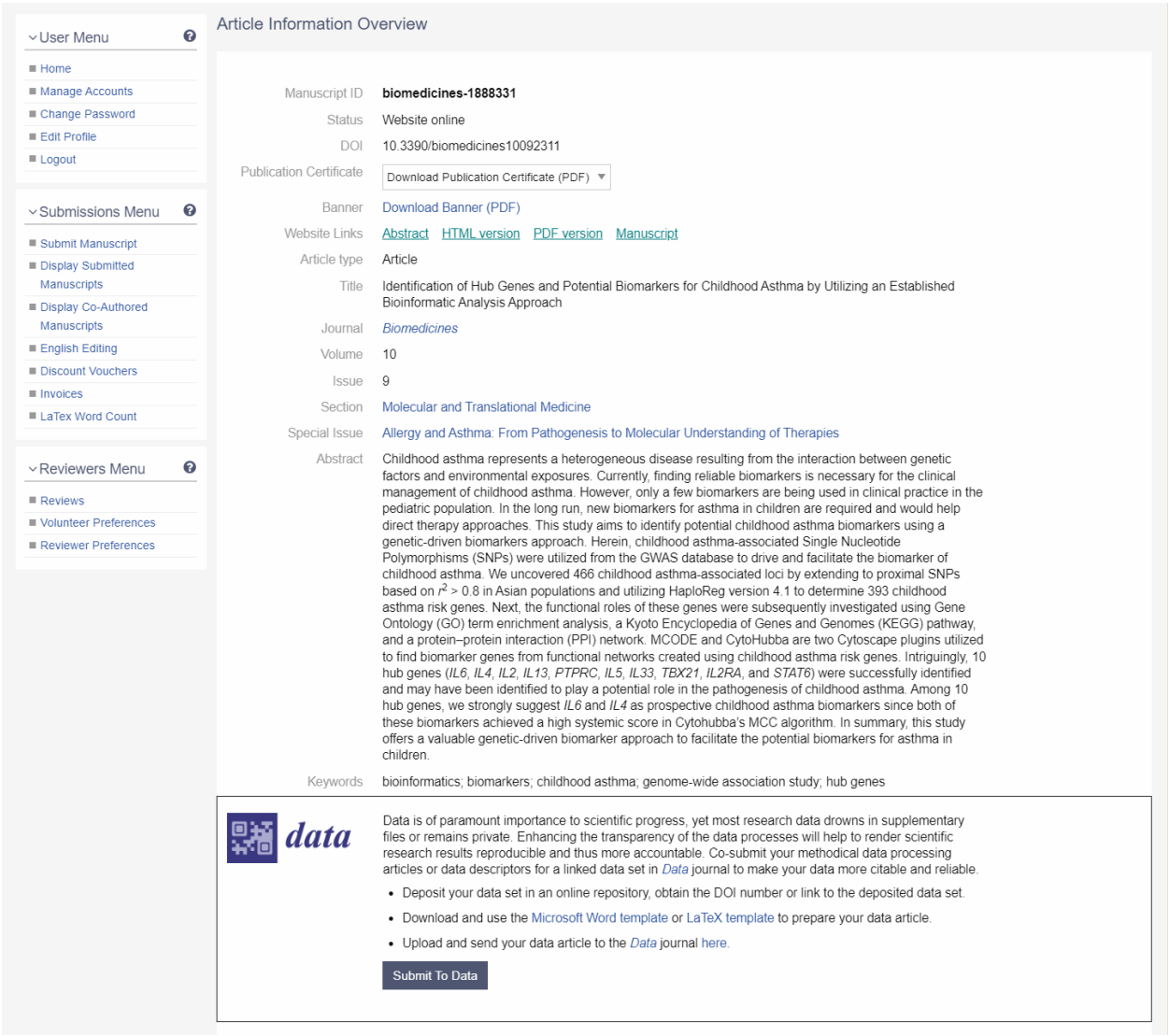
History Artikel

Identification of Hub Genes and Potential Biomarkers for Childhood Asthma by Utilizing an Established Bioinformatic Analysis Approach



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Article Information Overview

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Abstract	Childhood asthma represents a heterogeneous disease resulting from the interaction between genetic factors and environmental exposures. Currently, finding reliable biomarkers is necessary for the clinical management of childhood asthma. However, only a few biomarkers are being used in clinical practice in the pediatric population. In the long run, new biomarkers for asthma in children are required and would help direct therapy approaches. This study aims to identify potential childhood asthma biomarkers using a genetic-driven biomarkers approach. Herein, childhood asthma-associated Single Nucleotide Polymorphisms (SNPs) were utilized from the GWAS database to drive and facilitate the biomarker of childhood asthma. We uncovered 466 childhood asthma-associated loci by extending to proximal SNPs based on $r^2 > 0.8$ in Asian populations and utilizing HaploReg version 4.1 to determine 393 childhood asthma risk genes. Next, the functional roles of these genes were subsequently investigated using Gene Ontology (GO) term enrichment analysis, a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and a protein–protein interaction (PPI) network. MCODE and CytoHubba are two Cytoscape plugins utilized to find biomarker genes from functional networks created using childhood asthma risk genes. Intriguingly, 10 hub genes (*IL6*, *IL4*, *IL2*, *IL13*, *PTPRC*, *IL5*, *IL33*, *TBX21*, *IL2RA*, and *STAT6*) were successfully identified and may have been identified to play a potential role in the pathogenesis of childhood asthma. Among 10 hub genes, we strongly suggest *IL6* and *IL4* as prospective childhood asthma biomarkers since both of these biomarkers achieved a high systemic score in Cytohubba's MCC algorithm. In summary, this study offers a valuable genetic-driven biomarker approach to facilitate the potential biomarkers for asthma in children.
Keywords	bioinformatics; biomarkers; childhood asthma; genome-wide association study; hub genes

data

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Review Report

Reviewer 1	Review Report (Round 1) Review Report (Round 2)
Reviewer 2	Review Report (Round 1) Review Report (Round 2)

