

Review

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Linking childhood allergic asthma phenotypes with endotype through integrated systems biology: current evidence and research needs

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Abstract: Asthma and other complex diseases results from a complex web of interactions involving inflammation, immunity, cell cycle, apoptosis, and metabolic perturbations across multiple organ systems. The extent to which various degrees of the age at onset, symptom severity, and the natural progression of the disease reflect multiple disease subtypes, influenced by unique process of development remains unknown. One of the most critical challenges to our understanding stems from incomplete understanding of the mechanisms. Within this review, we focus on the phenotypes of childhood allergic asthma as the basis to better understand the endotype for quantitative define subtypes of asthma. We highlight some of the known mechanistic pathways associated with the key hallmark events before the asthma onset. In particular, we examine how the recent advent of multi-omic technologies and systems biology could help to clarify our current understanding of the pathway. We review how a large volume of molecular, genomic data generated by multi-omic technologies could be digested to identify cogent pathophysiologic molecular networks. We highlight some recent successes in application of these technologies within the context of other disease conditions for therapeutic interventions. We conclude by summarizing the research needs for the predictive value of preclinical biomarkers.

Keywords: allergic sensitization; asthma; atopic dermatitis; endotype; gene-environment interaction; phenotype; systems biology.

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Introduction

Asthma refers to a syndromic set of conditions, including respiratory constriction due to intermittent and reversible tissue inflammation, airway remodeling, and hyper-reactivity (1). A wide range of symptoms is associated with asthma, in terms of the age of onset, clinical profile, symptom severity, and response to treatments (1). Within the last 100 years, there has been a growing recognition that such syndromic definition of asthma fails to capture heterogeneous pathogenic mechanisms underlying multiple diseases (2, 3). For example, the traditional classification of asthma into allergic IgE-mediated and nonallergic non-IgE-mediated phenotypes fails to capture the intricate network of molecular drivers underlying a wider range of asthma subtypes (4). Such classifications reflect a growing desire within the asthma research community to characterize the causal web of asthma. In recognition of such a knowledge gap, a growing body of literature has made a distinction between asthma phenotype and endotype (3, 5). The notion of asthma ‘phenotype’ is defined as a collection of clinically observable symptoms, which may or may not reflect the underlying pathologic processes (6). Multiple pathophysiologic processes might be disguised as a singular disease entity through common symptoms (6). By contrast, the notion of ‘endotypes’ is built on pathogenetic mechanisms as the foundation for concrete sub-classifications of the disease (6).

Asthma and other complex diseases result from a web of interactions involving inflammation, immunity, cell cycle, apoptosis, and other metabolic perturbations (7). Current paradigm of the asthma development involves intricate interactions between host genetics (8) and environmental stressors (9). Before and first few years of life is likely to be particularly critical period during which epigenetic (9, 10), metabolomic (11), and transcriptomic (12–14) signaling mechanisms influence the robustness of the host’s underlying immune susceptibility (15).

Yet symptomatic definitions of the disease and symptoms management approaches have not been able to stop

the growing global prevalence of asthma nor alleviate its growing burden (4). That is, the identification of high-risk infants/toddlers, even when they do not manifest symptoms, represents urgent research need. Such identification must be paired with a plan to alter the high-risk toddlers' developmental trajectory away from asthma. Clarification of the relationship between phenotype and endotype is needed to break through such barrier. Phenotyping groups demographic, clinical, histologic traits, and pharmacologic therapy profile into concrete subgroups. Careful phenotyping is important not only for early identification of high-risk infants and children but also for the development of intervention strategies at the most opportune window of the affected individuals. For example, reduction in frequency and severity of eczematous inflammation in atopic toddlers through pharmacotherapy could arrest the progression of the symptoms toward asthma (4). In this regard, endotyping, in conjunction with deep phenotyping, has begun to identify biomarkers for pharmacotherapeutic purposes (4). In addition, careful endotyping is expected to clarify causal signaling pathway for particular asthma subtype (3, 6).

