Differential expression of microRNAs and their possible roles in patients with chronic idiopathic urticaria and active hives

Ching-Kow E. Lin, Ph.D.,¹ John S. Kaptein, Ph.D.,¹ and Javed Sheikh, M.D., F.A.A.A.A.I.²

ABSTRACT

Background: Chronic idiopathic urticaria (CIU) is a complicated skin disease with unknown pathophysiology. MicroRNAs (miRNA) have been shown to be active in cellular regulation. The goal of this pilot study was to examine whether miRNAs may be involved in the regulation of CIU or as biomarkers for CIU.

Methods: Four groups of three patients each were selected: patients with either active hives or no hives and with positive or negative chronic urticaria (CU) index results. MiRNAs were isolated from patient plasma and analyzed by using miRNA microarray technology to determine the amount of each of the 2567 known human miRNAs.

Results: A total of 16 miRNAs were found to be differentially expressed in patients with active hives. Among them, five (2355–3p, 4264, 2355–5p, 29c–5p, and 361–3p) were significantly increased in samples with positive CU index results, which could be useful biomarkers for patients with chronic autoimmune urticaria. The miRNA data bases were used to find the targets of these selected miRNA sequences. These potential targets were then compared against a list of 154 urticaria-related genes. Twenty-five genes were found to match. These included eight that were significantly downregulated and eight that were significantly upregulated; however, seven of the eight downregulated genes (FBXL20, OPHN1, YPEL2, STARD9, EZH1, KLHL24, ING4) and five of the eight upregulated genes (BYSL, PNO1, ADAMTS9, STEAP4, SRGN) have no reported roles in signaling. For the 13 genes with reported roles in signaling, the following pathways were found: transforming growth factor beta signaling pathway (NR3C1, KITLG, THBS1), glucocorticoid receptor signaling pathway (NR3C1, SELE, CCL2), p53 signaling pathway (CCNG2, THBS1, CCL2), p21-activated kinase pathway (PAK1IP1, KITLG, CCL2), phosphoinositol-3 kinase protein kinase B signaling pathway (NRC31, KITLG, THBS1, CCL2), and neuroactive ligand-receptor interaction (NRC31, HRH1, CHRM), which could play important roles in CIU.

Conclusion: A better understanding of those genes with undefined function and simultaneous quantitation of both miRNAs and messenger RNAs are needed to fully understand CIU disease.

(Allergy Rhinol 8:e67–e80, 2017; doi: 10.2500/ar.2017.8.0199)
Helicobacter pylori infection to CIU, but it has not been substantiated. Alterations in miRNA expression have been reported to be associated with several diseases, including asthma and allergic rhinitis, but it is unknown in CIU. A recent review indicates that miRNAs may be involved in skin disorders by influencing regulatory mechanisms of inflammation. In this study, we investigated whether the anticipated differential expression of miRNAs in CIU are similar or different from that shown in allergy and asthma.

Differential expression of miRNAs in CIU are similar or different from that shown in allergy and asthma. It is unknown in CIU whether the anticipated regulatory mechanisms of inflammation. miRNAs may be involved in skin disorders by influencing regulatory mechanisms of inflammation. In this study, we investigated whether the anticipated differential expression of miRNAs in CIU are similar or different from that shown in allergy and asthma.

After identifying the differentially expressed (DE) miRNAs in the patients with CIU and with active hives, the next approach was to find out which miRNAs could be affected by these miRNAs. We relied on miRNA data bases to identify potential targets.

Next, for validation of these potential miRNA targets, we compared them with reported urticaria-related genes. From “Gene expression profiles in chronic idiopathic (spontaneous) urticaria,” by Patel et al., and other sources, we created an urticaria gene panel, which consisted of 154 genes. We then compared these two gene groups with identified candidate miRNAs in CIU during active hives. Also, these selected candidate genes were subjected to several online gene function search engines such as the KEGG (Kanehisa Laboratories, Kyoto, Japan) gene pathway database and PathCards (Weizmann Institute of Science, Rehovot, Israel) to explore what pathways and cellular functions could be involved in CIU with active hives.

METHODS

Patients were recruited, during office visits, as part of a CIU study approved by Kaiser Permanente Institutional Review Board. Hive status was diagnosed at the time. Patients consented to have additional blood drawn for a chronic urticaria (CU) index test (a functional anti-FcεR test that supports an autoimmune basis for the disease) and other tests for further investigation. Subjects with no active hives and with negative CU index were invited to participate in the study as volunteers. The patients were divided into four groups, those with (1) active hives and with positive CU index results; (2) active hives and with a negative CU index; (3) a positive CU index but no active hives; and (4) normal, negative CU index and with no active hives. Three samples from each of the four groups were used in this pilot study. The plasma was separated from blood samples and kept frozen at −70°C. Isolation of miRNAs was performed by using Qiagen miRNeasy serum/plasma kit (Qiagen, Germantown, MD) in an RNase free environment. The isolated miRNAs were quantitated by using a Qubit microRNA assay kit (Molecular Probes by Life Technologies, Eugene, OR). The miRNA samples were stored at −70°C immediately and were processed by LC Sciences, LLC (Houston, TX) by using microarray technology for quantitation of the 2567 known human miRNAs.

Relative levels of miRNAs between the hive and the nonhive groups or among the four different groups of patients were determined and analyzed by using analysis of variance. DE miRNAs were selected based on the significance of the p value and at least twofold changes in the group mean. The potential targets of these identified miRNA sequences were then investigated by using various data bases, such as TargetScanHuman (Whitehead Institute for Biomedical Research, Cambridge, MA) and miRDB (Washington University, School of Medicine, St. Louis, MO). In general, hundreds to thousands of potential targets were proposed for each miRNA. Therefore, we used a more stringent selection criterion, such as three or more selected miRNAs that target a certain mRNA to consider it as a likely target. These potential targets were further narrowed to known CIU-related genes. Based on the report by Patel et al. of gene expression profiles in CIU that identified significant upregulation of 506 genes and downregulation of 51 genes in the CIU lesions and other online resources, we were able to generate a highly reliable urticaria-related gene panel, which consisted of 154 genes.