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Komentar reviewer 1 tahap 1:

Authors' Responses to Reviewer's Comments (Reviewer 1)

Author's Notes	Please see the attachment. Thank you
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Open Review	( ) I would not like to sign my review report (×) I would like to sign my review report
Quality of English Language	( ) English very difficult to understand/incomprehensible ( ) Extensive editing of English language and style required ( ) Moderate English changes required (×) English language and style are fine/minor spell check required ( ) I am not qualified to assess the quality of English in this paper
Comments and Suggestions for Authors	In this study, the authors focused on identifying potential biomarkers for childhood asthma based on candidate genes from the GWAS-identified loci. The authors identified IL6 and IL4 as biomarkers of potential childhood asthma. The results of this study are meaningful, but some questions remain. 1. Why was the CytoHubba tool used for biomarker discovery? What were the advantages of this method? Were there other more appropriate methods? These questions need to be explained clearly in the INTRODUCTION. 2. How can IL6 and IL4 be used as markers since they are widely distributed in the human body? 3. Biomarkers must correspond to their modification, e.g., gene mutations, overexpression of genes, amount of metabolite, etc. So what are the modifications corresponding to IL6 and IL4 as biomarkers? Does the presence of IL6 or IL4 indicate childhood asthma? Or do high levels of IL6 or IL4 indicate childhood asthma?
Submission Date	12 August 2022
Date of this review	26 Aug 2022 14:44:57

Komentar reviewer 2 tahap 1

Authors' Responses to Reviewer's Comments (Reviewer 2)

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(x) Extensive editing of English language and style required
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	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	( )	( )	(x)	( )
Are all the cited references relevant to the research?	(x)	( )	( )	( )
Is the research design appropriate?	(x)	( )	( )	( )
Are the methods adequately described?	(x)	( )	( )	( )
Are the results clearly presented?	(x)	( )	( )	( )
Are the conclusions supported by the results?	(x)	( )	( )	( )

Comments and Suggestions for Authors

The manuscript "Identification of hub genes and potential biomarkers for childhood asthma by utilizing an established bioinformatic analysis approach" describes a search for Single Nucleotide Polymorphisms (SNPs) associated with childhood asthma in Asian populations in a GWAS database. The reason for the search was the potential to find reliable genetic markers to identify asthma and start therapy earlier. Another reason to search for biomarkers was to find targets for new treatment options. The identified biomarkers were IL6 and IL4.

The methods are carefully performed and well described and the conclusions based on the results. However, there are some concerns with the study:

Major concerns:

The results are not surprising or new. We already knew this. How well do these biomarkers identify asthmatics to help start therapy earlier? With what rates do these biomarkers give false positives/negatives? The usefulness of the results needs to be further discussed.

Can IL6 and IL4 be used as therapy targets in children? This question needs to be discussed.

The goal is the Asian population, but can the same biomarkers be used in other populations? This needs to be addressed.

Minor concerns:

The language needs to be improved. Some examples:

Abstract: Hence, new biomarkers for diagnosing and predicting therapy responses for childhood asthma are emergence needed.

What do you mean by this sentence? Are biomarkers urgently needed, or what is the message here?

Line 53: However, childhoods with severe asthma exacerbation are still poorly understood and treated significantly.

What do you mean by this statement? Please rephrase to clarify the point.

Next sentence: In addition, high-dose inhaled corticosteroid therapy results in severe asthma patients who are unsatisfactory, with uncontrolled symptoms and frequent exacerbations.

The patients are not unsatisfactory, I hope. Please rephrase.

The introduction lacks focus and the language needs improvement.

Submission Date: 12 August 2022

Date of this review: 30 Aug 2022 12:15:59

Merespon terhadap saran dan masukan dari para reviewer:

September 6, 2022

Dear Editors,

We appreciate this opportunity to submit our revised manuscript entitled " **Identification of hub genes and potential biomarkers for childhood asthma by utilizing an established bioinformatic analysis approach,**" which we are submitting for consideration for publication as an Original Research article in "***Biomedicines"* **(Manuscript ID: biomedicines-1888331)**. We would like to thank the editor and reviewers for their constructive and insightful comments. Here, we are sending our revised manuscript in accordance with the comments given by the reviewers. We have carefully examined the raised questions and have answered or made changes accordingly. The revised parts are highlighted as a yellow color. We believe that the manuscript is greatly improved by the revisions. Thank you very much for your consideration of our work. Hope very much these revisions are adequate. We appreciate your assistance and are looking forward to hearing from you.

Sincerely yours,

Apt. Wirawan Adikusuma, M.Sc., Ph.D
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University of Muhammadiyah Mataram, Indonesia
Jl. KH. Ahmad Dahlan No.1, Pagesangan, Mataram, Nusa Tenggara Barat, 83115, Indonesia

Komentar dan saran reviewer 1 tahap 1

Reviewer 1

In this study, the authors focused on identifying potential biomarkers for childhood asthma based on candidate genes from the GWAS-identified loci. The authors identified IL6 and IL4 as biomarkers of potential childhood asthma. The results of this study are meaningful, but some questions remain.

