to be related to AD from GAD database, and SNPs (rs2781664 and rs2781663) in ARG1 are also known to be related to AD from the HGMD database. Therefore, AD specific SNPs can be useful for analyzing differentially altered genes as candidates of AD genetic markers (supplementary Table 5).

Our results mainly focused on profiling the Ade associated genes in a large scale DNA chip array, but do not include any detailed functional study. With DNA microarray, although we can obtain a great deal of genes that are overexpressed or down-regulated in AD disease, it is very difficult to choose the most important genes. Furthermore, it is hard to explain the meaning of those proteins. In this regard, interactomic tools such as performing PPI, KEGG, and SNP mapping may help to find the

Abbreviations: AD, atopic dermatitis; Ade, extrinsic type of AD; SAM, significance analysis of microarray; ECM, extracellular matrix; PPI, protein–protein interactions; PSIMAP, protein structural interactome MAP; PEIMAP, protein experimental interactome MAP.

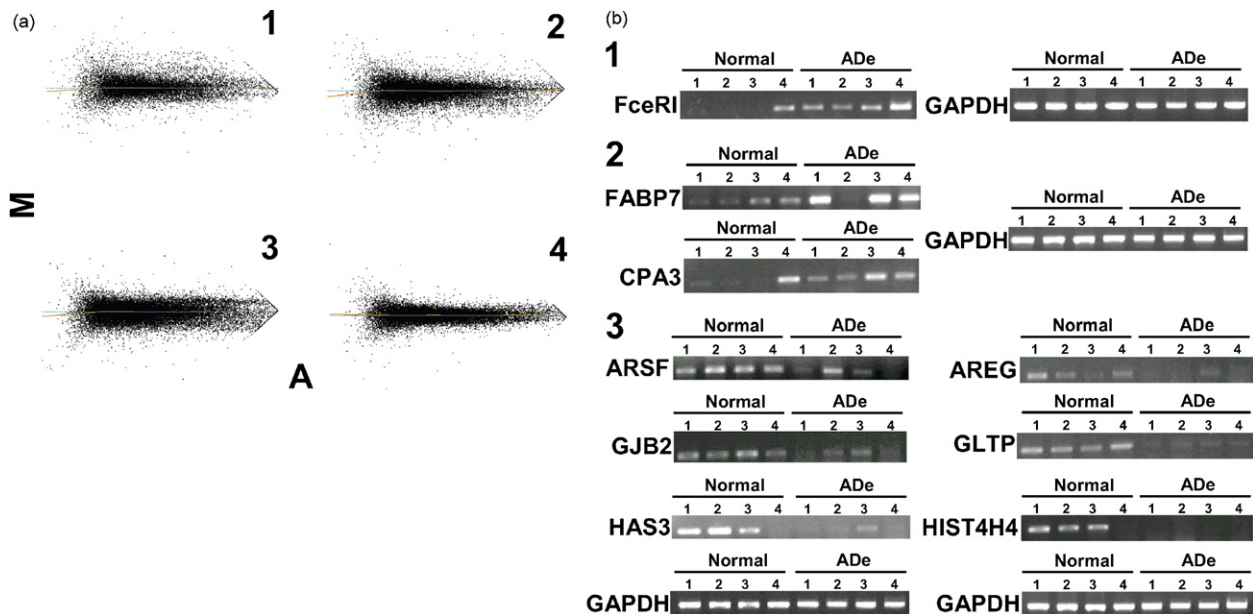


Fig. 1. cDNA microarray of ADe patients and validation by RT-PCR. (a) Plot of M vs. A. Labels 1–3, patient-pooling groups vs. the normal pooling group. Label 4, patient-pooling group 1 vs. the patient-pooling group 2. To minimize the patient-to-patient variations, candidate genes selected from (1) to (3) were reaffirmed by subtraction with the result of (4), in which the up-regulated and down-regulated candidates (more than twofold) were regarded as false positives. (b) RT-PCR results. PCRs were conducted with randomized pairing. The designed primers are listed in a [supplementary Table 2](#).

