Decreased microRNA 16 and 451a expression in hypertrophic adenoid tissue is associated with allergy

Zmniejszona ekspresja microRNA-16 i microRNA-451a w przeroście migdałka gardłowego związana jest z alergią

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Abstract

Introduction: MicroRNAs (miRNAs) regulate gene expression and play a role in many biological processes. Their imbalance may result in the development of numerous diseases, including allergy. Exact mechanisms causing allergic inflammation are still unclear, but recent studies show that miRNAs are involved in its pathogenesis. Adenoid hypertrophy (AH) and allergy often coexist, although the reason for that is still being investigated.

Aim: To compare the expression of several miRNAs in adenoid tissue and nasal mucosa from children with and without allergy and to investigate whether miRNA levels correlate with the patient’s allergy status.

Material and methods: Samples were taken from 37 patients and divided into two groups: allergic and non-allergic subjects. MiRNA was isolated from the adenoid tissue and nasal swabs collected during the adenoidectomy procedure, and transcribed into cDNA. MiRNA expression was measured with TaqMan MicroRNA Assays and analyzed with DataAssist software.

Results: MiR-16 and miR-451a expression was significantly decreased in the adenoid tissue of allergic children. Other miRNAs were not different between allergic and non-allergic patients. The expression of miRNA in the nasal mucosa did not differ between allergic and non-allergic patients.

Conclusions: MiRNAs are present in the adenoid tissue and have a distinct expression pattern in allergic patients compared to controls. This suggests that the molecular mechanism of AH formation in allergic patients is different and might explain why the allergy affects the prevalence of AH. Further studies are needed to better understand the role of miRNAs in the induction of allergic-type inflammation.

Key words
miRNA, allergy, adenoid hypertrophy, allergic rhinitis.
STRESZCZENIE

Wprowadzenie: MicroRNA (miRNA) regulują ekspresję genów i odgrywają rolę w wielu procesach biologicznych. Zaburzenie ich równowagi może skutkować rozwojem wielu chorób, w tym alergicznych. Dokładne mechanizmy powodujące powstawanie alergii są nadal nieznane, ale ostatnie badania pokazują, że miRNA są zaangażowane w jej patogenezę. Przerost migdałka gardlowego i alergia często współistnieją, chociaż przy czynu tego jest wciąż badana.

Cel: Porównanie ekspresji kilku miRNA w migdałku gardłowym i błonie śluzowej nosa u dzieci z alergią i bez alergii oraz zbadanie, czy poziomy miRNA korelują z diagnozą alergii u pacjenta.

Materiał i metody: Próbkę pobrano od 37 pacjentów podzielonych na dwie grupy: alergików i niebędących alergikami. miRNA wyizolowano z przerośniętej tkanki migdałka gardlowego oraz nablonka oddechowego uzyskanego z wymazów z nosa pobranych podczas adenoidektomii i przepisano na cDNA. Ekspresję miRNA mierzono za pomocą testów TaqMan MicroRNA i analizowano za pomocą oprogramowania DataAssist.

 Wyniki: Ekspresja miR-16 i miR-451a była istotnie obniżona w tkance gruczołowej dzieci z alergią. Inne miRNA nie różniły się między pacjentami alergicznymi i niealergicznymi. Ekspresja miRNA w błonie śluzowej nosa nie różniła się między pacjentami alergicznymi i niealergicznymi.

Wnioski: miRNA są obecne w migdałku gardłowym i różnią się ekspresją u pacjentów z alergią i bez niej. Sugeruje to, że molekularny mechanizm powstawania przerostu migdałka gardlowego u alergików jest inny i może wyjaśniać, dlaczego alergia wpływa na częstość jego występowania. Potrzebne są dalsze badania, aby lepiej zrozumieć rolę miRNA w indukowaniu nadwrażliwości alergicznej.

SŁOWA KLUCZOWE:
miRNA, alergia, przerost migdałka gardlowego, alergiczny nieżyt nosa.

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INTRODUCTION

Adenoid hypertrophy (AH) is a common childhood pathology, which causes the obstruction of upper airways that may eventually lead to sleep apnea, craniofacial abnormal growth, or conductive hearing loss and cognitive impairment [1]. Several studies underline higher incidence of AH among children with allergy, but underlying molecular mechanisms are still not fully understood [2].

