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# Identification of Novel Gene Expression Patterns and Genetic Mechanisms in Asthma affected Patients Treated with Budesonide

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**Abstract**

***Aim:*** *To identify the significant genes and pathways involved in asthma patients and asthma-affected patients treated with Budesonide is the aim of our research.*

***Materials and Methods:*** *DNA microarray analysis for asthma has been performed and significant DEGs are identified. Up-regulated genes and down-regulated genes were identified by GEO2R analysis. Gene-Gene interaction was predicted using the STRING. Gene Ontology was analyzed by using the STRING and FunRich. Hub genes were observed by using CytoHubba plugins of Cytoscape.* ***Results:*** *By analyzing GEO2R, 22 genes were upregulated genes and 16 genes were downregulated genes. We have obtained the gene comprising 28 nodes and 8 edges with an estimating clustering coefficient of 0.25 in pHBECs with not treated and pHBECs treated with Budesonide. Gene ontology has shown the 27 genes located in the large intestine as a COSMIC analysis more than other analyses. By using the CytoHubba plugin of Cytoscape, identified MMP3, TSLP, POSTN, ETS1, and SAA1 as hub genes.* ***Conclusion:*** *Due to this limitation, the medications that are brought into the market are not site-directed, and rather they showed random inhibitory actions. So we have developed a computational pipeline to identify the significant novel genes and novel pathways involved in the asthma patient and asthma-affected patient treated with Budesonide.*

**Key-words:** Asthma, Significant Genes, Budesonide, Primary Human Bronchial Epithelial Cells (pHBEC) Control (Non-stimulated), Primary Human Bronchial Epithelial Cells (pHBEC) Budesonide, Gene Ontology, and Hub Genes**,** Novel Genetic Mechanism, Molecular Biology, Genetic Analysis, Gene Expression.

## 1. Introduction

Asthma is a chronic inflammatory disorder of the airways. It is characterized by recurrent symptoms of breathing and reduction of airflow (Quirt et al. 2018). Asthma is a disorder with several variants of the condition (I. Agache et al. 2012). In children and adults, asthma is a non-communicative disorder. Asthma is a cause of specific gene-environment interactions with clinical heterogeneity as well as airway inflammation and remodeling type and intensity (Papi et al. 2018). Asthma is clinically characterized by the manifestation of cough, wheeze, breath loss on the respiratory tract, and hypersensitivity. Due to the worldwide increase in asthma prevalence, mortality and morbidity have increased rapidly. Asthma patients are not necessarily easily identified and may not be treated as optimally as possible (Murata and Ling 2012). Asthma is characterized by increased respiratory hyperresponsiveness, airway constriction, and remodeling of an inflammatory response in the respiratory system (Wall et al. 2018). Primary human bronchial epithelial cells (pHBEC) have decreased antioxidants and increased oxidative response (Vaughan et al. 2017). The NIH reported that COVID-19, which can affect the nose, mouth, and lung that might lead to serious problems for asthma patients ("Website" n.d.). The identification of the gene targets is highly important to develop a new drug. Gene mutations have a major effect on the risk of asthma development (Ioana Agache and Akdis 2019). Our research could be applicable to identifying the crucial genes which are upregulated and downregulated asthma patients and asthma-affected patients treated with Budesonide in the field of drug discovery.

Budesonide has been considered in clinical trials and is one of the inhaled corticosteroids used for the treatment of asthma (O'Connell 2002). Budesonide is a medicine used for the prevention and control, especially in the airways disease, gastrointestinal tract, and inflammatory diseases (Kalola and Ambati 2020). Budesonide is a potent anti-inflammatory medication. It binds and activates glucocorticoid receptor (GR) in the effector cell cytoplasm which allows the translocation in the bronchial nucleus of this budesonide-GR complex, which binds in both HDCA2 and with CBP (HAT) (Kalola and Ambati 2020; Adcock and Mumby 2017). This CBP (HAT) receptor prevents the inhibition of gene transcription which can lead to bronchoconstriction (Kalola and Ambati 2020). Budesonide is a highly topical glucocorticoid with low systemic bioavailability, and in contrast with other glucocorticoids decreases systemic effects (Brogden and McTavish 1992; Mostafa et al. 2019). The esterification mechanism increases the lipophilicity of budesonide to a larger extent than of other ICSs (O'Connell 2003). Budesonide inhalation suspension (BIS) is used in children aged 6 months to 5 years for the long-period treatment of asthma (Hvizdos and Jarvis 2000; Berger 2005).