Finally, by matching these miRNA targets and urticaria-related genes, a candidate gene set for CIU in active hives was found. These candidate genes were then subjected to online search engines such as KEGG pathway and PathCards to explore what pathways and cellular functions could be involved in CIU with active hives. In addition, the following three application programs were evaluated: (1) Diana Tool’s mirPath v.3 (Diana Lab, Athens, Greece), which allows direct pathways search from a set of miRNAs; (2) Qiagen’s Ingenuity Pathway Analysis (IPA), which uses its miRNA target filter to analyze miRNA data, prioritize mRNA targets, and examine miRNA–mRNA pairings to explore canonical pathways; and (3) Advaita Bioinformatics iPathwayGuide (Advaita Bioinformatics, Plymouth, MI), which performs meta-analyses of genes for predicted miRNA, pathways, and gene ontology (GO) analysis, to simulate the connection between the selected miRNAs, mRNAs, and their downstream effects on signal pathways and cellular functions. This approach may provide useful information that links miRNAs to possible causes or effects in CIU.

RESULTS

DE miRNAs During Active Hives

Our primary goal was to identify which miRNAs in circulating blood were DE in patients with CIU and with active hives. Twelve subjects were included in the study,
divided into four groups as described in the Methods section. The demographic details of these patients are shown in Table 1. DE miRNAs could be useful biomarkers for patients with active hives. To evaluate this, we compared blood samples from these 12 patients by using a microarray platform against the latest 2567 known mature human miRNAs. The relative miRNA level results are illustrated in the heat map in Fig. 1, which shows that miR-4649–5p and miR-6769a-5p differed the most between patients with hives and patients without hives with a \( p \) value of \( <0.01 \). We further analyzed these data by using analysis of variance to take the actual fold changes into consideration.

Twelve DE miRNAs were found to have at least twofold changes in miRNA expression between active hives and no hives. Among these, only miR-6769–5p showed an increase of more than twofold, whereas the other 11 miRNAs all showed decreases of more than twofold, as shown in Table 2. This was somewhat unexpected because, for miRNAs to act as inhibitors of mRNA function, these would have been expected to be increased in patients with CIU and with hives. Next, we compared the differential expression of miRNAs among the four groups to specifically evaluate the active hives with a positive CU index (i.e., CAU) group. When this CAU group was compared with the other groups, we found five DE miRNAs: 2355–3p, 2355–5p, 4264, 29c-5p, and 361–3p were all increased between 3- to 10-fold in mean values, as listed in Table 3. This result implied that genes targeted by these five miRNAs could be greatly inhibited and that they could be useful as biomarkers for the pathophysiology of CAU.\(^{29}\)

Potential mRNA Targets by the Selected DE miRNAs

Next, we investigated potential genes targeted by the miRNAs characteristic of active hives. Several miRNA target data bases, such as TargetScanHuman 7.1, TarBase, Diana-microT-CD, miRDB, were found by using a Wikipedia (The Free Encyclopedia, Wikimedia Foundation, San Francisco, CA) search.\(^{30}\) The number of potential targets identified by each databases differed, as shown in Table 4. We elected to use the TargetScanHuman 7.1 miRNA data base because it was used by Qiagen’s IPA simulation program and because it shows the largest number of possible targets for each miRNA. Because miRNA data bases predict targets based on sequence alone, there is some element of chance involved. To avoid this pitfall, we decided to consider only those potential targets that were reported to be related to CIU, as shown in Table 5, with the gene names and references listed for those matched gene symbols.

For each of the selected 16 DE miRNAs, we first created a gene set of potential targets found in the TargetScanHuman 7.1 miRNA data base because it was used by Qiagen’s IPA simulation program and because it shows the largest number of possible targets for each miRNA. Because miRNA data bases predict targets based on sequence alone, there is some element of chance involved. To avoid this pitfall, we decided to consider only those potential targets that were reported to be related to CIU, as shown in Table 5, with the gene names and references listed for those matched gene symbols.

For each of the selected 16 DE miRNAs, we first created a gene set of potential targets found in the TargetScanHuman 7.1 miRNA data base.\(^{19}\) We then compared them with the 154 urticaria-related genes. The matched genes, in rank order by the miRNA data base, are shown in Table 6. To weigh the importance of these selected targets, we counted the number of times that these genes were targeted by the selected DE miRNAs. The result is shown in Fig. 2. Among them, FBXL20 shows the highest count and is targeted by 13 of the 16 DE miRNAs, followed by EZN1 and OPHN1 as targets of 9 of the 16 DE miRNAs.

---

**Table 1 Demographics of patients with CIU**

| Patient Identifier | Age, y | Sex | Active Hives | CU Index (Fcβ antibody) | Response to Antihistamines | Atopy* | Group Notation |
|--------------------|-------|-----|--------------|-------------------------|-----------------------------|-------|---------------|
| 1                  | 40    | F   | Y            | 19 (H)                  | Y (high dose)               | N     | A1            |
| 2                  | 39    | F   | Y            | 13.3 (H)                | Y (high dose)               | Y     | A2            |
| 3                  | 41    | M   | Y            | >50 (H)                 |                             | N     | A3            |
| 4                  | 21    | M   | Y            | <2.0 (N)                | Y (high dose)               | Y     | B1            |
| 5                  | 33    | M   | Y            | <2.0 (N)                | Y                            | N     | B2            |
| 6                  | 20    | M   | Y            | <2.0 (N)                | Y                            | Y     | B3            |
| 7                  | 56    | M   | N            | 10.5 (H)                | Y                            | Y     | C1            |
| 8                  | 29    | F   | N            | 42 (H)                  | Y (high dose)               | N     | C2            |
| 9                  | 42    | M   | N            | 22.2 (H)                | Y                            | N     | C3            |
| 10                 | 66    | M   | N            | 6.2 (N)                 | N/A                         | Y     | D1            |
| 11                 | 61    | M   | N            | <2.0 (N)                | N/A                         | N     | D2            |
| 12                 | 29    | M   | N            | 4.8 (N)                 | N/A                         | N     | D3            |

CIU = Chronic idiopathic urticaria; CU = chronic urticaria; Fcβ = Fc region of immunoglobulin E; Y = yes; (H) = high; N = no; (N) = normal; N/A = not available; A1, A2, A3 = subjects 1, 2, and 3 in group A; B1, B2, B3 = subjects 1, 2, and 3 in group B; C1, C2, C3 = subjects 1, 2, and 3 in group C; D1, D2, D3 = subjects 1, 2, and 3 in group D.