MiRNAs are small, non-coding molecules that serve as gene regulators of many biological processes, such as cell differentiation, proliferation, apoptosis or angiogenesis [3]. Their role in allergic type of inflammation has recently been described in some studies [4]. Allergic type of inflammation is based on Th cell imbalance, where the cytokines secreted in Th2 type of inflammation dominate over cytokines produced in Th1 type [5]. Recent studies showed that particular miRNAs or their clusters regulate differentiation of Th cells into Th2 type. Several murine models demonstrated that either enhancing or silencing those miRNAs might rebuild Th cell balance [6].

Our assumption that specific miRNAs might take part in adenoid enlargement pathogenesis in allergic patients comes from the concept of the united airways disease (UAD). According to the latest evidence, the upper and lower respiratory tract mucosa in allergic patients have a similar potential to trigger the Th2 immune response and secrete cytokines stimulating the allergic type of inflammation in each of its compartments. IgE-mediated allergic inflammation is based on immunoglobulin class switching (from IgM to IgE) after antigen sensitization and secretion of specific cytokines, such as interleukin 4 (IL)-4 or IL-13, which are responsible for maintenance of the allergic response. This results in vasodilatation, bronchoconstriction and increased mucus production [7]. Immunoglobulin class switching is present in respiratory mucosa of patients with AR and asthma but also has been observed in the gastrointestinal tract in patients with food allergy [8]. Nguyen et al. compared several levels of cytokines in adenoids, middle ear fluid and torus tubarius biopsies, and found that the eosinophils, T lymphocytes and IL-4 mRNA levels were significantly higher in the allergic group. This proved the
Assumption that all three compartments share the allergic pattern of inflammation [9].

**AIM**

Therefore, we hypothesized that nasopharynx, with its adenoid tissue, is a part of the united airways and that the mechanism of AH formation in allergic patients might depend on a specific regulatory mechanism that is absent in non-allergic children. Taking into account the high regulatory potential of miRNAs and their involvement in allergic inflammatory processes, we sought to determine whether miRNA expression differs between patients with and without allergy, suggesting that the pathomechanism of tonsil enlargement in these two groups of patients might be distinct.

**MATERIAL AND METHODS**

The study was approved by the Bioethics Committee of Poznan University of Medical Sciences. Patients were recruited from inpatients at the Department of Pediatric Otolaryngology, Poznan University of Medical Sciences. The study was approved by the Poznan University of Medical Sciences Bioethics Committee. Subjects were from the Wielkopolska region of Poland, which is considered ethnically homogeneous. Written informed consent was obtained from a parent or legal guardian. The study group consisted of children diagnosed with adenoid hypertrophy.

Exclusion criteria included: craniofacial abnormalities, cleft palate, genetic syndromes, immune deficiencies, cystic fibrosis, immotile cilia syndrome, steroids, and antihistamine or leukotriene drug intake 2 weeks prior to the surgical procedure.

Full otolaryngologic examination was performed at the admission. Each child was carefully investigated for a history of allergic diseases using a detailed questionnaire. Children with positive skin prick tests or blood tests for food or inhalant allergens or with allergic rhinitis diagnosis based on ARIA [10] criteria or allergic asthma according to GINA [11] criteria were assigned to the allergy group and compared against children without allergic history. The allergy group consisted of 16 patients whereas the non-allergic group had 21 patients.

Samples were taken during the adenoidectomy procedure, before the tonsil removal. Nasal respiratory mucosa swabs were also taken. The material was then transferred to the laboratory and frozen at -80°C for further experiments.

Total RNA was isolated with miRCURY miRNA Isolation Kit – Cell and Plant (Exiqon), according to the manufacturer’s instructions and transcribed to cDNA with TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific). MiRNA expression was analyzed with TaqMan MicroRNA Assays and TaqMan Universal Master Mix II, no UNG (Thermo Fisher Scientific), according to the manufacturer’s protocol. MiRNA expression datasets were analyzed with DataAssist software v.3.01 after global normalization.

Pathway enrichment analysis was performed for miRNAs that had significant changes in expression. We selected validated target genes (via reporter assay) from miRTarBase (available at http://mirtarbase.mbc.nctu.edu.tw/php/index.php). To identify KEGG pathways, the list of validated targets was analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7.