Previously our team has a rich experience in working on various research projects across multiple disciplines (Sathish and Karthick 2020; Varghese, Ramesh, and Veeraiyan 2019; S. R. Samuel, Acharya, and Rao 2020; Venu, Raju, and Subramani 2019; M. S. Samuel et al. 2019; Venu, Subramani, and Raju 2019; Mehta et al. 2019; Sharma et al. 2019; Malli Sureshbabu et al. 2019; Krishnaswamy et al. 2020; Muthukrishnan et al. 2020; Gheena and Ezhilarasan 2019; Vignesh et al. 2019; Ke et al. 2019; Vijayakumar Jain et al. 2019; Jose, Ajitha, and Subbaiyan 2020). Now the growing trend in this area motivated us to pursue this project.

The major lacunae is that in other research they haven't studied the genes involved in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and treated with Budesonide in asthma. Due to this limitation, the medications that are brought into the market are not site-directed and rather they showed random inhibitory actions. So we have a computational pipeline to identify the significant genes and pathways involved in the asthma patient and asthma patient treated with Budesonide is the aim of our research.

## 2. Materials and Methods

### 2.1 Dataset

The expression gene dataset of primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and treated with Budesonide were computed from NCBI Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/). GSE161805 consists of 2 groups with 6 samples each. Grouping the dataset into 2 groups and classified the Group as primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide. In classification, each Group has 6 samples. GEO2R was used to identify the expressed genes (Ruan, Wang, and Li 2006) shown in Table 1.

Table 1 - Retrieving Gene Expression Dataset of Primary Human Bronchial Epithelial Cells (pHBEC) with not Treated and Primary Human Bronchial Epithelial Cells (pHBEC) Treated with Budesonide from GSE161805

| Group | Accession | Title | Source name | Subject/donor id | Cell type | Treatment | No of sample in each group |
|---|---|---|---|---|---|---|---|
| GSM4914821 | pHBEC, control, 6h, donor 1 | pHBEC_control_6h | donor 1 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | 6 |
| GSM4914822 | pHBEC, control, 6h, donor 2 | pHBEC_control_6h | donor 2 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | |
| GSM4914823 | pHBEC, control, 6h, donor 3 | pHBEC_control_6h | donor 3 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | |
| GSM4914824 | pHBEC, control, 6h, donor 4 | pHBEC_control_6h | donor 4 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | |
| GSM4914825 | pHBEC, control, 6h, donor 5 | pHBEC_control_6h | donor 5 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | |
| GSM4914826 | pHBEC, control, 6h, donor 6 | pHBEC_control_6h | donor 6 | primary human bronchial epithelial cells (pHBEC) | Control (non-stimulated) | 6h | |
| GSM4914827 | pHBEC, bud, 6h, donor 1 | pHBEC_bud_6h | donor 1 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | 6 |
| GSM4914828 | pHBEC, bud, 6h, donor 2 | pHBEC_bud_6h | donor 2 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | |
| GSM4914829 | pHBEC, bud, 6h, donor 3 | pHBEC_bud_6h | donor 3 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | |
| GSM4914830 | pHBEC, bud, 6h, donor 4 | pHBEC_bud_6h | donor 4 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | |
| GSM4914831 | pHBEC, bud, 6h, donor 5 | pHBEC_bud_6h | donor 5 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | |
| GSM4914832 | pHBEC, bud, 6h, donor 6 | pHBEC_bud_6h | donor 6 | primary human bronchial epithelial cells (pHBEC) | Budesonide | 6h | |

## 2.2. Identification of Significant Genes

In GEO2R analysis, the P-value cut was set greater than 0.05 ($0.05 < p \leq 1$) from the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated), and primary human bronchial epithelial cells (pHBEC) Budesonide (Udhaya Kumar et al. 2020) (S. et al. 2020).

### 2.3. Classification of Up-regulated and Down-regulated Genes

In GEO2R analysis, the upregulated genes (UG) (log FC $\geq 1$) and downregulated genes (DG) (log FC $\leq -1$) were identified from the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated), and primary human bronchial epithelial cells (pHBEC) Budesonide (Udhaya Kumar et al. 2020)(S. et al. 2020).

### 2.4 Gene-Gene Interaction

STRING database (Search Tool for the Retrieval of Interacting Proteins) is a web-based software used for Gene-Gene Interaction, and Functional Enrichment Analysis (https://string-db.org/). Upregulated genes and downregulated genes in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide were given as input in the STRING prediction to identify the gene-gene interactions (Mering et al. 2003).

### 2.5 Gene Enrichment Analysis

Upregulated genes and downregulated genes in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide were given as input in the STRING prediction to identify the Gene Ontology (Mering et al. 2003).