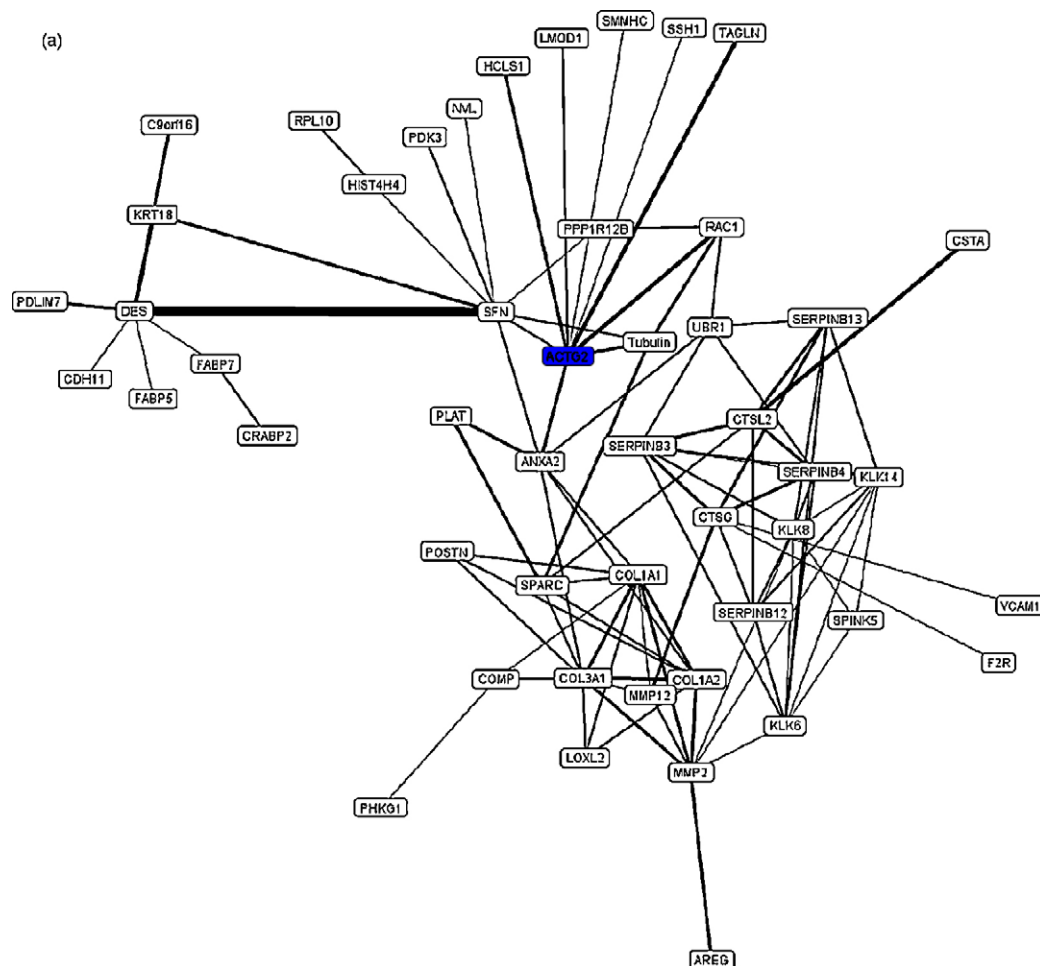


Fig. 2. PPI mappings: (a) PEIMAP; (b) PSIMAP; (c) overlapping map of both PEIMAP and PSIMAP. Labels with red and blue indicate the hub proteins. Red proteins are defined as proteins that interact with more than 25% of the total proteins in the network. Blue ones are proteins that interact with the number of protein between 20% and 25% of the total proteins in the network. Also, the thickness of the edge between two proteins represents the number of reference databases.

(b)