**RESULTS**

**PATIENT CHARACTERISTICS**

Samples were taken from 37 subjects (10 girls and 27 boys). Mean age was 6.22 ±2.4 years. Adenoid hypertrophy was more common in boys independently of allergy status (75% in allergic group and 71% in non-allergic group). Mean total IgE serum level was higher among allergic children (392.8 ±125.1 and 90 ±574.5 in non-allergic patients). Mean eosinophil count was also higher in the allergy group (6.2 ±2.3% in allergy group and 3.5 ±3.3% in non-allergic group). These results correspond to other studies underlining that IgE serum level as well as eosinophils is elevated in allergic patients. 69% of allergic children and 67% of non-allergic children attended kindergarten. 18.7% of allergic children and 14.2% of non-allergic children were under chronic exposure to tobacco smoke at home, which is a separate allergy factor (Table 1).

| Parameter                              | Allergic | Non-allergic |
|----------------------------------------|----------|--------------|
| Male                                   | 12       | 15           |
| Female                                 | 4        | 6            |
| Age, mean ± SD                         | 6.3 ±3.3 | 6.1 ±3.3     |
| Total IgE level, mean ± SD [kU/ml]     | 392.8 ±125.1 | 90 ±574.5   |
| Eosinophil count (%), mean ± SD        | 6.2 ±2.3% | 3.5 ±3.3%    |
| Exposure to tobacco smoke              | 3; 18.7% | 3; 14.2%     |
| Attending kindergarten                  | 11; 69%  | 14; 67%      |
Figure 1. Expression of selected miRNAs in nasal mucosa

A. miR-16 expression

B. miR-25 expression

C. miR-223 expression

D. miR-320 expression

E. miR-451 expression

No allergy Allergy

Group

Mean ± SE

Mean ± SD

FIGURE 1. Expression of selected miRNAs in nasal mucosa
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**FIGURE 2.** Expression of selected miRNAs in adenoid tissue.
MIRNA EXPRESSION ANALYSIS

We found that expression of analyzed miRNAs in nasal mucosa did not differ significantly between allergic and non-allergic patients with AH (Figure 1). In adenoid tissue, we observed significantly decreased expression of 2 miRNAs, miR-16 ($p = 0.048$) and miR-451a ($p = 0.019$), in allergic patients as compared to non-allergic children (Figure 2).

TARGET ANALYSIS

A list of validated target genes regulated by miR-16-5p or miR-451a was analyzed with the DAVID annotation tool to identify the most enriched pathways possibly regulated by this miRNA. Several pathways were significantly enriched for both miRNAs (Tables 2 and 3).

DISCUSSION

While several studies have debated whether allergy is a separate risk factor of AH, there is still insufficient molecular evidence supporting this hypothesis [12]. Nguyen et al. reported that IL-4 levels and eosinophil infiltration were increased in adenoids and middle ear effusions from allergic patients compared to controls [9]. Huo et al. linked adenotonsillar regrowth with the allergy status. They established levels of GATA3+ cells (Th2-type cells) and found that they were increased in the adenoids from the allergic subgroup [13]. To our knowledge, our study is the first to analyze miRNA expression in adenoid tissue in the pediatric population and whether it depends on allergy status.

Expression of 5 analyzed miRNAs (miR-320e, miR-16-5p, miR-451a, miR-223-3p, miR-25-3p) was not significantly different in nasal mucosa between allergic and non-allergic children, so these miRNAs are not likely to play a role in allergic inflammation in the nose. Interestingly, we found that miR-16 and miR-451a were significantly decreased in the adenoid tissue of allergic patients, suggesting that they may favor the expression of genes participating in adenoid hypertrophy in allergic patients. MiR-16 takes part in the allergic inflammation. Pangiban et al. demonstrated that several miRNAs, including miR-16, had different expression in patients with asthma and allergic rhinitis (AR) as compared to the controls and underlined their potential as future noninvasive biomarkers of allergic diseases [14]. Another study in asthmatic patients revealed the possible role of miR-16 in asthma exacerbation by regulating Th2 cytokine expression and favoring airway inflammation [15]. Yu et al. also demonstrated that miR-16 has the potential to serve as an asthma biomarker. Moreover, miR-16 regulates mRNA expression of adrenoreceptor $\beta 2$, which is an agonist receptor for bronchodilators; thus miR-16 may affect their efficacy [16].

Pathway analysis of predicted target genes for miR-16 and miR-451a have identified several potential regulatory mechanisms that might be involved in allergic inflammation. For example, the PI3K-Akt pathway, regulated by both the miRNAs, modulates airway inflammation and airway hyper-responsiveness [17]. MAPK signaling pathway contributes to the expression of proinflammatory genes [18]. Moreover, the neurotrophin signaling pathway modulates biological effects of infiltrated eosinophils in the allergic airways [19].