Within the present review, we focus on two particular phenotypes of childhood asthma, namely, allergic and noneosinophilic asthma. We examine damp indoor environment as the primary exposure context during early life and the extent to which the natural histories of the phenotypes build the foundation for clarifying specific asthma endotypes. The goal of this review is to summarize the current state of knowledge regarding how the childhood asthma phenotypes are influenced by an interaction between intermediate outcomes and environmental triggers (including air pollution and/or viral infection) in their transition from atopic diseases to respiratory illness. In particular, we survey how the bioinformatic and computing tools could help to meet our goal.

Phenotype characterization

Allergic asthma

One of the prerequisite conditions of allergic asthma diagnosis is confirmation of allergic sensitization (4). Typical approaches for diagnosing allergic asthma also include quantification of specific IgE antibodies, positive skin prick tests, and/or detection of eosinophilia. Allergic asthma is typically heralded by a collection of symptoms before age 6 (4). Such collection of allergy symptoms includes atopic dermatitis (AD), atopic rhinitis, and/or wheezing. Other risk factors of allergic asthma include family histories of

atopic diseases and cigarette smoke exposures. Children with early-onset wheezing symptoms have greater susceptibility to frequent infections and eczema compared with those children with later onset asthma (4, 16). Among the sensitized infants/toddler, respiratory viral infections (e.g. respiratory syncytial virus) modify subsequent asthma risk by moderating the regulatory T-cell function and interleukin-4 (IL-4) receptor pathway (4). During the same period, the early-onset wheezing symptoms are also associated with a largest decrement in lung function (17). Among wheezy children, mucosal epithelial cell death, thickening of basement membrane, predisposition for inflammatory response, and proliferation of eosinophil are particularly severe compared with the healthy controls (17).

Noneosinophilic asthma

Noneosinophilic asthma is typically associated with more severe symptoms (5). It is associated with proliferation of neutrophils and low concentration of airway eosinophil (3, 5). To date, it remains unclear whether noneosinophilic asthma represents a discrete subphenotype of asthma (5). An alternative possibility proposes that neutrophilic state merely reflects a stage within a spectrum of severity or transient response to airway infection (5). The children who suffer from neutrophilic asthma commonly demonstrate similar pattern of airway remodeling as the eosinophilic asthmatic children. In particular, thickened basement membrane, an elevated epithelial cell death, and a greater micro-vascularization are typically observed, just as in eosinophilic asthmatic children (3). In contrast to the eosinophilic asthmatics, those children with neutrophilic asthma display overexpression of interferon- β (IFN- β), IFN- λ 1/IL-29 and IFN-stimulated genes myxovirus resistance 1, oligoadenylate synthetase, and viperin in their sputum cells within innate immune cells (18). Additional biomarkers of neutrophilic response have been proposed as IL-8, IL-17A, leukotriene B₄, IL-32, pathogen-associated molecular patterns, and damage-associated molecular patterns (19).

Exposure characterization as foundation for endotyping

Home indoor dampness

The gestational period represents a critical window during which perturbation in immune system development could

initiate atopic sensitization, AD, rhinitis, and/or wheezing (20, 21). In particular, early-life pathologic processes is speculated to largely influence the phenotype of asthma (22).

Prenatal exposures to modern chemicals of indoor origin (emitted from building material, consumer product uses, and/or lifestyle-related products) within a damp home indoor environment (contributed by structural water damage, poor ventilation, and/or low temperature) contribute to altered development of fetal innate and adaptive immune system cells (21, 23–26). The indoor environment during early life (i.e. prenatal and first few years) is critical in the occurrence of childhood wheezing and asthma (27–29). Indoor environmental conditions are particularly critical for the occupant's health because the general population is estimated to spend >90% of their time indoors (30). Consequently, indoor air at home is estimated to deliver ~80% of total exposure for a child (31). Within the home environment, a wide array of volatile organic compounds (VOCs) are emitted and retained because of the lifestyle activities and/or building construction material. Particularly within damp and/or poorly ventilated indoor environment, known biogenic allergens, adjuvants, and irritants are mutually correlated (32–34). As a result, other unknown correlates of classic airborne allergens and indoor dampness need to be identified (29). For example, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TMPD-DIB, also known as TXIB) and 2,4-trimethyl-1,3-pentanediol monoisobutyrate (TMPD-MIB, also known as TexanolTM) are a few of the most common coalescing agents in latex (i.e. water-based) paints, print-ink, and PVC flooring material (35). They were present in toxic concentration range in temporary housing units constructed for the families displaced by Hurricane Katrina (36). Nonoccupational exposure to TMPD-DIB and TMPD-MIB in the general population is suspected to be high. For example, they are present in considerable concentrations in ambient air in the city of Los Angeles (35), newly painted and installed housing units (36–38), and in food packed in polystyrene and polypropylene cups (39). More generally, propylene glycol, glycol ether, and TexanolTM (PGTex) are also present in paints, cleaning agents, cosmetics, pharmaceuticals, food manufacturing, and food packaging (along with TMPD-DIB and TMPD-MIB) (39, 40). PGTex could induce allergic airway inflammation, heightened production of IL-12, and oxidative stress in allergen sensitized mice (41). Occupational exposures to painted surfaces have been associated with eye irritation, skin