1. Why was the CytoHubba tool used for biomarker discovery? What were the advantages of this method? Were there other more appropriate methods? These questions need to be explained clearly in the INTRODUCTION.

   Answer: We thank you for the reviewer's comment. An explanation about Cytohubba has been added to the manuscript. We used the CytoHubba tool for biomarker discovery in this study due to some reasons: We selected the CytoHubba plugin for biomarker discovery because CytoHubba provides a user-friendly interface to explore important nodes in biological networks. Besides, researchers could combine cytoHubba with other plugins into a novel analysis scheme. The network and sub-networks caught by this topological analysis strategy will lead to new insights on essential regulatory networks and protein drug targets for experimental biologists. CytoHubba provides 11 topological analysis methods, including Degree, Edge Percolated Component, Maximum Neighborhood Component, Density of Maximum Neighborhood Component, Maximal Clique Centrality and six centralities (Bottleneck, EcCentricity, Closeness, Radiality, Betweenness, and Stress) based on shortest paths. Among the eleven methods, we selected the MCC algorithm to predict essential proteins because it has a better performance on the precision of predicting essential proteins from the yeast PPI network [1]. In addition, several articles have been published using the CytoHubba to identify potential biomarkers in different diseases [2]–[5]. We have revised the manuscript and added the introduction part. The revised sentences are as below. "In addition, the Cytohubba plugin of Cytoscape provides 11 topological methods to identify some key genes. MCC was selected in this study to predict essential proteins from the yeast PPI network more accurately among the eleven methods. Several articles have been published using the CytoHubba to identify potential biomarkers in different diseases." **(line 79-83).**

2. How can IL6 and IL4 be used as markers since they are widely distributed in the human body?

   Answer: Many thanks for the reviewer's comments. A biomarker is a defining characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions. In asthma, the role of biomarkers has been largely studied in diagnosis, prediction of asthma severity, prognosis, and treatment response. Biomarkers may be useful in assessing and studying the biology of exacerbation, but a more precious function may be recognizing patients at increased risk for exacerbations. We identified IL6 and IL4 as potential childhood asthma biomarkers in our predictions. Interleukin 6 (IL6) was recently identified as a promising

biomarker for adult asthma in peripheral blood [6]. Nevertheless, IL6 has not been studied as a biomarker in childhood asthma [7]. IL6 is traditionally considered an inflammatory marker, along with TNFα and IL-1β, instead of a regulatory cytokine [8]. IL6 levels are associated with systemic inflammation, metabolic dysfunction, and greater asthma severity among lean and obese adults [6]. Elevated IL6 levels were associated with reduced lung function and heightened exacerbation risk. In addition, epithelial IL6 trans-signaling has been identified as a potential mechanism linked to asthma phenotypes characterized by increased airway inflammation [9]. Interestingly, IL6 increased exacerbation risk in children but did not affect lung function or other severity indicators as in adults. Children with frequent exacerbations and high IL6 levels may grow up to be adults with severe asthma [7]. Based on longitudinal analyses, children with higher plasma IL6 levels are related to obesity, metabolic syndrome, and greater asthma severity, with a risk for asthma exacerbation and decreased lung function [10]. IL4 is an important cytokine involved in asthma development [11] and it has been reported that airway hyperresponsiveness, eosinophil infiltration, and inflammation are all symptoms of bronchial asthma that IL4 likely mediates [12]. IL4 is a potent activator of inflammation and is involved in developing fibrosis during Th2 inflammation [13]. In asthma patients, Th2 is hyperactive, causing a rise in IL4 and immunoglobulin E (IgE), which stimulates the growth and activation of eosinophilic granulocytes, which then secretes a variety of inflammatory mediators, leading to bronchial chronic inflammation and asthma [14]. In addition, in asthmatic children, levels of IL4 were significantly higher among atopic asthmatics than nonatopic asthmatics [15], [16]. IL4 is an anti-inflammatory protein that can modulate inflammation, thus preventing asthma and vice versa [11]. In particular, we would like to emphasize that our method of genetic-driven biomarkers ultimately provided a candidate list of the biomarker in childhood asthma to be used in clinical outcome predictions. Nevertheless, further research is needed to confirm these findings.

3. Biomarkers must correspond to their modification, e.g., gene mutations, overexpression of genes, amount of metabolite, etc. So what are the modifications corresponding to IL6 and IL4 as biomarkers? Does the presence of IL6 or IL4 indicate childhood asthma? Or do high levels of IL6 or IL4 indicate childhood asthma?

   Answer: We sincerely thank you for the reviewer's comments. It's an essential point of this study. To identify the expression of IL6 and IL4 in asthma in human tissues, we used expression quantitative trait loci (eQTL) via the GTEX portal database (http://www.gtexportal.org/home/), which contains the expression levels of genes in various tissues. As shown in **Figure 1**, we identified that IL6 and IL4 are highly expressed in the lung. The high level of IL6 and IL4 reduced lung function and heightened exacerbation risk. IL6 and IL4 could be potential biomarkers for asthma. However, in the GTex portal databases, we couldn't identify the expression of a gene specific to childhood or adults. Hence, further research is needed to confirm this finding in clinical applications.