MiR-451 plays a role in pathogenesis of various cancers, but its exact role in allergic inflammation is still not fully understood. Macrophages activated by reactive oxygen species have altered miR-451 expression, suggesting its important role in macrophage maturation [20]. Chung et al. investigated the role of miR-451 in a mouse model of allergic asthma and found that its levels are significantly decreased, affecting macrophage activation in lungs. Macrophages isolated from mice’s lungs had increased levels of CCL17 and sirtuin-2, indicating miR-451 function in regulating the allergic response [21].

Identifying the altered expression of particular miRNAs in allergic patients could help to understand the pathogenesis of AH formation, linking it to allergic inflammation. Further miRNAs studies would enable the mechanisms underlying morbidities to be explained and could possibly lead to the development of biomarkers or even therapeutic options in cases where current therapy is unfortunately insufficient.

There are two major limitations to this study that are going to be addressed in future research. The primary limitation is the small sample size. Another limitation is the lack of target gene verification addressed at altered miRNAs, which will be dealt with in a future study.

CONCLUSIONS

We have documented that miRNAs are expressed in adenoid tissue in children and that the expression of two of them, miR-16 and miR-451a, differs between allergic and non-allergic patients. These miRNAs may be involved in adenoid hypertrophy formation in allergic patients. Further studies are needed to better understand their exact role in the induction of allergic-type inflammation.