### 2.6 Refined Gene Enrichment Analysis

FunRich database (functional enrichment analysis) is a software tool used for the analysis of the Gene Ontology and pathways. Enrichment analysis contains cellular components (CC), molecular function (MF), biological processes (BP), biological pathways, a protein domain, a site of expression, transcription factor, clinical phenotype and cosmic analysis. Upregulated genes and downregulated genes in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide were given as input in the FunRich database (Pathan et al. 2015).

### 2.7 Hub Gene Identification

CytoHubba gives a fast interface for the analysis of the molecular interaction network. String interaction in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide were given as input in CytoHubba (Chin et al. 2014).

### 2.8 Integrated Gene Enrichment

CluePedia includes Gene ontology in the analysis of the pathways interaction. In CluePedia, Ontologies pathways like Biological pathways, Cellular component, Immune system process, Molecular function, Protein domains, KEGG, Reactome, and Wikipathways are selected. String interaction in the primary human bronchial epithelial cells (pHBEC) Control (non-stimulated) and primary human bronchial epithelial cells (pHBEC) Budesonide were given as input in CluePedia (Bindea, Galon, and Mlecnik 2013).

## 3. Results

### 3.1 Identification of Significant Genes

In GEO2R analysis, we have observed 38 significant genes in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Table. 2.

Table 2 - A number of Significant Genes and Upregulated and Downregulated Genes from Primary Human Bronchial Epithelial Cells (pHBEC) with not Treated and Primary Human Bronchial Epithelial Cells (pHBEC) Treated with Budesonide

| | |
|---|---|
| Total number of significant genes | 38 |
| Total number of upregulated genes | 22 |
| Total number of downregulated genes | 16 |

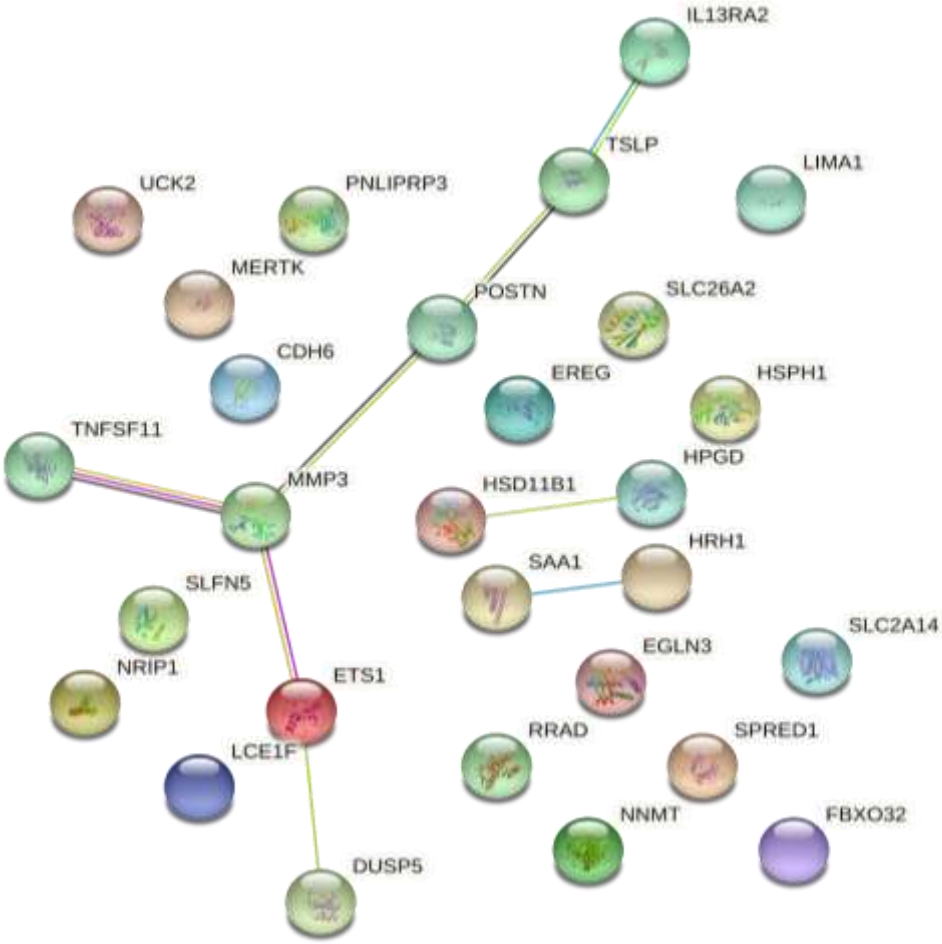
### 3.2 Classification of Up-regulated and Down-regulated Genes

In GEO2R analysis, we have observed 22 upregulated genes and 16 downregulated genes in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Table. 2.