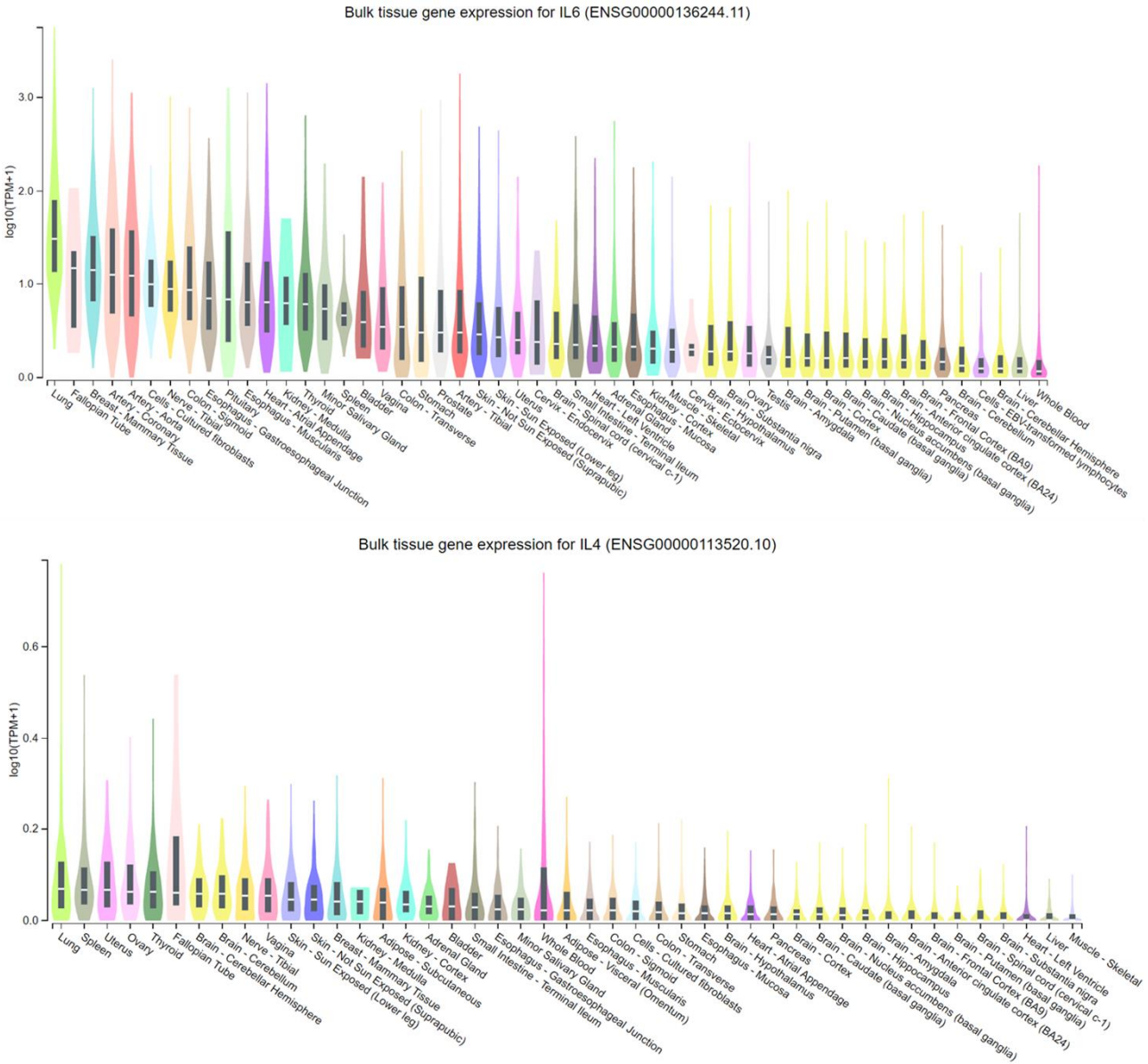

Figure 1. The expression of IL6 and IL4 in different human tissue according to GTex portal database

References

[1] C.-H. Chin, S.-H. Chen, H.-H. Wu, C.-W. Ho, M.-T. Ko, and C.-Y. Lin, "cytoHubba: identifying hub objects and sub-networks from complex interactome.," *BMC Syst Biol*, vol. 8 Suppl 4, no. Suppl 4, p. S11, 2014, doi: 10.1186/1752-0509-8-S4-S11.

[2] Z. Chen, Z. Zhong, W. Zhang, G. Su, and P. Yang, "Integrated Analysis of Key Pathways and Drug Targets Associated With Vogt-Koyanagi-Harada Disease," *Front Immunol*, vol. 11, Dec. 2020, doi: 10.3389/fimmu.2020.587443.

[3] L. Qian *et al.*, "Integrated Bioinformatics-Based Identification of Potential Diagnostic Biomarkers Associated with Diabetic Foot Ulcer Development," *J Diabetes Res*, vol. 2021, 2021, doi: 10.1155/2021/5445349.

[4] C. Zhao *et al.*, "Identification of significant gene biomarkers of low back pain caused by changes in the osmotic pressure of nucleus pulposus cells," *Sci Rep*, vol. 10, no. 1, Dec. 2020, doi: 10.1038/s41598-020-60714-y.

[5] X. Zhang, Z. Wang, L. Hu, X. Shen, and C. Liu, "Identification of Potential Genetic Biomarkers and Target Genes of Peri-Implantitis Using Bioinformatics Tools," *Biomed Res Int*, vol. 2021, 2021, doi: 10.1155/2021/1759214.

[6] M. C. Peters *et al.*, "Plasma IL6 levels, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts," *Lancet Respir Med*, vol. 4, no. 7, pp. 574–584, 2017, doi: 10.1016/S2213-2600(16)30048-0.Plasma.