itching, obstructive airway problems, and frequent urination in a longitudinal follow-up studies (42–47). To date, US Environmental Protection Agency posed a significant new rule on 14 of PGTex with known risks (48). Because of lack of data, 11 other PGTex, TMPD-DIB, and TMPD-MIB are presumed safe. Thus, the current data gap regarding human exposures to commercially available PGTex and their risk on unborn children represents the most critical barrier for policy development. An improved understanding of prenatal exposure to indoor synthetic pollutants and the risks on prolonging the Th1/Th2 cell imbalance represents the main challenge to aid interventions, which limit risks during pregnancy.

Prenatal and postnatal immune programming

During pregnancy, multiple hits on the maternal immune system from home indoor exposure to VOCs, and other insults (e.g. obesity, atopy, secondhand smoke, and/or season) could activate innate immune cells (e.g. natural killer cells, macrophages, and neutrophils). Such ordained hits on the maternal immune system promulgate inflammation and resultant oxidative stress, further skewing the maternal T cells toward T helper type 2 (Th2) development (49), while suppressing T regulatory (T_{reg}) cells and the associated Foxp3 gene expression (50). Suppression of maternal T_{reg} cells, in turn, leads to an unbalanced development of cytokines produced by the Th1 and Th2 cells as markers of preclinical, immunotoxic consequences in newborns. Activated Th2 cells, which release IL-4, IL-5, and IL-13, have been associated with asthma symptoms. Increased IL-4 production heightens the IgE level, which arms mast cells and basophils for their detrimental allergen-induced release of bronchoconstrictor mediators (51). IL-5 increases the presence of eosinophils (51), and IL-13 increases bronchoconstriction. Therefore, the environmental factors, which promote a Th1-Th2 imbalance in the prenatal or early postnatal period, in particular, T-cell polarization toward Th2 reactivity, may contribute to the risk of allergic disease. However, the Th2 activities do not account for the increased inflammation, which suggests that additional innate and adaptive immune cells likely are involved in asthma. Genetic associations have also connected inflammation and atopy with asthma (52). Unclear events preceding Th1/Th2 imbalance represent critical research need to an improved understanding of the etiologic web of processes (53).

Timing and sequence of natural disease course as foundation for establishing endotype

Atopic march

Clinically, diagnosable asthma is typically preceded by distinct atopic phases during childhood (54, 55). AD, allergic rhinitis, and asthma occur in 20–40% of the children across the so-called ‘westernized’ countries (54). The notion of the atopic march describes coordinated presentation of allergic illnesses, typically starting with AD during infancy, followed by allergic rhinitis and/or wheezing during the first few years of life, and confirmed by clinician-diagnosed asthma during childhood (54).

AD represents itchy and inflamed skin injuries, associated with compromised epidermal barrier layer, immune dysfunction, and IgE-associated reaction to food and environmental allergens (54). Atopic dermatitis is an inflammatory skin condition, which appears commonly during the first few years of life. Atopic dermatitis first appears during the first year of life in 60% of the children, and during the first 5 years in 85% of the same population (55). The national prevalence of the childhood AD is estimated between 0.3% and 20.5% (56, 57). Within the United States, the prevalence of atopic dermatitis is reported between 9% and 18% (58). Approximately 50% of children with allergic rhinitis and/or wheezing have preceding symptoms or the diagnosis of atopic dermatitis (54).