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| Category               | Pathway                                      | No. of genes | Fold enrichment | P-value | P corrected |
|------------------------|----------------------------------------------|--------------|----------------|---------|-------------|
| KEGG                   | PI3K-Akt signaling pathway                   | 20           | 7.7            | < 0.001 | < 0.001     |
| KEGG                   | Proteoglycans in cancer                      | 16           | 10.6           | < 0.001 | < 0.001     |
| KEGG                   | MicroRNAs in cancer                          | 17           | 7.9            | < 0.001 | < 0.001     |
| KEGG                   | Pathways in cancer                           | 19           | 6.4            | < 0.001 | < 0.001     |
| KEGG                   | Prostate cancer                              | 11           | 16.5           | < 0.001 | < 0.001     |
| KEGG                   | Melanoma                                     | 10           | 18.6           | < 0.001 | < 0.001     |
| KEGG                   | Hepatitis B                                  | 11           | 10             | < 0.001 | < 0.001     |
| KEGG                   | Acute myeloid leukemia                       | 8            | 18.9           | < 0.001 | < 0.001     |
| KEGG                   | Colorectal cancer                            | 8            | 17.1           | < 0.001 | < 0.001     |
| KEGG                   | Pancreatic cancer                            | 8            | 16.3           | < 0.001 | < 0.001     |
| KEGG                   | Glioma                                       | 8            | 16.3           | < 0.001 | < 0.001     |
| KEGG                   | Signaling pathways regulating pluripotency of stem cells | 10          | 9.4            | < 0.001 | < 0.001     |
| KEGG                   | Small cell lung cancer                       | 8            | 12.5           | < 0.001 | < 0.001     |
| KEGG                   | Measles                                      | 9            | 9              | < 0.001 | < 0.001     |
| KEGG                   | Central carbon metabolism in cancer          | 7            | 14.5           | < 0.001 | < 0.001     |
| KEGG                   | p53 signaling pathway                        | 7            | 13.8           | < 0.001 | < 0.001     |
| KEGG                   | Chronic myeloid leukemia                     | 7            | 12.9           | < 0.001 | < 0.001     |
| KEGG                   | Focal adhesion                               | 10           | 6.4            | < 0.001 | < 0.001     |
| KEGG                   | Rap1 signaling pathway                       | 10           | 6.3            | < 0.001 | < 0.001     |
| KEGG                   | Neurotrophin signaling pathway               | 8            | 8.8            | < 0.001 | < 0.001     |
| KEGG                   | Ras signaling pathway                        | 10           | 5.9            | < 0.001 | < 0.001     |
| KEGG                   | Endometrial cancer                           | 6            | 15.3           | < 0.001 | < 0.001     |
| KEGG                   | Non-small cell lung cancer                   | 6            | 14.2           | < 0.001 | < 0.001     |
| KEGG                   | HIF-1 signaling pathway                      | 7            | 9.6            | < 0.001 | < 0.001     |
| KEGG                   | VEGF signaling pathway                       | 6            | 13             | < 0.001 | 0.001       |
| KEGG                   | HTLV-I infection                             | 10           | 5.2            | < 0.001 | 0.001       |
| KEGG                   | Bladder cancer                               | 5            | 16.1           | < 0.001 | 0.001       |
| KEGG                   | Cell cycle                                   | 7            | 7.5            | < 0.001 | 0.002       |
| KEGG                   | MAPK signaling pathway                       | 9            | 4.7            | < 0.001 | 0.003       |
| KEGG                   | T cell receptor signaling pathway            | 6            | 7.9            | 0.001   | 0.004       |
| KEGG                   | mTOR signaling pathway                       | 5            | 11.4           | 0.001   | 0.004       |
| KEGG                   | Apoptosis                                    | 5            | 10.7           | 0.001   | 0.005       |
| KEGG                   | Toxoplasmosis                                | 6            | 7.2            | 0.001   | 0.006       |
| KEGG                   | Renal cell carcinoma                         | 5            | 10             | 0.001   | 0.006       |
| KEGG                   | Thyroid hormone signaling pathway            | 6            | 6.9            | 0.002   | 0.007       |
| KEGG                   | Sphingolipid signaling pathway               | 6            | 6.6            | 0.002   | 0.008       |
| KEGG                   | Hepatitis C                                  | 6            | 6              | 0.003   | 0.012       |
| KEGG                   | FoxO signaling pathway                       | 6            | 5.9            | 0.003   | 0.012       |
| KEGG                   | Viral carcinogenesis                         | 7            | 4.5            | 0.004   | 0.015       |
| Category     | Pathway                                           | No. of genes | Fold enrichment | P-value | P corrected |
|--------------|---------------------------------------------------|--------------|-----------------|---------|-------------|
| KEGG         | ErbB signaling pathway                            | 5            | 7.6             | 0.004   | 0.015       |
| KEGG         | Jak-STAT signaling pathway                        | 6            | 5.5             | 0.004   | 0.016       |
| KEGG         | Hippo signaling pathway                            | 6            | 5.3             | 0.005   | 0.018       |
| KEGG         | Choline metabolism in cancer                      | 5            | 6.5             | 0.006   | 0.023       |
| KEGG         | Chagas disease (American trypanosomiasis)         | 5            | 6.4             | 0.007   | 0.025       |
| KEGG         | Tuberculosis                                      | 6            | 4.5             | 0.010   | 0.033       |
| KEGG         | Epstein-Barr virus infection                      | 5            | 5.4             | 0.012   | 0.041       |
| KEGG         | AMPK signaling pathway                             | 5            | 5.4             | 0.013   | 0.041       |
| KEGG         | Fc epsilon RI signaling pathway                   | 4            | 7.8             | 0.014   | 0.043       |
| KEGG         | B cell receptor signaling pathway                 | 4            | 7.7             | 0.014   | 0.044       |
| KEGG         | Adipocytokine signaling pathway                   | 4            | 7.6             | 0.015   | 0.045       |
| KEGG         | Leishmaniasis                                     | 4            | 7.5             | 0.015   | 0.046       |
| KEGG         | Prolactin signaling pathway                       | 4            | 7.5             | 0.015   | 0.046       |
| KEGG         | Wnt signaling pathway                             | 5            | 4.8             | 0.019   | 0.054       |
| KEGG         | Insulin signaling pathway                         | 5            | 4.8             | 0.019   | 0.054       |
| KEGG         | Thyroid cancer                                    | 3            | 13.7            | 0.019   | 0.055       |
| KEGG         | TGF-β signaling pathway                           | 4            | 6.3             | 0.024   | 0.067       |
| KEGG         | African trypanosomiasis                           | 3            | 12              | 0.025   | 0.067       |
| KEGG         | NF-κB signaling pathway                           | 4            | 6.1             | 0.026   | 0.071       |
| KEGG         | Progesterone-mediated oocyte maturation           | 4            | 6.1             | 0.026   | 0.071       |
| KEGG         | Allograft rejection                               | 3            | 10.7            | 0.030   | 0.080       |
| KEGG         | Transcriptional misregulation in cancer           | 5            | 4               | 0.035   | 0.089       |
| KEGG         | Estrogen signaling pathway                        | 4            | 5.3             | 0.037   | 0.093       |
| KEGG         | Type 1 diabetes mellitus                          | 3            | 9.4             | 0.038   | 0.095       |
| KEGG         | Influenza A                                       | 5            | 3.8             | 0.039   | 0.096       |
| KEGG         | Toll-like receptor signaling pathway              | 4            | 5               | 0.043   | 0.100       |
| KEGG         | TNF signaling pathway                             | 4            | 4.9             | 0.044   | 0.100       |
| KEGG         | Herpes simplex infection                         | 5            | 3.6             | 0.046   | 0.110       |
| KEGG         | Insulin resistance                               | 4            | 4.9             | 0.046   | 0.110       |
| KEGG         | Serotonergic synapse                              | 4            | 4.8             | 0.049   | 0.110       |
| KEGG         | Malaria                                           | 3            | 8.1             | 0.051   | 0.110       |
| KEGG         | Amyotrophic lateral sclerosis (ALS)               | 3            | 7.9             | 0.053   | 0.110       |
| KEGG         | Basal cell carcinoma                              | 3            | 7.3             | 0.060   | 0.130       |
| KEGG         | Natural killer cell mediated cytotoxicity         | 4            | 4.3             | 0.061   | 0.130       |
| KEGG         | Long-term depression                              | 3            | 6.6             | 0.073   | 0.150       |
| KEGG         | Osteoclast differentiation                        | 4            | 4               | 0.073   | 0.150       |
| KEGG         | Inflammatory bowel disease (IBD)                  | 3            | 6.2             | 0.081   | 0.160       |
| KEGG         | RIG-I-like receptor signaling pathway             | 3            | 5.7             | 0.095   | 0.190       |
| KEGG         | Oxytocin signaling pathway                        | 4            | 3.5             | 0.099   | 0.190       |
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The data that support the findings of this study are available from the corresponding author, upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**TABLE 3.** Results of pathway analysis of validated target genes for hsa-miR-451a in DAVID software (bolded \( p \)-value indicates significance after multiple testing correction with Benjamini procedure)

