Genomic mosaicism: A neglected factor that promotes variability in asthma diagnosis

Thiago de Bittencourt Buss, Leonardo Mroginski, Gabriel Moojen de Jesus, Sofia Forcellini, Ana Vitória Lucion Didonè, Fabiana Quoos Mayer, José Eduardo Vargas

Laboratório de Genética e Biologia Molecular, Instituto de Ciências Biológicas (ICB), Universidade de Passo Fundo (UPF), Campus I, km 171, BR 285, P.O. Box 611, CEP 99001-970 Passo Fundo, RS, Brazil

A B S T R A C T

Introduction

Asthma is a complex disease with a broad range of clinical variation, which affects all ages, especially children. The most common symptoms include variable limitation of expiratory airflow, bronchial hyperresponsiveness and airway inflammation accompanied by recurrent episodes of wheezing, coughing, dyspnea and chest tightness [1]. Approximately 300 million people suffer from asthma around the world, regardless of their economic, social and cultural level. For these reasons this disease is one of the most registered chronic lung disorders with a high prevalence [2–5]. Consequently, this pathology is considered a significant public health problem worldwide. Despite advances in effective therapy, this condition is associated with great health expenditures. A study analyzed the biennial health cost associated with acute asthma disease in the United States and revealed that the average hospitalization cost was USD $6875 for an asthmatic individual [6]. Other fact to consider is the consequent economic burden associated with asthma control, comorbidities and co-founders, such as obesity, aspirin-exacerbated respiratory disease, or cigarette smoking, which continue to increase and promote asthma development [7–9].

At cellular level, multiple immunological cells and cellular mediators promote bronchial inflammation, which corresponds to the most important physiopathological mechanism of asthma [10,11]. Approximately two-thirds of patients with asthma have an allergic component, in which elevated IgE levels are detected with concomitant allergic diseases from early stages of life. The other third of asthmatic patients presents non allergic asthma, most of them later in life. In this sense, asthma classification can be based on a cluster of characteristics of the disease in children or adults [9]. However, the use of endotypes helped to obtain more precise diagnostics for asthmatic patients. The endotypes include specific immune cell types and biological pathways that promote characteristic profiles for the patients, as reviewed by Scherzer and Grayson, 2018. In this sense, patients with asthma can be also classified based on their endotype into TH2-high or TH2-low subgroups. Patients with a TH2-high-based disease have eosinophils in sputum secretion and high levels of pro-inflammatory interleukins (IL) 4, IL-5, and IL-13 from bronchoalveolar lavage (BAL) fluid and...
bronchial tissue; moreover, the patients are steroid responsiveness. On the other hand, patients with TH2-low endotype have a different cytokine profile in BAL fluid and lung tissue accompanied of a lower response to corticosteroids [9].

Is important to emphasize that other T cell subtypes are also involved in the asthma immunopathology, such as TH1, TH17, Th22 and regulatory T cells, as reviewed by Martín-Orosco 2017 [12]. Therefore, the immune response in asthma is considered highly heterogeneous, which may induce biases for classification using endotypes. In addition, there are a high number of comorbidities that can alter asthma endotypes, i.e. viral infections or overlapping with other progressive lung disease as chronic obstructive pulmonary disease [13,14].

Furthermore, asthma also has a complex genetic basis involving environmental factors and individual variabilities [9,15,16]. In this sense, the term “asthma” has been suggested to be out of date, being necessary to perform more studies to find a common process to understand this disease as a unique pathology [17]. We consider that the genetic variability itself can contribute to define key mechanisms to describe this disease. In our hypothesis, we believe in a potential role of genomic mosaicism (GM) in asthma disorder. Mosaicism is defined as the presence of two or more cellular populations with distinct genomic backgrounds in an individual. Currently, GM effects are underestimated and its influence is practically ignored in asthma pathology. Recent evidences suggest that GM in an individual do not necessarily promotes pathologies, but is a multifactorial condition. In fact, GM has lately been explored in the brain, reporting that somatic mutations in this tissue may contribute to neuronal diversity [18–20]. Extrapolating to asthma, could genetic mosaicism be a neglected variable with potential to improve diagnostics?