[7] D. J. Jackson *et al.*, "Serum IL-6: A Biomarker in Childhood Asthma?," *Journal of Allergy and Clinical Immunology*, vol. 145, no. 6, pp. 1701–1704, 2021, doi: 10.1016/j.jaci.2020.01.021.Serum.

[8] W. A. Neveu *et al.*, "Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function," *Respir Res*, vol. 11, no. 28, pp. 1–10, 2010.

[9] Z. Jevnikar *et al.*, "Epithelial IL-6 trans-signaling defines a new asthma phenotype with increased airway inflammation.," *J Allergy Clin Immunol*, vol. 143, no. 2, pp. 577–590, Feb. 2019, doi: 10.1016/j.jaci.2018.05.026.

[10] P. Permaul *et al.*, "The association of plasma IL-6 with measures of asthma morbidity in a moderate-severe pediatric cohort aged 6-18 years," *J Allergy Clin Immunol Pract*, vol. 9, no. 7, pp. 2916-2919.e2, doi: 10.1016/j.jaip.2021.02.047.

[11] R. Wang, H. Jin, S. Shang, X. Liu, S. Chen, and Z. Jin, "Associations of IL-2 and IL-4 Expression and Polymorphisms With the Risks of Mycoplasma pneumoniae Infection and Asthma in Children &," *Arch Bronconeumol*, vol. 51, no. 11, pp. 571–578, 2015.

[12] G. Takayama *et al.*, "Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals.," *J Allergy Clin Immunol*, vol. 118, no. 1, pp. 98–104, Jul. 2006, doi: 10.1016/j.jaci.2006.02.046.

[13] A. Munitz, E. B. Brandt, M. Mingler, F. D. Finkelman, and M. E. Rothenberg, “Distinct roles for IL-13 and IL-4 via IL-13 receptor alpha1 and the type II IL-4 receptor in asthma pathogenesis.,” *Proc Natl Acad Sci U S A*, vol. 105, no. 20, pp. 7240–7245, May 2008, doi: 10.1073/pnas.0802465105.

[14] E. Truyen *et al.*, “Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients.,” *Thorax*, vol. 61, no. 3, pp. 202–208, Mar. 2006, doi: 10.1136/thx.2005.052399.

[15] P. S. Thomas *et al.*, “Exhaled breath condensate in pediatric asthma: promising new advance or pouring cold water on a lot of hot air? a systematic review.,” *Pediatr Pulmonol*, vol. 48, no. 5, pp. 419–442, May 2013, doi: 10.1002/ppul.22776.

[16] C. M. H. H. T. Robroeks *et al.*, “Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma.,” *Clin Exp Allergy*, vol. 37, no. 9, pp. 1303–1311, Sep. 2007, doi: 10.1111/j.1365-2222.2007.02788.x.

[17] S. Sánchez-García, A. Habernau Mena, and S. Quirce, “Biomarkers in inflammometry pediatric asthma: utility in daily clinical practice.,” *Eur Clin Respir J*, vol. 4, no. 1, p. 1356160, 2017, doi: 10.1080/20018525.2017.1356160.

[18] A. Chiappori, L. De Ferrari, C. Folli, P. Mauri, A. M. Riccio, and G. W. Canonica, “Biomarkers and severe asthma: A critical appraisal,” *Clinical and Molecular Allergy*, vol. 13, no. 1, pp. 1–11, 2015, doi: 10.1186/s12948-015-0027-7.

[19] M. Y. Hachim *et al.*, “Derangement of cell cycle markers in peripheral blood mononuclear cells of asthmatic patients as a reliable biomarker for asthma control,” *Sci Rep*, vol. 11, no. 1, pp. 1–24, 2021, doi: 10.1038/s41598-021-91087-5.

[20] A. H. Cui, J. Zhao, S. X. Liu, and Y. S. Hao, “Associations of IL-4, IL-6, and IL-12 levels in peripheral blood with lung function, cellular immune function, and quality of life in children with moderate-to-severe asthma,” *Medicine (United States)*, vol. 96, no. 12, 2017, doi: 10.1097/MD.0000000000006265.

[21] X.-H. Chen, S. Huang, and D. Kerr, *Biomarkers in clinical medicine*, vol. 163. IARC Sci Publ, 2011.

Reviewer 2

The manuscript "Identification of hub genes and potential biomarkers for childhood asthma by utilizing an established bioinformatic analysis approach" describes a search for Single Nucleotide Polymorphisms (SNPs) associated with childhood asthma in Asian populations in a GWAS database. The reason for the search was the potential to find reliable genetic markers to identify asthma and start therapy earlier. Another reason to search for biomarkers was to find targets for new treatment options. The identified biomarkers were IL6 and IL4.