The epidermal layer plays a critical role not only in sensing the immediate environment but also in defending the host against environmental insults (e.g. toxins and irritants). A compromised epidermal structure is suspected to play a critical role in the onset of AD. In particular, an unbalanced development of the immune system within the epidermal layer is suspected to contribute to a sequential appearance of AD, allergic rhinitis, and/or asthma (59). It is unknown whether the three diseases arise from distinct etiologic origins, contributed by both genetic predisposition as well as the environmental exposures (55). Some researchers have speculated that the coordinated appearances of the symptoms denote a common underlying pathway (55). The extent to which the early life exposures and host susceptibility gives rise to asthma subtypes remains unknown (54). To date, the etiological relevance of temporal sequence of atopic march, viral infection and the associated risk

of progression toward childhood asthma remain inadequately characterized (60–62).

Compared with the healthy children, the children with an early-onset (i.e. < 6 months of age) AD are at a significantly elevated risk of developing allergic rhinitis and/or asthma at a later age. Among AD affected children, those who also show IgE sensitization toward common allergens (i.e. extrinsic AD) are at an elevated risk of atopic march compared with the IgE-negative children (i.e. intrinsic AD) (63). Furthermore, the severity of the AD symptoms is robustly correlated with the subsequent development of asthma (55). Therefore, AD could represent an intermediate outcome within the pathogenic pathways of asthma. Transformation of atopic diseases into asthma could be prevented by reduction in the frequency and severity of inflammatory AD flare-ups.

Transition of atopic march to asthma

Multiple birth cohort and cross-sectional studies have replicated the observation that early-onset (i.e. first manifestation at < 3 years of age) and/or poorly controlled AD are associated with a significantly greater likelihood of developing allergic rhinitis and/or asthma (64). Those who develop AD during the first year are significantly more likely to develop subsequent allergic sensitization and/or persistent wheezing by 6 years of age. IgE sensitization among the AD-positive children (i.e. extrinsic) was the most robust predictor for subsequent persistent wheezing, hyperreactivity of the airway, and allergic asthma (64). However, atopic dermatitis alone may not be sufficient to predict the progression toward asthma. A large proportion of AD-positive infants typically resolve by age 3, in absence of any additional predisposing conditions (64). By contrast, a subset with a severe AD in conjunction with an allergic sensitization could progress toward an asthma development at later years (64).

Viral infection

Respiratory viral infection with human arbovirus, respiratory syncytial virus, and/or rhinovirus represents the most prevalent infections during the first 2 years of life (65). In majority of the infected children, infection is contained within the upper respiratory tract, and the corresponding symptoms remain mild (66). However, within a small subset, the infection spreads to lower respiratory tract, thereby becoming severe enough to require

hospitalization (66). Those children who recover from lower respiratory viral infection often experience wheezing symptoms as sequelae (66). However, it remains unknown whether the intermittent to persistent wheezing represents the sentinel event for IgE-mediated asthma. The mildly infected infants/children may also develop recurrent wheezing symptoms at older age (66). In a small prospective follow-up study, the children with a history of respiratory syncytial virus-induced bronchiolitis during infancy were significantly more likely to develop current asthma, current asthma/recurrent wheezing, or allergic rhinoconjunctivitis at 13 years of age (67). Such likelihood was accompanied not only by allergic sensitization as measured by serum IgE antibodies during the first few years of life (67). Children with a history of bronchiolitis hospitalization during infancy were at a significantly greater risk of lung function deficit, heightened airway reactivity, and reduced bronchodilator response in a number of small follow-up studies (67). In children with preexisting allergies, the viral infection may precipitate their underlying susceptibilities to allergen sensitization, inflammation, and heightened airway responsiveness and obstruction.

Putative mechanisms

Innate immune system plays an important role in recognizing and mounting preliminary defense mechanisms against the infection (68). This is followed by adaptive immune response, which sustains longer lasting inflammation (68). Viral infection could transition atopic march symptoms into asthma by inducing aberrant innate immune system function. Respiratory viral infection have been shown to induce more robust and sustained provocation of chronic activation of the innate immune system, compared to an allergen challenge (68).