*Indicates patients who had a history of either allergic rhinitis or food allergy with a previous positive allergy testing result.
These three genes, along with five other genes (CCNG2, YPEL2, KLHL24, STARD9, and ING4), which were targeted by at least three different DE miRNAs, were reported as the most differentially downregulated genes in CIU. These three genes (BYSL, THBS1, and PNO1) were found to be targeted by seven DE miRNAs, followed by five other genes (ADAMTS9, STEAP4, CCL2, SELE, and SRGN), which were targeted by at least three different DE miRNAs were reported by Patel et al. as the most differentially expressed genes.
### Table 2  Differential expression of miRNAs between patients with active hives and patients negative for hives by ANOVA analysis

| hsa-miR          | p Value   | Positive for Hives, mean ± SD | Negative for Hives, mean ± SD | Fold Changes       |
|------------------|-----------|-------------------------------|--------------------------------|--------------------|
| miR-6769a-5p     | 0.005454  | 17.91 ± 8.82                  | 5.75 ± 4.25                    | 3.12-fold increase |
| miR-3691-3p      | 0.000903  | 1.97 ± 1.47                   | 9.34 ± 3.59                    | 4.75-fold decrease |
| miR-1184         | 0.007058  | 2.85 ± 2.79                   | 12.28 ± 6.25                   | 4.31-fold decrease |
| miR-302c-5p      | 0.009217  | 3.16 ± 4.04                   | 12.42 ± 5.94                   | 3.93-fold decrease |
| miR-6799-3p      | 0.002512  | 4.35 ± 5.32                   | 16.88 ± 5.51                   | 3.88-fold decrease |
| miR-4733-5p      | 0.007494  | 4.56 ± 4.53                   | 13.51 ± 4.75                   | 2.97-fold decrease |
| miR-205-5p       | 0.007383  | 9.06 ± 9.65                   | 25.28 ± 6.89                   | 2.79-fold decrease |
| miR-3187-3p      | 0.003867  | 10.59 ± 9.16                  | 28.71 ± 7.56                   | 2.71-fold decrease |
| miR-6800-3p      | 0.006485  | 8.40 ± 6.24                   | 19.18 ± 4.52                   | 2.28-fold decrease |
| miR-3180         | 0.003655  | 11.46 ± 6.09                  | 25.31 ± 6.62                   | 2.21-fold decrease |
| miR-4649-5p      | 0.007991  | 21.71 ± 9.48                  | 46.71 ± 17.25                  | 2.15-fold decrease |
| miR-1910-5p      | 0.007885  | 11.75 ± 6.10                  | 23.73 ± 6.43                   | 2.02-fold decrease |

miRNA = MicroRNA; ANOVA = analysis of variance; hsa-miR = Homo sapiens related miRNA; SD = standard deviation.

---

### Table 3  Differential expression of miRNAs in different groups of patients by ANOVA analysis

| hsa-miR          | p Value   | Active Hives with Positive CU Index, mean ± SD | Active Hives but Negative CU Index, mean ± SD | Negative for Hives with Positive CU Index, mean ± SD | Negative for Hives and Negative CU Index, mean ± SD | Group Specificity |
|------------------|-----------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------------|---------------------------------------------------|-------------------|
| miR-2355-3p      | 0.000245  | 54.45 ± 9.76                                  | 5.56 ± 9.62                                   | 5.93 ± 7.65                                       | 6.28 ± 7.17                                       | A*                |
| miR-4264         | 0.001642  | 11.00 ± 1.30                                  | 1.53 ± 1.78                                   | 1.84 ± 1.87                                       | 2.54 ± 3.12                                       | A*                |
| miR-2355-5p      | 0.006449  | 12.37 ± 3.04                                  | 1.66 ± 2.69                                   | 3.11 ± 4.27                                       | 1.25 ± 1.63                                       | A*                |
| miR-29c-5p       | 0.006870  | 131.15 ± 25.91                                | 29.31 ± 30.52                                 | 45.55 ± 32.63                                     | 36.62 ± 21.52                                     | A*                |
| miR-361-3p       | 0.015906  | 96.48 ± 33.88                                 | 33.41 ± 17.60                                 | 33.80 ± 18.23                                     | 32.07 ± 9.30                                     | A*                |
| miR-549a         | 0.000022  | 1.40 ± 2.41                                   | 12.87 ± 1.71                                  | 0.18 ± 0.29                                       | 0.58 ± 0.93                                       | B#                |
| miR-99b-3p       | 0.001337  | 14.49 ± 9.52                                  | 71.61 ± 19.93                                 | 14.68 ± 10.43                                     | 26.33 ± 3.45                                     | B#                |
| miR-548bb-3p     | 0.001470  | 0.02 ± 0.02                                   | 5.20 ± 2.33                                   | 0.11 ± 0.10                                       | 0.20 ± 0.27                                       | B#                |
| miR-939-3p       | 0.001582  | 3.34 ± 4.48                                   | 33.40 ± 8.01                                  | 15.85 ± 7.05                                       | 12.63 ± 1.88                                     | B#                |
| miR-377-5p       | 0.001766  | 13.12 ± 4.30                                  | 29.73 ± 1.65                                  | 10.63 ± 4.91                                       | 9.72 ± 5.84                                       | B#                |
| miR-379-3p       | 0.004437  | 8.23 ± 7.85                                   | 24.61 ± 6.29                                  | 7.29 ± 4.03                                       | 1.30 ± 1.61                                       | B#                |
| miR-4764-3p      | 0.000153  | 25.40 ± 16.60                                 | 14.68 ± 6.96                                  | 118.90 ± 16.51                                    | 31.57 ± 20.63                                     | C§                |
| miR-1305         | 0.000055  | 0.47 ± 0.77                                   | 0.28 ± 0.48                                   | 2.16 ± 2.39                                       | 12.32 ± 2.11                                     | D¶                |
| miR-8061         | 0.000069  | 0.56 ± 0.93                                   | 1.04 ± 1.34                                   | 0.01 ± 0.01                                       | 6.49 ± 0.75                                       | D¶                |
| miR-3163         | 0.000159  | 0.13 ± 0.16                                   | 0.01 ± 0.01                                   | 1.07 ± 1.84                                       | 10.57 ± 2.88                                     | D¶                |
| miR-1304-5p      | 0.000539  | 3.79 ± 3.41                                   | 1.16 ± 1.61                                   | 4.04 ± 2.15                                       | 17.10 ± 3.70                                     | D¶                |
| miR-1183         | 0.002170  | 5.61 ± 3.86                                   | 1.81 ± 2.38                                   | 3.69 ± 4.39                                       | 19.83 ± 4.98                                     | D¶                |
| miR-567          | 0.002564  | 1.70 ± 0.88                                   | 0.36 ± 0.63                                   | 1.30 ± 1.05                                       | 7.64 ± 1.12                                       | D¶                |
| miR-524-5p       | 0.002971  | 2.26 ± 3.56                                   | 1.09 ± 1.75                                   | 3.74 ± 4.07                                       | 13.23 ± 0.36                                     | D¶                |
| miR-1307-3p      | 0.004398  | 572.20 ± 108.67                               | 783.22 ± 173.33                               | 454.35 ± 213.08                                   | 1732.38 ± 563.17                                  | D¶                |
| miR-3187-5p      | 0.006410  | 9.13 ± 6.02                                   | 4.45 ± 4.93                                   | 9.84 ± 3.99                                       | 25.65 ± 6.27                                     | D¶                |
| miR-1237-5p      | 0.009264  | 68.33 ± 47.49                                 | 70.90 ± 11.76                                 | 82.33 ± 25.84                                     | 175.67 ± 31.38                                    | D¶                |