Hypothesis

Our hypothesis suggests that the genomic mosaicism increases the complexity and variability of cell biology of asthma. This genetic variability has potential to uncover patterns that could help to perform more precise asthma diagnosis, updating current “endotypes” or establishing a new classification. Our hypothesis is represented in Fig. 1.

For an easier analysis of our hypothesis, we support it in two interrelated points: mosaicism in asthmatics as a probable phenomenon and the major difficulties of screening technologies to explore genetic variability from tissues of asthmatics, a crucial factor to be improved in diagnose.

Genomic mosaicism in asthmatics: a probable phenomenon

Multiple genetic components are involved to the development of asthma. More than 100 genes have their participation recognized in the individual susceptibility [21]. In addition, familial background contributes to about 35% to 70% of total risk, according studies based on twins [22,23]. Taking into account the modifying genes and also environmental factors leads to a complex picture of this disorder [21,24,25]. To resolve this challenge, many studies are investigating genetic variations to define the genetic background of asthma. For this objective, two major approaches are used, genetic linkage analysis or genetic (allelic) association analysis. According to a recent meta-analysis, between 2007 and 2016 twenty-five genome-wide association studies (GWAS) have been published, the largest with a sample size of 157,242 individuals [26,27]. Between 2017 and 2018, only five studies were performed. In the majority of studies susceptibility variants were identified providing mechanistic insights into asthma pathogenesis, which play a role in the regulation of immune cell functions.

Although single nucleotide polymorphisms (SNPs) promotes genomic variations in all cells of a individual, there is a plethora of genetic variations at all genomic scales encircling the mosaicism term. GM can be caused by small insertions or by copy number variations (CNVs), which correspond to deletions or duplications of genomic regions composed by few kilobases in length, and other genomic alterations from large structural variants to aneuploidies in different sets of cells of a individual, as reviewed by Biesecker and Spinner, 2013 [28]. Considering all these variants, only one study, published by Walsh et al. 2010 [29], correlates directly the absence of TCR-gamma copies with the presence of asthma, suggesting a possible role of GM. The gamma-delta T-Cells are inflammation inhibitors, which promote bronchial inflammation when reduced. Based on this evidence and the high quantity of genetic alterations included in the GM concept, the crucial question is: why are there so few cases of mosaicism reported in asthmatics?

GM is classically associated with diseases, such as Tyrosinemia type.

Fig. 1. Schematic representation showing a variable of genomic mosaicism in a central point, which can update current asthma classification based on endotypes/phenotypes or to generate a new system for asthma diagnosis based on individual genetic diversity.
I [30], Lesch-Nyhan [31], Conradi-Hunermann-Happle [31], Adenosine deaminase deficiency [32], Wiskott-Aldrich syndrome [32], Hemophilia A [34], Hemophilia B [35], Marfan syndrome [36], Pseudoachondroplasia [37], Duchenne muscular dystrophy [38], Congenital myotonic dystrophy [39], Neurofibromatosis type I [40], Neurofibromatosis type II [41], Tuberous sclerosis [42], Bullous ichthyosiform erythroderma [43], Incontinentia pigmenti [44], Friedreich ataxia [45] and some cancer types as reviewed by Fernández Díaz et al, 2015 [46], among other somatic disorders. In the level of mosaicism aneuploidy, only a few trisomies are compatible with human life, such as trisomy of chromosomes 13, 18, 21, and X, varying in prevalence, percentages of cells affected, and phenotypes. [47].

Largely, the literature stigmatizes the mosaicism as only responsible for diseases. In this sense, are mosaic mutations rare events? Are all these mosaic mutations promoters of diseases or any disorder? Recently, Lichtenstein (2018), explains that mosaicism is an unavoidable natural phenomenon; he considers the interplay between the genome of each daughter cell, suggesting that mosaicism occurs after the first division of the zygote, and is amplified in subsequent cell cycles [48].

An in vitro study using primary fibroblast cells obtained from seven different patients supports Lichtenstein’s model, as they verified that approximately 30% of the fibroblasts have CNVs in their genomes [49]. Likewise, Lichtenstein predicted that the average frequency of cells with a somatic mutation should be approximately the number of cellular generations since the zygote stage multiplied by the mutation rate per cell division. For 30 generations from an adult fibroblast back to the zygote, a 30% frequency of mutated cells is detected. Based on this rationale, this phenomenon could occur naturally in bronchial cells or the immune system cells, generating a wide and varied repertoire of immune responses in healthy individuals or asthmatic patients.