The methods are carefully performed and well described and the conclusions based on the results. However, there are some concerns with the study:

Major concerns:

1. The results are not surprising or new. We already knew this. How well do these biomarkers identify asthmatics to help start therapy earlier? With what rates do these biomarkers give false positives/negatives? The usefulness of the results needs to be further discussed.
   Answer: We sincerely thank the reviewer's comments and suggestions. The role of biomarkers in asthma diagnosis, prediction of asthma severity, prognosis, and treatment response has been extensively studied. Using biomarkers to assess the likelihood of an exacerbation might be more effective than evaluating and researching the biology of exacerbations. A reliable genetic marker could identify and treat childhood asthma more quickly. The discovery of new genetic targets can also provide new insights into the pathophysiology of childhood asthma [1]. The discovery of new biomarkers may aid in categorizing patients, therapeutic responses, and clinical outcome prediction [2]. Most biomarkers for asthma are confined to the Th2 phenotype, and no useful biomarkers for severe asthma have been confirmed in both children and adults [3]. Our study found that IL6 and IL4 are potential biomarkers for childhood asthma based on a bioinformatic approach. Increased levels of IL6 and IL4 could reduce lung function [4]. IL-6 and IL4 represent further promising markers. Research on genetic predisposition, through genome-wide association studies or specific gene findings, highlighted an increasing number of genetic polymorphisms involved. Many works reported genetic overlap between previously described asthma susceptibility genes and exacerbation-associated loci. In particular, we would like to emphasize that our method of genetic-driven biomarkers ultimately provided a candidate list of the biomarker in childhood asthma to be used in clinical outcome predictions. However, we realize that our study has limitations. The biomarker found in this pipeline has not been validated. Nevertheless, further research is required to verify this finding.

2. Can IL6 and IL4 be used as therapy targets in children? This question needs to be discussed.
   Answer: Many thanks for the reviewer's comments. Through a genetic-driven biomarkers approach, we identified that IL6 and IL4 could be potential target therapy for childhood asthma. A reduction in lung function could be caused by elevated levels of IL6 and IL4. IL6 and IL4 represent further promising markers for childhood asthma. However, IL-6 was associated with asthma exacerbation risk asthma in adults but not with symptoms or lung function in children. Interestingly, IL6 increased exacerbation risk in children but did not affect lung function or other severity indicators as in adults. It's possible that children with frequent exacerbations and high IL6 levels may grow up to be adults with severe asthma [5]. Based on

longitudinal analyses, children with higher plasma IL6 levels are related to obesity, metabolic syndrome, and greater asthma severity, with a risk for asthma exacerbation and decreased lung function [6]. IL4 is an important cytokine involved in asthma development [7] and it has been reported that airway hyperresponsiveness, eosinophil infiltration, and inflammation are all symptoms of bronchial asthma that IL4 likely mediates [8]. IL4 is a potent activator of inflammation and is involved in developing fibrosis during Th2 inflammation [9]. In asthma patients, Th2 is hyperactive, causing a rise in IL4 and immunoglobulin E (IgE), which stimulates the growth and activation of eosinophilic granulocytes, which then secretes a variety of inflammatory mediators, leading to bronchial chronic inflammation and asthma [10]. In addition, in asthmatic children, levels of IL4 were significantly higher among atopic asthmatics than nonatopic asthmatics [11,12]. IL4 is an anti-inflammatory protein that can modulate inflammation, thus preventing asthma and vice versa [7]. Particularly, our genetic-driven biomarker approach ultimately provided a candidate list for the biomarker to be used in clinical outcome prediction in childhood asthma. However, it will be helpful to carry out more studies from animal models and clinical trials to determine the mechanisms of IL6 and IL4 as targeted therapy for asthma childhood.

3. The goal is the Asian population, but can the same biomarkers be used in other populations? This needs to be addressed.
   Answer: We thanks for the reviewer comments. Biomarkers play an important role in disease treatment, prognosis, and management in many different ways. Several common diseases are very heterogeneous, as the same disease may show different phenotypes, may be caused by different genetic mechanisms and may respond differently to the same treatment. In general, the same biomarker could be used in all populations. Biomarkers can indicate a variety of health or disease characteristics, including the level or type of exposure to an environmental factor, genetic susceptibility, genetic response to environmental exposures, markers of subclinical or clinical disease, or indicators of response to therapy [13].

Minor concerns:

The language needs to be improved. Some examples:

1. Abstract: Hence, new biomarkers for diagnosing and predicting therapy responses for childhood asthma are emergence needed. What do you mean by this sentence? Are biomarkers urgently needed, or what is the message here
   Answer: Thank you for the reviewer comments. We have revised the sentence to " In the long run, new biomarkers for asthma in children are required and would help direct therapy approaches." **(line 16-17)**

2. Line 53: However, childhoods with severe asthma exacerbation are still poorly understood and treated significantly. What do you mean by this statement? Please rephrase to clarify the point.
   Answer: Many thanks for the reviewer's comment and suggestion. We have revised the sentence to "However, the causes of and effective therapy for childhood severe asthma exacerbations remain poorly known." **(line 60-61)**

3. Next sentence: In addition, high-dose inhaled corticosteroid therapy results in severe asthma patients who are unsatisfactory, with uncontrolled symptoms and frequent exacerbations. The patients are not unsatisfactory, I hope. Please rephrase.
   Answer: Thank you for the reviewer's comment and suggestions. We have rephrased it to "severe asthma unresponsive to inhaled corticosteroids (ICS) therapy resulting in poor symptom control and increased exacerbations." **(line 61-62).**

4. The introduction lacks focus and the language needs improvement.
   Answer: We sincerely thank the reviewer for taking the time to review our work. We have revised the manuscript accordingly and requested professional proofreaders to improve the overall language quality.

References

1. Sánchez-García, S.; Habernau Mena, A.; Quirce, S. Biomarkers in Inflammometry Pediatric Asthma: Utility in Daily Clinical Practice. *Eur Clin Respir J* **2017**, *4*, 1356160, doi:10.1080/20018525.2017.1356160.

2. Chiappori, A.; De Ferrari, L.; Folli, C.; Mauri, P.; Riccio, A.M.; Canonica, G.W. Biomarkers and Severe Asthma: A Critical Appraisal. *Clinical and Molecular Allergy* **2015**, *13*, 1–11, doi:10.1186/s12948-015-0027-7.