miRNA = MicroRNA; ANOVA = analysis of variance; hsa-miR = Homo sapiens related miRNA; CU = chronic urticaria; SD = standard deviation.

*Active hives, positive CU index.

#Active hives, negative CU index.

§Negative for hives, positive CU index.

¶Normal, negative for hives and negative CU index.
upregulated genes in CIU. In addition, 11 genes not known to be DE in CIU were also targeted by at least three different DE miRNAs. In all, we found 25 (when considering \( CHRM1, CHRM2, CHRM3 \) as one \( CHRM \) family) of 49 targets as common targets by three or more selected DE miRNAs. These were considered as essential CIU candidate genes in active hives.

**Role of Candidate Genes Targeted by the Selected DE miRNAs in Signal Pathways and Cellular Functions**

With having found these 25 candidate genes for active hives, the question arises regarding what their roles are in signal pathways and cellular functions, and whether they can shed some light on understanding the mechanism of CIU. We first looked into the bioinformatics of each of these genes by using the KEGG pathway data base and the PathCards data base. Surprisingly, seven of the eight reported downregulated genes (\( FBXL20, EZH1, OPHN1, YPEL2, KLHL24, STARD9, ING4 \)) and five of the eight upregulated genes (\( BYSL, PNO1, ADAMTS9, STEAP4, SRGN \)) reported by Patel et al.\(^{21} \) have no known roles in signaling pathways and, hence, are unknown as to their subsequent cellular functions. The missing information about these 12 genes is a major setback for understanding the cause and mechanism of CIU because they are not only 12 of 16 most differentially regulated genes in CIU but, also, 9 of these 12 genes account for 18 of the most common targets by at least five or more DE miRNAs.

Only one significantly downregulated gene, \( CCNG2 \), and three significantly upregulated genes, \( THBS1,SELE, \) and \( CCL2 \), are known to be involved in certain signaling pathways that may be relevant to immune pathophysiology of CIU, which, in turn, are related to a variety of cellular functions, as shown in Table 7. However, 9 of the remaining 25 candidate genes (\( PA2G4, PAK1IP1, NR3C1, KITLG, HRH1, CHRM, MS4A2, PTGS1, SELP \)), which are uncertain of their differential expression status, are known to be associated with certain pathways, but most of their roles in cellular functions remain uncharacterized.

Because every pathway consists of many genes, enzymes, and proteins, we specifically searched for those pathways that engaged at least 3 members of these 13 candidate genes with known functions because it could
Table 5  A list of 154 CIU-related genes with gene names shown for those potential microRNA targets*

| Gene Symbol | Gene Name | Reference No. |
|-------------|-----------|---------------|
| ADAMTS9     | ADAM metalloepitidase with thrombospondin type 1 motif, 9 | 21 |
| ADRB1       | Adrenoceptor β 1 | 21 |
| ALOX5       | Arachidonate 5-Lipoxygenase | 23 |
| BYSL        | Bystin-like | 21 |
| CCL2        | Chemokine (C-C motif) ligand 2 | 21 |
| CCNG2       | Cyclin G2 | 21 |
| CD69        | CD69 molecule | 21 |
| CHRM1, 2, 3 | Cholinergic receptors, muscarinic 1, 2, 3 | 22,25 |
| CPN1        | Carboxypeptidase N, polypeptide 1 | 23 |
| DCAF6       | DD51 and CUL4 associated factor 6 | 21 |
| EZH1        | Enhancer of zeste homolog 1 (Drosophila) | 21 |
| FBXL20      | F-box and leucine-rich repeat protein 20 | 21 |
| Fcer1a      | Fc fragment of IgE receptor 1α | 23 |
| HNMT        | Histamine-N-methyltransferase | 23 |
| HRH1, H2    | Histamine receptors H1, H2 | 23,27 |
| HTR2C       | 5-Hydroxytryptamine (serotonin) receptor 2C, G protein-coupled | 21 |
| ING4        | Inhibitor of growth family, member 4 | 21 |
| ITGB2       | Integrin, β 2 (complement component 3 receptor 3 and 4 subunit) | 23 |
| KIT         | V-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog | 23 |
| Kitlg        | KIT ligand | 23 |
| KLHL24      | Kelch-like 24 (Drosophila) | 21 |
| MS4A2       | Membrane-Spanning 4-domains, subfamily A, member 2, Fcer1b | 27 |
| Mti1        | Metallothionein 1M | 21 |
| NLRP3       | NLR family, pyrin domain containing 3 | 23 |
| NR3C1       | Nuclear receptor subfamily 3, group C, member 1, glucocorticoid receptor | 22 |
| Npr2        | Natriuretic peptide receptor B/guanylate cyclase B | 21 |
| Ophn1       | Oligophrin 1 | 21 |
| Osmr        | Oncostatin M receptor | 21 |
| Pa2g4       | Proliferation-associated 2G4, 38 kDa | 21 |
| Pak1p1      | Pak1 interacting protein 1 | 21 |
| Pno1        | Partner of NOB1 homolog (Saccharomyces cerevisiae) | 21 |
| Ptgs1       | Prostaglandin-endoperoxide synthase 1 | 23 |
| S100a8      | S100 calcium binding protein A8 | 21 |
| Sele        | Selectin E | 21 |
| Selpl       | Selectin P (antigen Cd62) | 21 |
| SLC25A27    | Solute carrier family 25, member 27 | 21 |
| Srgn        | Serylglycin | 21 |
| Stard9      | StAR-related lipid transfer (START) domain containing 9 | 21 |
| Steap4      | STEAP family member 4 | 21 |
| Thbs1       | Thrombospondin 1 | 21 |
| Tnc         | Tenacin C | 21 |
| Uap1        | UDP-N-acetylglucosamine pyrophosphorylase 1 | 21 |
| Yepel2      | Yippee-like 2 (Drosophila) | 21 |

CIU = Chronic idiopathic urticaria.