### 3.3 Gene-Gene Interaction

In STRING analysis, we have observed the gene comprised of 28 nodes and 8 edges with an estimating clustering coefficient of 0.25 in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Fig. 1.

Fig. 1 - Gene Gene Interaction Obtained from STRING Analysis for Primary Human Bronchial Epithelial Cells (pHBEC) with not Treated and Primary Human Bronchial Epithelial Cells (pHBEC) Treated with Budesonide



### 3.4 Gene Enrichment Analysis

In STRING analysis, we have predicted Gene Ontology as 61 biological processes, and 2 cellular components in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Table. 3.

Table 3 - Gene Ontology Obtained from STRING Analysis for (pHBEC) with not Treated and (pHBEC) Treated with Budesonide. In the Biological Process, 20 Genes are a Response to Stimulus and in Cellular Components, 8 Genes are Present in Extracellular Space

| Analysis | Team description | Gene count |
|---|---|---|
| Biological Process | Response to stimulus | 20 |
| Cellular Components | Extracellular space | 8 |

### 3.5 Refined Gene Enrichment Analysis

In FunRich analysis, we analyzed the 9 genes involved in the cytoplasm as a cellular component, 3 genes involved in molecular functions unknown, Transporter activity and Catalytic activity as a molecular function, 9 genes involved in the signal transduction as a biological process, 10 genes involved in ErbB receptor signaling pathway involved as a biological pathways, 9 genes involved in the signal peptide and transmembrane domain as a protein domain, 21 genes involved in HUVEC as a site of expression, 12 genes involved in SPI as a transcription factor, 3 genes involved in Abdomen, Abducted thumbs and great toes and Absent or minimally ossified vertebral bodies as a clinical phenotype and 27 genes involved in the large intestine as a cosmic in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Table. 4.

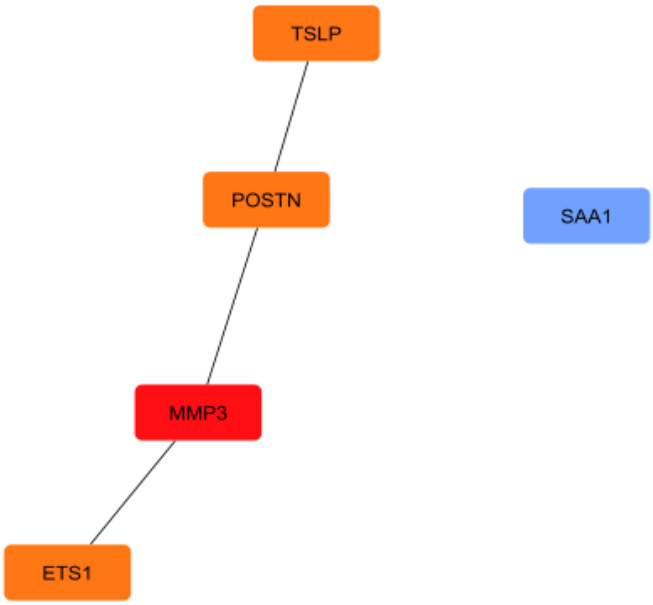
Table 4 - Gene Enrichment Analysis Obtained from FunRich for Primary Human Bronchial Epithelial Cells (pHBEC) with not Treated and Primary Human Bronchial Epithelial Cells (pHBEC) Treated with Budesonide

| Analysis | Analysis detail | No of the genes in the dataset |
|---|---|---|
| Cellular component | Cytoplasm | 9 |
| Molecular function | Molecular functions unknown, Transporter activity and Catalytic activity | 3 each |
| Biological process | Signal transduction | 9 |
| Biological pathway | ErbB receptor signaling pathway | 10 |
| Protein domain | Signal peptide and transmembrane domain | 9 each |
| Site of expression | HUVEC | 21 |
| Transcription factor | SPI | 12 |
| Clinical phenotype | Abdomen, Abducted thumbs and great toes, and Absent or minimally ossified vertebral bodies | 3 each |
| COSMIC | Large intestine | 27 |

### 3.6 Hub Gene Identification

In the CytoHubba plugin of Cytoscape, we identified MMP3, TSLP, POSTN, ETS1, and SAA1 as hub genes in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide shown in Fig. 2.