3. Hachim, M.Y.; Elemam, N.M.; Ramakrishnan, R.K.; Salameh, L.; Olivenstein, R.; Hachim, I.Y.; Venkatachalam, T.; Mahboub, B.; Al Heialy, S.; Hamid, Q.; et al. Derangement of Cell Cycle Markers in Peripheral Blood Mononuclear Cells of Asthmatic Patients as a Reliable Biomarker for Asthma Control. *Sci Rep* **2021**, *11*, 1–24, doi:10.1038/s41598-021-91087-5.

4. Cui, A.H.; Zhao, J.; Liu, S.X.; Hao, Y.S. Associations of IL-4, IL-6, and IL-12 Levels in Peripheral Blood with Lung Function, Cellular Immune Function, and Quality of Life in Children with Moderate-to-Severe Asthma. *Medicine (United States)* **2017**, *96*, doi:10.1097/MD.0000000000006265.

5. Jackson, D.J.; Bacharier, L.B.; Calatroni, A.; Gill, M.A.; Hu, J.; Liu, A.H.; Wheatley, L.M.; Gern, J.E.; Gruchalla, R.S.; Hershey, G.K.K.; et al. Serum IL-6: A Biomarker in Childhood Asthma? *Journal of Allergy and Clinical Immunology* **2021**, *145*, 1701–1704, doi:10.1016/j.jaci.2020.01.021.Serum.

6. Permaul, P.; Mas, M.C.P.; Ma, C.R.P.; Carlos, J.; Mph, C.; Ly, N.P.; Mph, S.K.R.; Ross, K.; Fitzpatrick, A.; Israel, E.; et al. The Association of Plasma IL-6 with Measures of Asthma Morbidity in a Moderate-Severe Pediatric Cohort Aged 6-18 Years. *J Allergy Clin Immunol Pract 9*, 2916-2919.e2, doi:10.1016/j.jaip.2021.02.047.

7. Wang, R.; Jin, H.; Shang, S.; Liu, X.; Chen, S.; Jin, Z. Associations of IL-2 and IL-4 Expression and Polymorphisms With the Risks of Mycoplasma Pneumoniae Infection and Asthma in Children &. *Arch Bronconeumol* **2015**, *51*, 571–578.

8. Takayama, G.; Arima, K.; Kanaji, T.; Toda, S.; Tanaka, H.; Shoji, S.; McKenzie, A.N.J.; Nagai, H.; Hotokebuchi, T.; Izuhara, K. Periostin: A Novel Component of Subepithelial Fibrosis of

Bronchial Asthma Downstream of IL-4 and IL-13 Signals. *J Allergy Clin Immunol* **2006**, *118*, 98–104, doi:10.1016/j.jaci.2006.02.046.

9. Munitz, A.; Brandt, E.B.; Mingler, M.; Finkelman, F.D.; Rothenberg, M.E. Distinct Roles for IL-13 and IL-4 via IL-13 Receptor Alpha1 and the Type II IL-4 Receptor in Asthma Pathogenesis. *Proc Natl Acad Sci U S A* **2008**, *105*, 7240–7245, doi:10.1073/pnas.0802465105.

10. Truyen, E.; Coteur, L.; Dilissen, E.; Overbergh, L.; Dupont, L.J.; Ceuppens, J.L.; Bullens, D.M.A. Evaluation of Airway Inflammation by Quantitative Th1/Th2 Cytokine MRNA Measurement in Sputum of Asthma Patients. *Thorax* **2006**, *61*, 202–208, doi:10.1136/thx.2005.052399.

11. Thomas, P.S.; Lowe, A.J.; Samarasinghe, P.; Lodge, C.J.; Huang, Y.; Abramson, M.J.; Dharmage, S.C.; Jaffe, A. Exhaled Breath Condensate in Pediatric Asthma: Promising New Advance or Pouring Cold Water on a Lot of Hot Air? A Systematic Review. *Pediatr Pulmonol* **2013**, *48*, 419–442, doi:10.1002/ppul.22776.

12. Robroeks, C.M.H.H.T.; van de Kant, K.D.G.; Jöbsis, Q.; Hendriks, H.J.E.; van Gent, R.; Wouters, E.F.M.; Damoiseaux, J.G.M.C.; Bast, A.; Wodzig, W.K.W.H.; Dompeling, E. Exhaled Nitric Oxide and Biomarkers in Exhaled Breath Condensate Indicate the Presence, Severity and Control of Childhood Asthma. *Clin Exp Allergy* **2007**, *37*, 1303–1311, doi:10.1111/j.1365-2222.2007.02788.x.

13. Chen, X.-H.; Huang, S.; Kerr, D. *Biomarkers in Clinical Medicine*; IARC Sci Publ, 2011; Vol. 163;.

Komentar reviewer 1 tahap 2

Authors' Responses to Reviewer's Comments (Reviewer 1)

Author's Notes: We sincerely thank you for the reviewer's comments and suggestions.

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Comments and Suggestions for Authors

Submission Date: 12 August 2022
Date of this review: 07 Sep 2022 13:11:25

Komentar reviewer 2 tahap 2

Authors' Responses to Reviewer's Comments (Reviewer 2)

Author's Notes	Please see the file attached. Thank you
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Comments and Suggestions for Authors

The changes made to the manuscript have improved the understanding, but there are still errors in the language. Please do not use contracted form as this is a scientific text. There are also some sentences in need of explanation:

Line 44: "amongst the top 20 countries in the world." What do you mean by top 20 countries? Top in regard to what?