*Other CIU-related genes not listed are the following: ACE, ADORA3, ATR, BCL2, CCL11 (Eotaxin), CCL13, CCL17 (TARC), CCL22 (MDC), CCL24 (Eotaxin-2), CCL26 (Eotaxin-3), CCL5 (RANTES), CCL8 (MCP-2), CCR1, CDKN1A, CH25H, CISH, CLC, CLEC4A, CMA1, CSF1, CSF2 (GM-CSF), CTS1, CYP2C9, CyshTR1, E2F4, FARS6, FGR2A, GARS, GNL3, GPR44 (CRTH2), HDAC2, HSP90AA1, HSP90, HSPA8, HSP90, HSPD1, IARS, ICAM1, ICOS, IFNAR, IFNAR2, IFNFL (IL10), IFN1, IFNGR2, IGHE, IL-12A, IL-12B, IL-13, IL-17A, IL-18, IL1R1, IL1RL1, IL-21, IL-25 (IL-17E), IL-3, IL-31, IL-33, IL-4, ILAR, IL-5, IL-9, IPO5, IRF1, JAK3, KPN1, KPN2, KPN4, KPNB1, LARS, LTRC45, MAP3K14, MARS, MARS2, MCE, MMP12, MTA2, MYC, MYD88, NFKB1, PDCC1, PMCH, PTGER4, PTGN2, PTGPI2, RAN, RANBP2, RANBP3, RNAS53, SGK1, SMARCA2, SOCS2, SOCS3, SRA1, STAT1, STAT3, TARS, TGFB1, TGFB3, TGFB1, TNFAIP6, TNFRSF1A, TNF-α, TNSF4 (OX40L), TSLP, VCAM1, WARS, YARS, and YPEL2NPR2.

boost their involvement due to possible synergic effects. Based on this consideration, we found the following pathways in transforming growth factor β (TGF-β) signaling pathway (via NRC31, KITLG, THBS1, CCL2), glucocorticoid receptor signaling pathway (NR3C1, SeL, CCL2), p53 signaling pathway...
(CCNG2, THBS1, CCL2), p21-activted kinase (PAK) pathway (PAK1IP1, KITLG, CCL2), phosphoinositide-3 kinase and protein kinase B (PI3K-Akt) signaling pathway (KITLG, CHRM, THBS1), and neuroactive ligand-receptor interaction (NRC31, HRH1, CHRM) all fit into this category and could be relevant in the pathophysiology of active hives in CIU.

In addition, we tried three pathway simulation programs:

1. Qiagen Bioinformatics IPA program. We uploaded our selected 16 DE miRNAs into the IPA program and found that the IPA automatically identified MS4A2 and HRH1 genes as the pivotal link for urticaria. This program seems to be ideal for the miRNA-mRNA analysis if both comparable differential expression results for miRNA and mRNA are available.

2. Advaita’s iPathwayGuide. This program analyzes RNA genes and predicts pathways based on the KEGG pathway data base as well as cellular function assessment based on GO biologic process analysis. We uploaded the selected 25 candidate genes and found it only returned limited information, such as SELECT and CCL2 involved in the tumor necrosis factor (TNF) signaling pathway and in the regulation of leukocyte migration. This program will be convenient to show the overall effect of the selected genes if all of them have defined roles in signal pathways and cellular functions.

3. Diana-miRPathv3.0. This program provides a free tool to directly decipher miRNA function for potential pathways and genes regulated by the selected miRNAs. We uploaded the selected five DE miRNAs specific for CAU.

Table 6  Matched candidate genes between CIU-related genes and miRNA targets for each of the selected miRNAs in active hives

| miR          | Matched Genes                                                                 |
|--------------|-------------------------------------------------------------------------------|
| miR-2355–3p  | ADAMTS9,* OPHN1,* YPEL2,* SRGN,* NLRP3,* CCNG2, CD69, CHRM3, FBXL20, NR3C1, THBS1 |
| miR-2355–5p  | SELE,* CHRM1,* BYSL,* CCL2,* STAR9,* NR3C1, FBXL20, OPHN1, KLHL24, MS4A2, PNO1  |
| miR-29c-5p   | CHRM3,* PNO1,* STAR9,* TNC                                                    |
| miR-361–3p   | OPHN1,* PA2G4,* ING4,* CCNG2,* EZHI, ITGB2, KIT, NLRP3, FBXL20, CHRM1, YPEL2, NR3C1, PTGS1 |
| miR-4264     | YPEL2,* STEAP4,* THBS1,* BYSL,* OPHN1,* FBXL20,* PTGS1, UAP1, EZHI, PNO1      |
| miR-6769a-5p | PA2G4,* HRH2,* ITGB2, CHRM2, OPHN1, EZHI, ADRB1, FBXL20, STEAP4, BYSL, PTGS1 |
| miR-1184     | THBS1,* OPHN1,* SELP,* YPEL2,* HRH1,* S100A8, CHRM5, ADAMTS9, FBXL20, EZHI, CHRM3, PTGS1 |
| miR-1910–5p  | BYSL,* ING4,* OPHN1,* FBXL20,* KITLG,* HTR2C, CHRM5, ALOX5, STAR9, KLHL24, THBS1, MS4A2, PNO1 |
| miR-205–5p   | ADAMTS9,* CHRM2,* EZHI,* SELE,* SELP,* SLC25A27, ADRB2, CHRM3, KIT, OPHN1, HRH1, KITLG, KLHL24, THBS1, NR3C1, PKI1IP1, FBXL20 |
| miR-302c-5p  | CCL2,* PAK1IP1,* MT1M, KLHL24, THBS1, ADAMTS9, YPEL2, CHRM3, ADRB1, FBXL20, CHRM2, SLC25A27, HRH1, MS4A2, SELE, STEAP4, HNMT, KITLG, CCNG2 |
| miR-3187–3p  | BYSL,* SRGN,* OPHN1,* HRH1, NPR2, NR3C1, FBXL20, CHRM3, EZHI, HRH3, HRH4, STAR9, CCNG2, CPN1, MS4A2, PTGS1 |
| miR-3691–3p  | SRGN,* CCL2,* ING4,* PA2G4,* HRH2,* KITLG,* FBXL20, ADAMTS9, CHRM3, NR3C1, CCNG2, MS4A2, PNO1 |
| miR-4649–5p  | CHRM2,* BYSL,* KLHL24,* THBS1*                                              |
| miR-4733–5p  | FBXL20,* SELP,* OSMR, DCAF6, HNMT, HTR2C, CHRM2, EZHI, KITLG, NR3C1, PANK1IP1, PNO1, TNC, CCNG2 |
| miR-6799–3p  | YPEL2,* EZHI,* STAR9,* CHRM1,* BYSL, PTGS1, CHRM3                            |
| miR-6800–3p  | EZHI,* HRH1,* PNO1,* FBXL20,* STEAP4,* NR3C1,* HRH4, PTGS1, CCNG2            |