Fig. 2 - Hub Gene Identification from CytoHubba for Primary Human Bronchial Epithelial Cells (pHBEC) with not Treated and Primary Human Bronchial Epithelial cells (pHBEC) Treated with Budesonide. The Genes are MMP3, TSLP, POSTN, ETS1, and SAA1 as Hub Genes




### 3.7 Integrated Gene Enrichment

In the CluePedia plugin of Cytoscape, we haven't identified the pathway involved in primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide because there is no well-defined interaction and pathway involved here (Bindea, Galon, and Mlecnik 2013).

## 4. Discussion

The microarray dataset was retrieved from NCBI Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) with ID GSE161805. By using GEO2R, differentially expressed genes (DEGs) were identified among primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide. A total of 38 significant genes (SG), 22 upregulated genes (UG), and 16 downregulated (DG) were identified from

GEO2R analysis. TNFSF11, MMP3, ETS1, POSTN, TSLP, DUSP5, IL13RA2, HSD11B1, HPGD, SAA1, and HRH1 genes were shown interaction in STRING whereas MMP3, TSLP, POSTN, ETS1, and SAA1 genes were identified as a hub gene by CytoHubba.

The hub genes are MMP3, TSLP, POSTN, ETS1, and SAA1. Matrix metalloproteinase 3 (MMP3), according to gene ontology, takes part in metallopeptidase activity and calcium ion binding (Yuan et al. 2010). An effective strategy to prevent respiratory disorders might be an MMP inhibitor (Vandenbroucke, Dejonckheere, and Libert 2011). In Thymic Stromal Lymphopoietin (TSLP), gene ontology explained that this gene interacts with cytokine activity (He et al. 2009). In comparison with healthy controls, asthmatic epithelial cells have increased the Thymic Stromal Lymphopoietin (TSLP) protein secretion (Moorehead et al. 2020). In Periostin (POSTN), gene ontology explained that this gene includes cell adhesion molecule binding and heparin-binding (Coutu et al. 2008). Periostin is expressed in the lungs of idiopathic respiratory fibrosis patients, and its serum concentrations will estimate drug outcomes (Izuhara et al. 2016). ETS Proto-Oncogene 1, Transcription Factor (ETS1), gene ontology explained that this gene interacts with DNA-binding transcription factor activity and transcription factor binding (Lamber et al. 2008). In Serum Amyloid A1 (SAA1), gene ontology explained that this gene includes chemoattractant activity and heparin-binding (Carty et al. 2009). Thus, these hub genes can further be used for computer-aided drug design to develop a drug for asthma.

The interacting genes observed from the CytoHubba plugin of Cytoscape analysis have shown that these hub genes are related to primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide but in some studies, these genes are involved in other diseases. MMP3 gene is involved in many cardiovascular diseases such as Coronary heart disease (CHD) (Pawlik et al. 2017). The POSTN gene is involved in cancer and it is important for developing drugs for POSTN function (González-González et al. 2019). However, these genes related to primary human bronchial epithelial cells (pHBEC) with not treated and primary human bronchial epithelial cells (pHBEC) treated with Budesonide was not in the existing literature and also the analyses performed in this study.

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Vijayashree Priyadharsini 2019; Ezhilarasan, Apoorva, and Ashok Vardhan 2019; Ramesh et al. 2018; Mathew et al. 2020; Sridharan et al. 2019; Pc, Marimuthu, and Devadoss 2018; Ramadurai et al. 2019). We hope this study adds to this rich legacy.

The major limitation of our study is that these genes and pathways need to be confirmed by wet lab techniques such as western blot and RT-PCR before they can be clinically applied. Only the significant genes were identified but the reason for the upregulated and downregulated is not studied. Studying the mutations and post-translational modifications are further essential to understand the role of these genes in the disease. We have developed a computational pipeline to identify the significant genes and pathways involved in the asthma patient and asthma patient treated with Budesonide. Our research could be applicable to identifying the crucial genes which are upregulated and downregulated asthma patients and asthma-affected patients treated with Budesonide in the field of drug discovery. Based on the identified genes, the results can be further taken to computer-aided drug design to develop a drug for asthma.

## 5. Conclusion

Asthma is a chronic inflammatory disease of the airways. Thus sorted the significant genes and the upregulated genes and downregulated genes during the normal condition, and during the condition treated with Budesonide, and that was analyzed by GEO2R. Due to this limitation, the medications that are brought into the market are not site-directed and rather they showed random inhibitory actions. As the result developed a computational pipeline for identifying the novel significant genes and pathways involved in the asthma patient and treated with Budesonide, and the identified genes can use computer-aided drug design to develop a drug for asthma.

## Declarations

### Conflict of Interest

The authors of this paper declare no conflict of interest.

### Author Contribution

Author DR was involved in data collection, data analysis, manuscript writing. Author MD was involved in conceptualization, guidance and critical review of manuscript.

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