Line 63: What is GINA?

Line 81 and 220: What is MCC/MCC algorithm?

Submission Date	12 August 2022
Date of this review	07 Sep 2022 09:08:15

Merespon komentar reviewer tahap 2

September 8, 2022

Dear Editors,

We appreciate this opportunity to submit our revised manuscript entitled " **Identification of hub genes and potential biomarkers for childhood asthma by utilizing an established bioinformatic analysis approach,**" which we are submitting for consideration for publication as an Original Research article in "***Biomedicines"* **(Manuscript ID: biomedicines-1888331)**. We would like to thank the editor and reviewers for their constructive and insightful comments. Here, we are sending our revised manuscript in accordance with the comments given by the reviewers. We have carefully examined the raised questions and have answered or made changes accordingly. The revised parts are highlighted as a yellow color. We believe that the manuscript is greatly improved by the revisions. Thank you very much for your consideration of our work. Hope very much these revisions are adequate. We appreciate your assistance and are looking forward to hearing from you.

Sincerely yours,

Apt. Wirawan Adikusuma, M.Sc., Ph.D
Department of Pharmacy,
University of Muhammadiyah Mataram, Indonesia
Jl. KH. Ahmad Dahlan No.1, Pagesangan, Mataram, Nusa Tenggara Barat, 83115, Indonesia

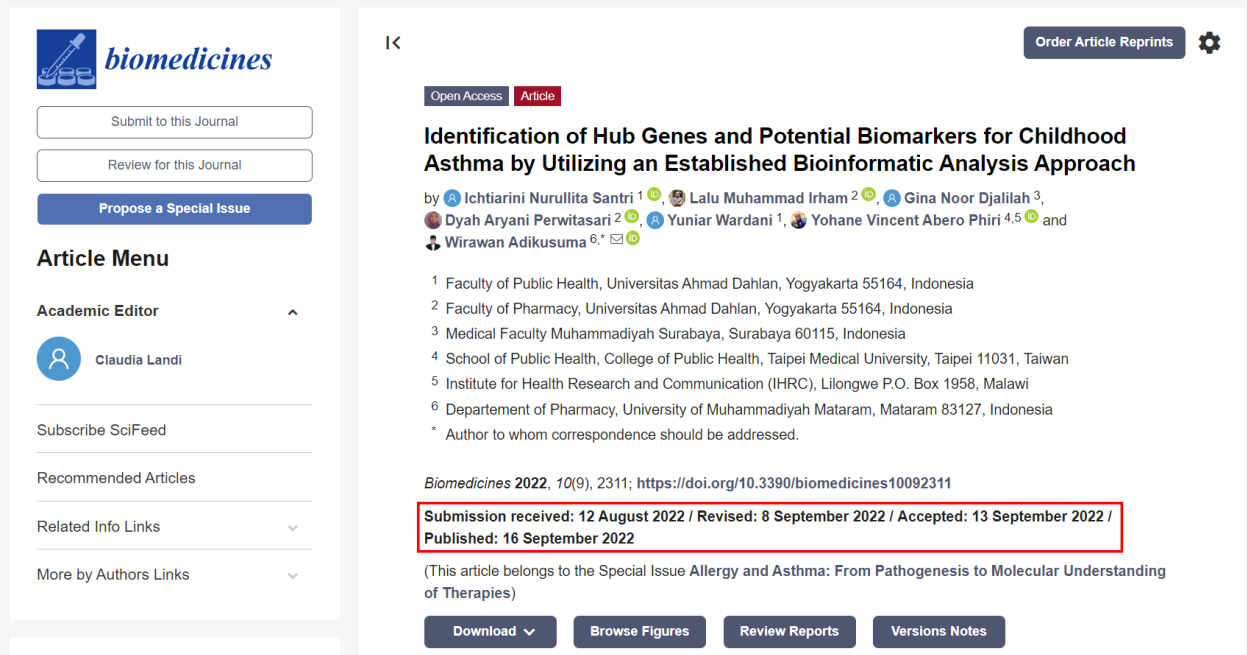
The changes made to the manuscript have improved the understanding, but there are still errors in the language. Please do not use contracted form as this is a scientific text. There are also some sentences in need of explanation:

1. Line 44: "amongst the top 20 countries in the world." What do you mean by top 20 countries? Top in regard to what?
   Answer: Many thanks for the reviewer's comments. We have revised the sentence to be "Asthma is the most prevalent chronic disease affecting children, and it is among the top 20 conditions in the world for disability-adjusted life years in children." **(line 43-45)**

2. Line 63: What is GINA?
   Answer: We thank the reviewer's comments. Gina is Global Initiative for Asthma. We have added it in the introduction part **(line 64)**

3. Line 81 and 220: What is MCC/MCC algorithm?
   Answer: We sincerely thank the reviewer's comments. MCC is a maximal clique centrality method to predict essential proteins more accurately from the yeast PPI network **(line 81–82).**

Artikel accepted 13 September 2022 dan terbit pada tanggal 16 September 2022



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Identification of Hub Genes and Potential Biomarkers for Childhood Asthma by Utilizing an Established Bioinformatic Analysis Approach

by Ichtiarini Nurullita Santri [1], Lalu Muhammad Irham [2], Gina Noor Djalilah [3], Dyah Aryani Perwitasari [2], Yuniar Wardani [1], Yohane Vincent Abero Phiri [4,5] and Wirawan Adikusuma [6,*]

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