CIU = Chronic idiopathic urticaria; miRNA = microRNA.
*Higher-ranked genes.
into the program and found that it returned 965 genes that can be targeted by these miRNAs. It also listed four KEGG pathways and 18 GO categories. Overall, this program was a good start to look for the roles of miRNAs in pathways and cellular functions based on miRNAs alone; however, without knowing their roles within specific genes related to the disease, the results will seem to be too broad to explain the actual mechanisms that define that disease.

Due to the lack of information about involvement of certain genes in pathways and because we do not have parallel mRNA differential expression results, these simulation programs could not be applied to provide answers about CIU disease in this study. Nevertheless, because of the complexity of the miRNA network, a simplified diagram is shown in Fig. 3 to illustrate the links between the DE miRNAs found in this pilot study and their candidate targets as well as between these miRNAs to their possible roles in signal pathways and cellular functions.

**DISCUSSION**

miRNA is a new player in the field of molecular biology. Since the first discovery of lin-4 in 1993,32 miRNA research has been booming and the volume of publications has increased greatly. However, although thousands of miRNAs have been found and tens of thousands of miRNA reviews have been reported, the real functions of these small noncoding miRNAs remain largely unsettled. The main reason could be in the difficulty of defining their roles directly and effectively. Nevertheless, deregulation of miRNAs were found to be associated with human diseases, e.g., miR-21 was reported in chronic lymphocytic leukemia33 and allergic diseases.14 The broad posttranscrip-
| Gene Name | Canonical Pathways in which Gene is Involved | Cellular Functions in which Gene is Involved* |
|-----------|-----------------------------------------------|-----------------------------------------------|
| CCNG2     | p53 signaling pathway§ FoxO signaling pathway§ Wnt signaling pathway§ Mitotic G1-G1/S phases§ | Cell cycle arrest, cellular senescence or apoptosis§ Cellular growth and proliferation (A)¶ |
| PA2G4     | Cell cycle: G1/S checkpoint regulation¶ | Cellular growth and proliferation (A)¶ |
| PAK11P1   | Cell cycle: G1/S checkpoint regulation¶ PAK Pathway§ | Cellular growth and proliferation (A)¶ |
| NR3C1     | Neuroactive ligand-receptor interaction§ Glucocorticoid pathway§ IL-2, 6 signaling pathways§ Proteolysis putative SUMO-1 pathway§ Nuclear receptor transcription pathway§ TGF-β signaling pathways§ SIDS susceptibility pathways§ Prolactin signaling pathway§ | |
| KITLG     | Ras signaling pathway§ Rap1 signaling pathway# Phospholipase D signaling pathway§ PI3K-Akt signaling pathway§ Cytokine-cytokine receptor interaction# Kit Receptor signaling pathway§ NF-kB family pathway§ TGF-β pathway§ Phospholipase-C pathway§ CREB pathway§ GPCR pathway§ PAK pathway§ | |
| HRH1      | Calcium signaling pathway§ Neuroactive ligand-receptor interaction§ | |
| CHRM1, 2, 3 | Calcium signaling pathway§ cAMP signaling pathway§ PI3K-Akt signaling pathway§ Neuroactive ligand-receptor interaction# | |
| THBS1     | TGF-β signaling pathway§ Rap1 signaling pathway# | Cellular growth and proliferation (A)¶ Hematologic system development and function (B)¶ |
For 4 of these 13 candidate genes, the CCNG2 gene was significantly downregulated and the 3 others (THBS1, SELE, CCL2) were significantly upregulated, as reported by Patel et al.21 CCNG2 is one of several genes, including MDM2, COP-1, PIRH-2, WIP1, SIAH1, TP73, that act as a p53 negative feedback regulator; p53 activation is induced by a number of stress signals, including DNA damage and oxidative stress. Because of its role via the p53 pathway, CCNG2 is reported to be associated with cell cycle arrest, cellular apoptosis, cellular growth, and proliferation functions. THBS1 is also involved in p53 signaling pathway; however, it plays a different role in inhibition of angiogenesis and metastasis. Upregulation of THBS1 does not seem to be in conflict with downregulation of CCNG2, which could imply collaboration in their effects on p53 activation. Also, THBS1 is also involved in the TGF-β signaling pathway, Rap1 signaling pathway, and PI3K-Akt signaling pathway. Thus, not only can an miRNA target multiple mRNAs, but a certain mRNA can also partic-