| Category | Pathway | No. of genes | Fold enrichment | \( P \)-value | \( P \)-corrected |
|----------|---------|--------------|----------------|-------------|---------------|
| Kegg     | mTOR signaling pathway | 4 | 31.6 | 0.000 | 0.012 |
| Kegg     | PI3K-Akt signaling pathway | 6 | 8 | 0.000 | 0.017 |
| Kegg     | Hepatitis B | 5 | 15.8 | 0.000 | 0.02 |
| Kegg     | Fox0 signaling pathway | 4 | 13.7 | 0.002 | 0.067 |
| Kegg     | NOD-like receptor signaling pathway | 3 | 24.6 | 0.006 | 0.13 |
| Kegg     | Acute myeloid leukemia | 3 | 24.6 | 0.006 | 0.13 |
| Kegg     | Chronic myeloid leukemia | 3 | 19.1 | 0.009 | 0.17 |
| Kegg     | TNF signaling pathway | 3 | 12.9 | 0.019 | 0.18 |
| Kegg     | HTLV-I infection | 4 | 7.2 | 0.013 | 0.19 |
| Kegg     | Toll-like receptor signaling pathway | 3 | 13 | 0.019 | 0.19 |
| Kegg     | HIF-1 signaling pathway | 3 | 14.3 | 0.016 | 0.2 |
| Kegg     | AMPK signaling pathway | 3 | 11.2 | 0.025 | 0.2 |
| Kegg     | Chagas disease (American trypanosomiasis) | 3 | 13.2 | 0.018 | 0.2 |
| Kegg     | Neurotrophin signaling pathway | 3 | 11.5 | 0.024 | 0.21 |
| Kegg     | MAPK signaling pathway | 4 | 7.3 | 0.013 | 0.21 |
| Kegg     | Insulin signaling pathway | 3 | 10 | 0.031 | 0.23 |
| Kegg     | Jak-STAT signaling pathway | 3 | 9.5 | 0.034 | 0.24 |
| Kegg     | Non-alcoholic fatty liver disease (NAFLD) | 3 | 9.1 | 0.037 | 0.24 |
| Kegg     | Pathways in cancer | 4 | 4.7 | 0.042 | 0.26 |
| Kegg     | Tuberculosis | 3 | 7.8 | 0.049 | 0.27 |
| Kegg     | Influenza A | 3 | 7.9 | 0.047 | 0.27 |
| Kegg     | Thyroid cancer | 2 | 31.6 | 0.057 | 0.3 |
| Kegg     | Prion diseases | 2 | 27 | 0.067 | 0.32 |
| Kegg     | Ras signaling pathway | 3 | 6.1 | 0.075 | 0.34 |
| Kegg     | Bladder cancer | 2 | 22.4 | 0.08 | 0.35 |
| Kegg     | Type II diabetes mellitus | 2 | 19.1 | 0.093 | 0.39 |
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