Within a mouse model, an acute viral infection induces chronic airway injury even after complete clearance of viral infection (68). The type of T cells produced as a secondary reaction from viral infection may contribute to the pathogenesis of various inflammatory conditions, including asthma and chronic obstructive pulmonary disease (COPD), to diabetes (68). Hallmark features of such injuries include an elevated airway mucus production, sensitivity toward methacholine challenge (68). Such changes were driven primarily by macrophages, which, in turn, were under the control of CD4[−] and CD4⁺ natural killer T (NKT) cells (68). CD4nNKT sustained and led to a long-term production of IL-13, and sustained level of IL-13 was required to recruit lung macrophages (68). Even

after viral infection clears, activated macrophages were capable of stimulating chronic mucous cell activation and airway hyperreactivity within the lung (68). Such observation has also been made based on the tissue samples of persons with COPD, and the number of innate NKT cells was also elevated.

The mechanisms of sustained inflammation were investigated among allergic sensitized children who were hospitalized with acute severe viral infection (69). Among the sensitized children, viral infection sets off a cascading reaction in which type 1 IFN induces a 10-fold increase in the number of circulating FcεRIα-loaded monocytes/dendritic cells (DC) (69). The state of acute asthma exacerbation was associated with an activation of in response to viral infection an heightened expression of C-C chemokine receptor type 2 (CCR2), responsible for attracting DC and monocytes to the affected airway (69). Furthermore, an up-regulation of FcεRIα was also associated with an increased expression of the FcεRIγ-chain, which anchors FcεRIα -chain on the cell surface. Such anchoring is important because it allows the circulation of the cells across the epithelia to blood and/or bone marrow. Such elevation of FcεRIα expression on monocytes/DC promotes a sustained activation of migrating Th2 memory cells (69).

Multiscale systems biology

Genome wide association studies: the foundations

Overall, there is dearth of highly reproduced susceptibility loci associated with asthma susceptibility and/or severity. This might be contributed by environmental modulations (e.g. epigenomic influences). Thus, careful functional genomic studies of the asthma endotype could improve the current understanding regarding the role of the genomics.

Genome-wide association studies (GWAS) represents the mostly utilized domain to pinpoint genetic loci associated with asthma severity outcomes as a whole or with specific symptoms. To date, there are several GWAS on allergic rhinitis (70): National Human Genome Research Institute catalogs one GWAS of allergic rhinitis comparing 33 for asthma and 61 diabetes (71), Andiappan et al. report no significant GWAS loci for 942 Chinese subject, Ramasamy et al. report one genome-wide significant locus in a GWAS meta-analysis of 12,898 Europeans (71), and Hinds et al. report 16 genome-wide significant loci for

self-reported allergy in a GWAS meta-analysis of subjects of European ancestry (72). To a broader context, Torger-son et al. (73) report five asthma-susceptible loci from 5416 individuals in diverse North American population, replicated in additional 12,649 individuals from the same ethnic groups. Although significant advances are made in GWAS, there are still barriers to translate the findings into clinically actionable information. Some of such barriers include (i) emphasis on common SNPs tend to miss high-impact rare SNPs, (ii) small effect sizes and missing heritability, and (iii) difficulty with understanding noncoding regions (74). The candidate genes or genome-wide association studies have identified an incomplete picture of the mediators, receptors, or cytokines involved in asthma pathogenesis (19).

Systems biology approach: dissecting complexity of asthma

Coexpression network approach

Recent efforts to overcome the limitations of GWAS involve the integration of GWAS with transcriptomic data to further elucidate the functional role of the relevant susceptibility loci. Gene-based coexpression network analyses together with expression single nucleotide polymorphism (eSNP) provide a powerful framework by pinpointing GWAS loci for eSNPs and mapping these GWAS hit genes to relevant modules/interactome representing disease-driven functional pathways (13, 70). Bunyavanich et al. (70) report mitochondria module from CD4+ lymphocytes gene expression data, showing an effect of the GWAS hits. In the absence of such genotype data, coexpression network analyses alone can further provide meaningful interactomes of genes associated to asthma symptoms. Morrow et al. (13) report putative hub genes from top five modules mostly associated with COPD exacerbation, including IGHM, KLRD1, AFF3, GPR56, GBP1, and GBP5. These genes are proposed as potential key regulators of the underlying functional pathways of B-cell receptor signaling pathway, protein ubiquitination, and cell adhesion molecules (13). Ding et al. (75) utilized a case-control study of 13 lesional AD against 12 nonlesional AD from microarray data (GSE32924) at Gene Expression Omnibus to perform a combination of differentially expressed genes (DEG) analysis, followed by DEG coexpression network analyses and miRNA-TF-DEG regulatory network analyses to pinpoint candidate genes STRA13, PSENEN, and NAP1L2 as presumed critical regulators of AD.