### Table 7  Continued

| Gene Name | Gene Involvement in Canonical Pathways | Gene Involvement in Cellular Functions* |
|-----------|---------------------------------------|----------------------------------------|
| p53 signaling pathway§ | | Immune cell trafficking (C)¶ |
| PI3K-Akt signaling pathway§ | | Cell to cell signaling and interaction (C)¶ |
| Ras signaling pathway§ | | Tissue development (C)¶ |
| Integrin pathway§ | | Cellular movement (D)¶ |
| SELE | Glucocorticoid receptor signaling¶ | Cellular growth and proliferation (A)¶ |
| | Cell adhesion molecules§ | Hematologic system development and function (B)¶ |
| | cGMP-PKG signaling pathway# | Immune cell trafficking (C)¶ |
| | Metabolic pathways# | Cell to cell signaling and interaction (C)¶ |
| | TNF signaling pathway§ | Tissue development (C)¶ |
| | NOD-like receptor signaling pathway# | Cellular movement (D)¶ |
| | NF-kB signaling pathway§ | Inflammatory response (D)¶ |
| | Selenium pathway§ | Regulation of leukocyte migration¶ |
| | p53 pathway§ | | |
| | IL-23 signaling pathway§ | | |
| | TWEAK pathway§ | | |
| | Toll-like receptor signaling pathway§ | | |
| | Integrin pathway§ | | |
| | TGF-β pathway§ | | |
| | PAK pathway§ | | |
| CCL2 | Hepatic fibrosis, hepatic stellate cell activation¶¶ | Cellular growth and proliferation (A)¶ |
| | Glucocorticoid receptor signaling¶ | Hematologic system development and function (B)¶ |
| | Cytokine-cytokine receptor interaction# | Immune cell trafficking (C)¶ |
| | Chemokine signaling pathway# | Cell-to-cell signaling and interaction (C)¶ |
| | TNF signaling pathway§§ | Tissue development (C)¶ |
| | NOD-like receptor signaling pathway# | Cellular movement (D)¶ |
| | NF-kB signaling pathway§§ | Inflammatory response (D)¶ |
| | Selenium pathway§ | Infectious disease (E)¶ |
| | p53 pathway§ | Regulation of leukocyte migration¶ |
| | IL-23 signaling pathway§ | | |
| | TWEAK pathway§ | | |
| | Toll-like receptor signaling pathway§ | | |
| | Integrin pathway§ | | |
| | TGF-β pathway§ | | |
| | PAK pathway§ | | |

CIU = Chronic idiopathic urticaria; NF-κB = nuclear factor kappa light chain enhancer of activated B cells.

*Other CIU related genes which also involved in this cellular function as reported by Patel (21) are: (A) ALOX5, CD69, OSMR, S100A8, SGRN, SLC25A27, TNC; (B) ALOX5, CD69, S100A8; (C)CD69, S100A8; (D) ALOX5, CD69, S100A8; (E) ALOX5, CD69, S100A8, UIAP1.

#From Ref. 24.
§From Ref. 25.
¶From Ref. 21.
||From Ref. 27.
ipate in multiple signaling pathways. Therefore, for similar reasons, as we reasoned, potential targets should be targeted by three or more selected miRNAs to be a trustworthy candidate gene, a signaling pathway should also comprise ≥3 different genes from these 13 candidate genes to be a trustworthy canonical signaling pathway. With this assumption, p53 signaling seemed to be one of the valid pathways in CIU by means of CCNG2, THBS1, and CCL2 contribution.

**Figure 3.** Simplified diagram, illustrating linkage between selected microRNAs (miRNA) and their potential target messenger RNAs (mRNA) and potential signaling pathways and cell functions. Differentially expressed miRNAs in active hives are placed together in either increased (five miRNAs as potential chronic autoimmune urticaria [CAU] biomarkers are shown in bold) or decreased boxes in the left-hand side. Likewise, the candidate target mRNAs are grouped as either down- or upregulated, or in the unknown boxes in the middle. Due to the complexity of the miRNA-mRNA network, only connections among miRNAs and the chosen candidate genes that were targets of three or more different miRNAs are shown in solid lines if they are in accordance (i.e., increased miRNA expression that corresponded to mRNAs decreased in chronic idiopathic urticaria [CIU], or decreased miRNA expression that corresponds to mRNAs increased in CIU) or in dashed lines if the status between them is unknown. Similarly, only pathways (in round boxes and italic) and possible cellular functions (in rectangular boxes) were shown if they were involved with at least three of the candidate genes with known functions.

SELE is reported to be involved in glucocorticoid receptor signaling, the cyclic 3',5'-guanosine monophosphate dependent protein kinase G signaling pathway, metabolic pathways, and TNF signaling pathway. CCL2 is also reported to be associated with glucocorticoid receptor signaling, the TNF signaling pathway, chemokine signaling pathway, nucleotide-binding, oligomerization domain-like receptor signaling pathway, NF-κB signaling pathway, p53 signaling pathway, and others.
When comparing the roles of \( \text{SELE} \) and \( \text{CCL2} \), we found that two common pathways in glucocorticoid receptor signaling and TNF signaling were shared by these two genes but with different attributes. For example, in the TNF signaling pathway, \( \text{SELE} \) is related to cell adhesion in the same group with \( \text{ICAM1}, \text{VCAM1} \); however, \( \text{CCL2} \) is related to leukocyte recruitment propelled by \( \text{CCL5}, \text{CCL20} \), and other chemokine motifs. The other nine selected essential genes (\( \text{PA2G4}, \text{PAK1IP1}, \text{NR3C1}, \text{KITLG}, \text{HRH1}, \text{CHRM1,2,3}, \text{MS4A2}, \text{PTGS1}, \text{SEL} \)) are unknown regarding their differential expression status in CIU. \( \text{PA2G4} \) and \( \text{PAK1IP1} \) were reported by Patel et al. \(^{21} \) to be involved in G1/S checkpoint regulation in cell cycle. Along with the \( \text{CCNG2} \) gene, which is also involved in mitotic G1-G1/S phases, these three genes are related to cell growth and proliferation.

Three genes (\( \text{NR3C1}, \text{HRH1}, \text{CHRM1,2,3} \)) are related to neuroactive ligand-receptor interaction reported in the KEGG pathway data base, but their roles are different. \( \text{NR3C1} \) is associated with cortisol in glucocorticoid receptor signaling, \( \text{HRH1} \) is associated with histamine, and \( \text{CHRM1, CHRM2, and CHRM3} \) are associated with acetylcholine. \( \text{CHRM1} \) and \( \text{CHRM2} \) are also associated with the PI3K-Akt signaling pathway through the chemokine signaling pathway down to G protein-coupled receptors. The PI3K-Akt signaling pathway is also linked to \( \text{THBS1} \) through extracellular matrix receptor interaction and to \( \text{KITLG} \), which acts as a growth factor through \( \text{KIT} \), a receptor tyrosine kinase. Also, three genes (not shown in Table 7) are the following: \( \text{PTGS1} \) is involved in metabolic pathway but has a different role from that of \( \text{SELE} \) in the metabolic pathway; \( \text{SEL} \), like \( \text{SELE} \) which is shown in Table 7, is a cell adhesion molecule involved in leukocyte migration; and \( \text{MS4A2} \) is a lone essential gene that links to the FceRI signaling pathway, phospholipase D signaling pathway, and sphingolipid signaling pathway.