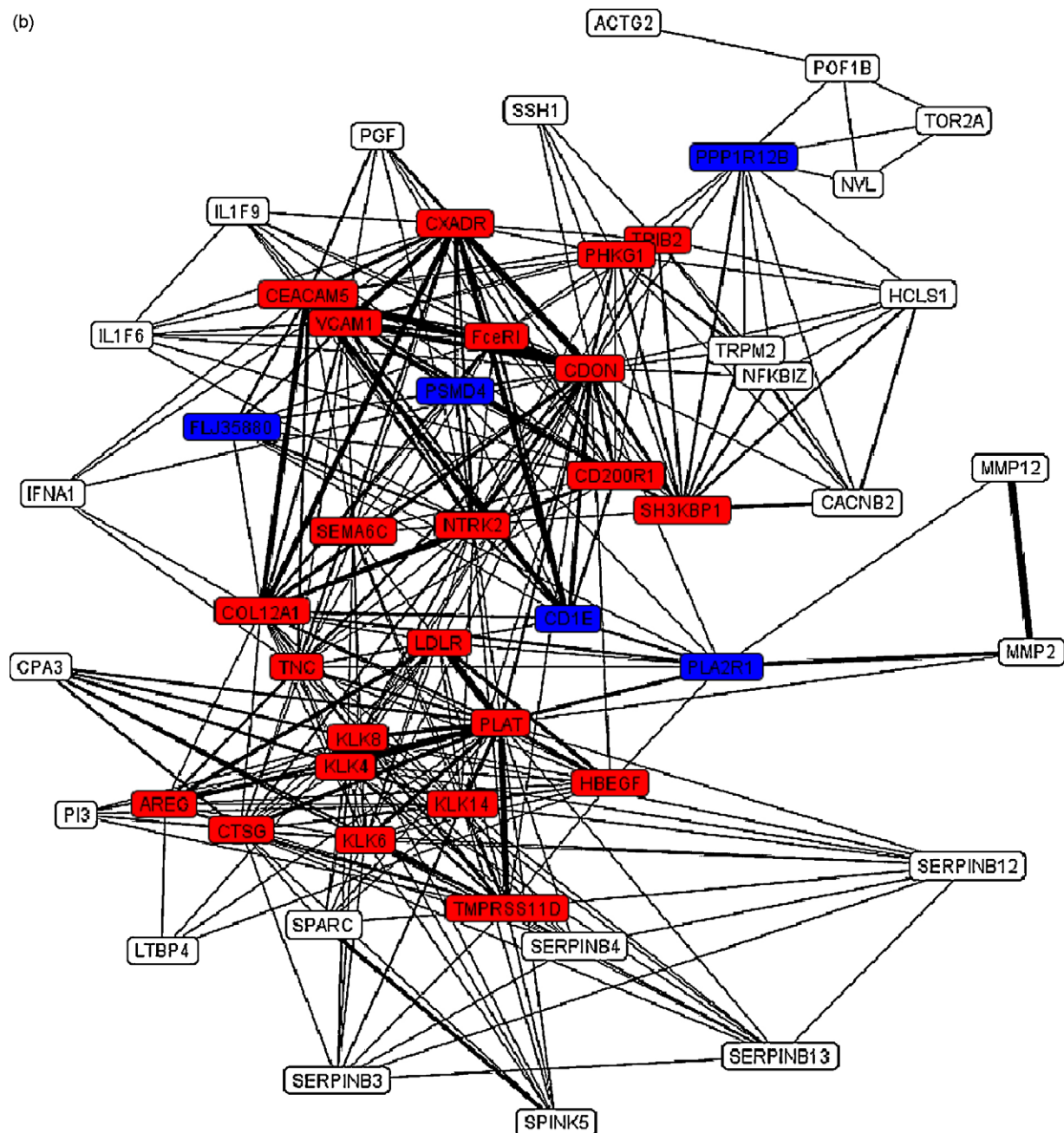


Fig. 2. (Continued).

core proteins among the detected candidate genes [6]. Since the next step of functional studies is a time- and cost-consuming process, the number of target proteins must be limited; hence, to make the right choice, computational prediction on the basis of microarray results could be critical. The candidate genes listed in

the PPI maps may provide important information to piece together the complex nature of AD disease. The computational prediction of interacting partners of the detected genes using PPI mapping in this study can be useful for conducting further functional studies of AD.

(c)