Gene set enrichment analysis

Gene set enrichment analyses (GSEA) (76) provides another means to identify dysregulated functions and pathways from querying *a priori* knowledge-based databases, such as Kyoto Encyclopedia of Genes and Genomes, Gene Ontology, BioCarta, Reactome Pathway Database, Molecular Signature Database, and aforementioned collections of GWAS hits (8). The advantages of GSEA is that it effectively identifies the robust list of signatures by leveraging known pathways by means of projecting list of differentially expressed genes to these pathways. Croteau-Chonka et al. (77) report 583 gene sets from a study of 1170 adult asthmatic and controls, each with gene expression data derived from either whole blood or unstimulated CD4+ T-lymphocytes (CD4). These include signatures of eosinophilic and granulocytic inflammatory signals for suboptimal control groups, and immature lymphocytic patterns for optimal control groups. GSEA does limit the discovery of critical regulators within the context of known pathways only. It is often accompanied by coexpression network analyses to perform unsupervised module detection and to identify enriched pathways in these modules to further guide the data inference (12, 13, 70).

Inflammations modulated by epigenomic regulation

To further address molecular mechanisms of asthma beyond functional domain, epigenome studies particularly address the environmental impacts on asthma progression. Environmental factors, which have been examined, include prenatal NO₂ air pollution exposure (10), prenatal exposure to maternal stress (9), postnatal exposure to air pollution (78), and obesity (79). These recent investigations identified differentially methylated regions (DMR) and related key epigenetic mechanisms altered in children. Candidate gene studies from DMR report oxidative stress, chronic inflammation, and antioxidant defense pathways in airways (10, 78–80).

Integrative approach to asthma

Efforts to integrate multi -omics data have recently emerged. Protein-protein interaction (PPI) networks from knowledge-based databases such as STRING and human protein reference database (HPRD) have provided useful platform to integrate gene expressions with protein interactions. DEGs of corticosteroid resistance in asthma have been mapped onto PPI networks, identifying interactions

of inflammatory environment with steroid resistance (81). The only integrative network approach to date combining metabolomics, transcriptomics, epigenomics, and genomics (11) has examined metabolomics as a regulatory core affecting asthma control. A conditional Gaussian-Bayesian network (CGBN) was conducted across mRNA, CpG sites, and SNPs (11). On the basis of the CGBN model, the authors report four SNPs (rs9522789, rs7147228, rs2701423, and rs759582) and two metabolites (monoHETE_0863 and sphingosine-1-phosphate), which predict asthma control with AUC score of 95% (11).

Conclusion

An important unanswered goal of endotype research is the identification biomarkers of early, intermediate health outcomes. Careful endophenotypic characterization of atopic diseases, wheezing symptoms, and lower respiratory viral infection during the first few years of life is critical for clarifying whether the previously mentioned conditions are distinct illness or single one. An added benefit of endotyping includes an identification of high-risk infants and/or toddler at earliest possible period and curving the progression toward an established illness. Most studies to date have been largely restricted to transcriptomic data only. Furthermore, they are largely dependent on the guidance by *a priori* knowledge, further limiting the scope of the models. A promising direction is to perform unbiased, large-scale integrative studies of multi-omics data as the molecular interplay among these axes remains largely underexplored. These integrative approaches have already demonstrated a potential to dissect the complexity and to identify the underlying regulators of other complex diseases such as Alzheimer's disease (82), type I diabetes (83), and breast cancer (84) and may provide a useful strategy to fulfill these unmet needs.

Conflict of interests: All authors declare that they have no competing interests.

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