When taking the 13 candidate genes together, we found the following six pathways in the TGF-\( \beta \) signaling pathway (\( \text{NRC31, KITLG, THBS1, CCL2} \)), glucocorticoid receptor signaling pathway (\( \text{NR3C1, SELE, CCL2} \)), p53 signaling pathway (\( \text{CCNG2, THBS1, CCL2} \)), p21-activated kinase (PAK) pathway (\( \text{PAK1IP1, KITLG, CCL2} \)), phosphoinositide-3 kinase and protein kinase B (PI3K-Akt) signaling (\( \text{KITLG, CHRM, THBS1} \)), and neuroactive ligand-receptor interaction (\( \text{NRC31, HRH1, CHRM} \)) all contain at least 3 of these chosen 13 genes that could play important roles in active hives.

CONCLUSION

From the GO biologic process analysis by IPA and iPathwayGuide simulation programs, \(^{27,28} \) these 13 genes and their affiliated signaling pathways were found to be associated with several biologic functions. For example, the three significantly upregulated genes \( \text{THBS1, SELE, and CCL2} \) were reported to be associated with many cellular functions, including cell-to-cell signaling and interaction, cellular movement, tissue development, regulation of leukocyte migration, immune cell trafficking, inflammatory response, and hematologic system development and function, as shown in Fig. 3. Overall, the GO biologic process analysis in the disease of CIU pointed to cellular movement, cell migration, cell-cell interaction, and cell growth of the immune cells; however, it is still unclear as to the exact role in CIU mechanisms because the functions of the 12 most prominent CIU-related genes are still unknown.

ACKNOWLEDGMENTS

We thank John Chase, M.D., for his contribution in recruiting patients and providing the needed patient information for this research project.

REFERENCES

1. Magen E, Zueva E, Mishal J, and Schlesinger M. The clinical and laboratory characteristics of acute spontaneous urticaria and its progression to chronic spontaneous urticaria. Allergy Asthma Proc 37:394–399, 2016.
2. Greaves MW. Chronic idiopathic urticaria. Curr Opin Allergy Clin Immunol 3:363–368, 2003.
3. Azkur D, Civelek E, Toyran M, et al. Clinical and etiologic evaluation of the children with chronic urticaria. Allergy Asthma Proc 37:450–457, 2016.
4. Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116:281–297, 2004.
5. Lewis BP, Shih IH, Jones-Rhoades MW, et al. Prediction of mammalian microRNA targets. Cell 115:787–798, 2003.
6. Maziere P, and Enright AJ. Prediction of microRNA targets. Drug Discov Today 12:452–458, 2007.
7. Friedman RC, Farh KK, Burge CB, and Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19:92–105, 2009.
8. Kozomara A, and Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42(Database issue):D68–D73, 2014.
9. Lewis BP, Burge CB, and Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20, 2005.
10. Baek D, Villén J, Shin C, et al. The impact of microRNAs on protein output. Nature 455:64–71, 2008.
11. Ortiz-Quintero B. Cell-free microRNAs in blood and other body fluids, as cancer biomarkers. Cell Prolif 49:281–303, 2016.
12. Singh R, Ramanasubramanian B, Kanji S, et al. Circulating microRNAs in cancer: Hope or hype? Cancer Lett 381:113–121, 2016.
13. Rasooly MM, Moey NA, and Kirshenbaum AS. Helicobacter pylori: A significant and treatable cause of chronic urticaria and angioedema. Nurse Pract 40:1–6, 2015.
14. Lu TX, and Rothenberg ME. Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases. J Allergy Clin Immunol 132:3–13; quiz 14, 2013.
15. Rebane A, and Aksis CA. MicroRNAs in allergy and asthma. Curr Allergy Asthma Rep 14:424, 2014.
16. Pua HH, and Ansel KM. MicroRNA regulation of allergic inflammation and asthma. Curr Opin Immunol 36:101–108, 2015.
17. Panganiban RP, Wang Y, Howrylak J, et al. Circulating microRNAs as biomarkers in patients with allergic rhinitis and asthma. J Allergy Clin Immunol 137:1423–1432, 2016.

18. Mannucci C, Casciaro M, Minciullo PL, et al. Involvement of microRNAs in skin disorders: A literature review. Allergy Asthma Proc 38:9–15, 2017.

19. Agarwal V, Bell GW, Nam JW, and Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife 4, 2015.

20. Wong N, and Wang X. miRDB: An online resource for microRNA target prediction and functional annotations. Nucleic Acids Res. 43(Database issue):D146–D152, 2015.

21. Patel OP, Giorno RC, Dibbern DA, et al. Gene expression profiles in chronic idiopathic (spontaneous) urticaria. Allergy Rhinol (Providence) 6:101–110, 2015.

22. MalaCards: Human Disease Database, Weizmann Institute of Science, Rehovot, Israel. Available online at http://www.malacards.org/card/urticaria

23. GeneAnalytics: Gene set analysis, LifeMap Sciences, Alameda, CA. Available online at http://geneanalytics.genecards.org/

24. KEGG: Kyoto Encyclopedia of Genes and Genomes, Kanehisa Laboratories, Kyoto University, Kyoto, Japan. Available online at http://www.genome.jp/kegg/pathway.html

25. PathCards: Pathway unification database, Weizmann Institute of Science, Rehovot, Israel. Available online at http://pathcards.genecards.org/

26. Vlachos IS, Zagganas K, Paraskevopoulou MD, et al. DIANA-miRPath v3.0: Deciphering microRNA function with experimental support. Nucleic Acids Res 43:W460–W466, 2015.

27. IPA, Qiagen, Redwood City, CA. Available online at http://www.ingenuity.com/products/ips#/?tab=overview

28. iPathwayGuide, Advaita Bioinformatics, Plymouth, MI. Available online at https://apps.advaitabio.com/oauth-provider/login

29. Goh CL, and Tan KT. Chronic autoimmune urticaria: Where we stand? Indian J Dermatol 54:269–274, 2009.

30. MicroRNA and microRNA Target Database. Available online at https://en.wikipedia.org/wiki/MicroRNA_and_microRNA_target_database

31. Gene Ontology Consortium. Available online at http://www.geneontology.org/

32. Lee RC, Feinbaum RL, and Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854, 1993

33. Musilova K, and Mraz M. MicroRNAs in B-cell lymphomas: How a complex biology gets more complex. Leukemia 29:1004–1017, 2015.

34. U.S. National Institutes of Health. Available online at https://clinicaltrials.gov/ct2/results?term=MicroRNA&Search=Search

35. Suojalehto H, Lindstrom I, Majuri M, et al. Altered microRNA expression of nasal mucosa in long-term asthma and allergic rhinitis. Int Arch Allergy Immunol 163:168–178, 2014. □