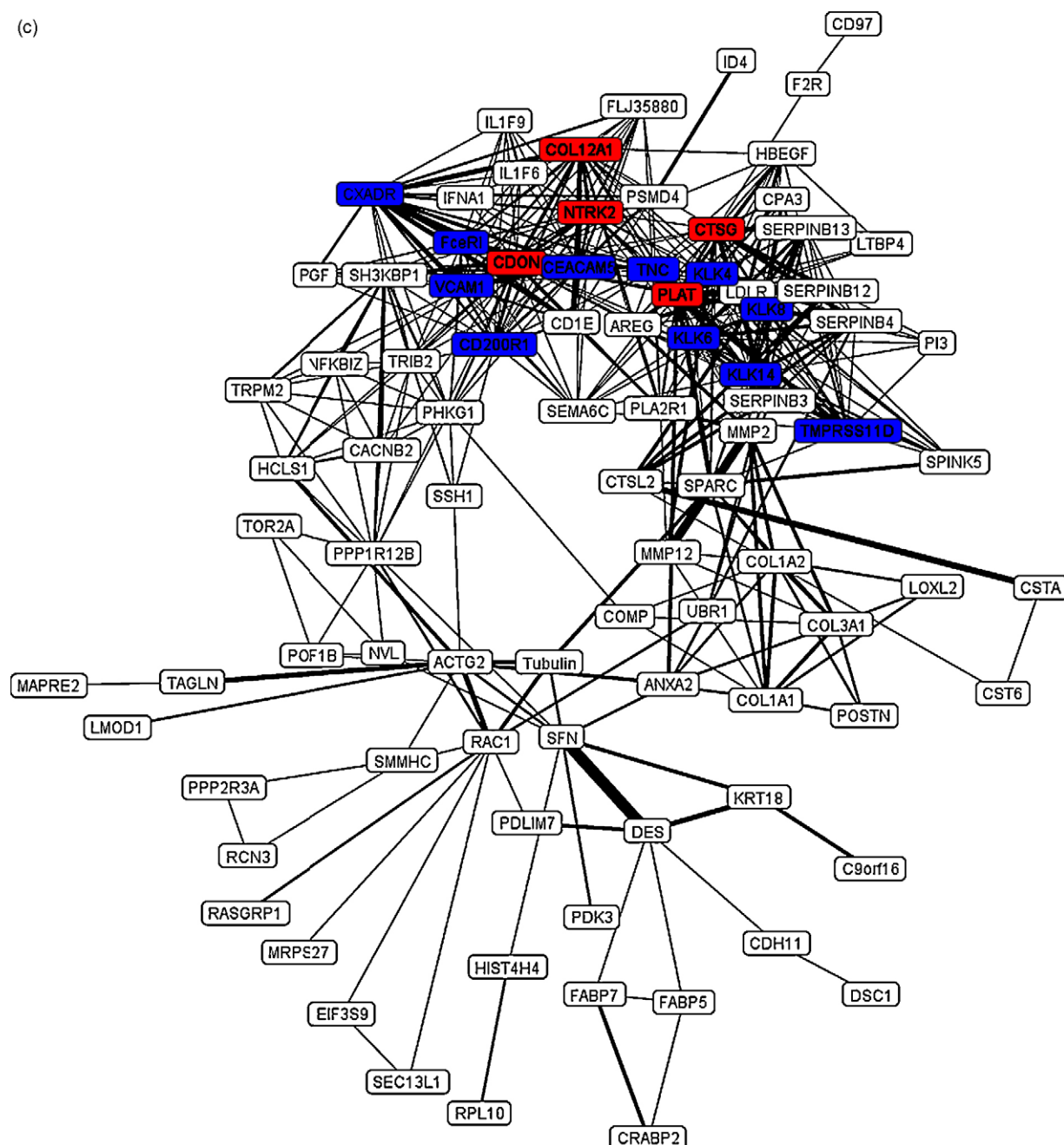


Fig. 2. (Continued).

Acknowledgements

This study was supported by the grants of the Korea Health 21 R&D Project (Ministry of Health, Welfare and Family Affairs, Republic of Korea, 01-PJ3-PG6-01GN12-0001 and A030003). Dr. Jong Bhak was supported by a grant from the KRIBB Research Initiative Program of Korea. Dr. Fei Zou was supported by a grant from the National Basic Research Program of China (No. 2006CB504100).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jdermsci.2008.08.004](https://doi.org/10.1016/j.jdermsci.2008.08.004).

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Zhi-Rong Lü¹
Yong-Doo Park
Fei Zou*

*Department of Environmental Health,
School of Public Health and Tropical Medicine,
Southern Medical University,
Guangzhou 510515, PR China*

Tae-Ho Park¹
Kea-Jeung Kim
*Department of Dermatology, Kangbuk Samsung Hospital,
Seoul, Republic of Korea*

Eil-Soo Lee
Jun-Mo Yang**
*Department of Dermatology,
Sungkyunkwan University School of Medicine,
Samsung Medical Center, Seoul, Republic of Korea*

Daeui Park
Byoung-Chul Kim
Seong-Woong Cho
Jong Bhak
*Korean BioInformation Center (KOBIC),
KRIBB, Daejeon, Republic of Korea*

*Corresponding author. Tel.: +86 2061648301; fax: +86 2061648324
E-mail address: zoufei_dean@hotmail.com
(F. Zou)

**Corresponding author. Tel.: +822 3410 3541; fax: +822 3410 3869
E-mail address: junmo.yang@samsung.com
(J.-M. Yang)

These authors are contributed equally to this study.

5 May 2008