Prior knowledge confirm that the immune system represents a clear example of mosaicism involving normal cells of the body, with clear functional consequences through the generation of antigen receptors, and selection and survival of immune cells [50–52]. In a similar manner, several reports within the last 20 years have identified multiple forms of somatically produced GM, wherein brain cells, especially neurons, which showed diverse alterations in their DNA, distinct from the cells of germline. The first single-cell study performed using 110 human frontal cortex neurons verified that 13% to 41% of these cells contained at least one megabase-scale de novo CNV [53]. Similar studies confirmed that de novo CNVs occurs in at least 10% of neurons; however in these works fewer neuronal genomes were analyzed [54,55]. In the genome, the presence of CNVs of multiples neurons can be common with a varied number of cell populations maintained by clonal proliferation [55]. This data has strong impact in the cerebral physiology affecting the diversity and function of neurons, as well as on the understanding of pathologies, particularly considering the increased prevalence of sporadic brain diseases, which are not promoted by inherited mutations.

Using similar experimental strategies, the interplay between asthma and mosaicism would be explored, but there are difficulties to be resolved.

Major difficulties of genomic mosaic analysis from tissues of asthmatic patients

Several genomic methods can be applied to study mosaicism, such as next generation sequencing (NGS), massively-parallel sequencing, array comparative genomic hybridization, allele-specific PCR, high-resolution melting (HRM) analysis, pyrosequencing, Sanger sequencing, SNaPshot and immunohistochemistry [47,56]. Each technology/method has advantages and drawbacks. However, all have common limitations when GM is explored. There are reviews or computational applications that carefully describe these limitations [56–61]. Based on these articles, we list the hardest drawbacks to overcome:

- To detect a somatic mutation is dependent on its frequency within a cell population;
- To detect mosaicism in a tissue is necessary to analyze multiple tissues within an individual;
- To use DNA sequencing, small pools of cells, single cells, or clonally reprogrammed cells are required to identify or to validate low frequent somatic mutations. However, clonal cell expansion can promote mutations during the process;
- To minimize the uneven read depths of the genome, strong algorithms are required in high sequencing depths. Also, algorithms are impractical when the paired control samples are unavailable;
- To analyze haplotypes through phasing methods, data from other individuals are necessary, which is dependent on genetic inheritance to identify somatic mutations by considering the relation between germline variant mutations and somatic mutations. The high cost and low per-base pair accuracy are factors that limit this method;
- To exclude errors in the process with low frequency variants, all results obtained based on mosaicism research need good validation methods.

In addition, other limitations emerge by consequence of the nature of asthma, as well as the difficult access to bronchial tissue or to choose a cellular fraction representative of pulmonary inflammation environment. In asthmatics, various biological materials may be tested. Primarily, low invasive procedures are recommended for sample collection; if possible, peripheral blood sample is a good option, as several studies explore asthma using this type of sample [62–64]. However, blood is a mixture of cells, which can be very challenging, when the focus is to evaluate other tissues, i.e. lungs. In this sense, BAL and sperm are good alternatives to reflect lung environment [56,65,66].

Still, GM could be extended for all tissues involved in asthma pathology, thus, bronchopsia is also recommend to be analyzed to explore individual genetic diversity in a in situ evaluation.

Conclusion

Currently, asthmatic patients are classified based on their endotypes. However, this classification is poor, considering the complexity of asthma pathology. It is probable that cells of innate and adaptive immune systems or bronchial cells have genomic mosaicism. This phenomenon generates a genetic background variable in each patient, promoting phenotypical diversity. We purpose that genomic mosaicism is a biological event with priority to be investigated in asthmatic patients. The new data obtained considering genetic variability can help to complement current endotype or to establish a new classification based on phenotypic consequences of mosaicism in asthmatic patients, which can provide, in a nearly future, personal care.

Conflict of interest statement

The authors declare that no competing interests exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.04.009.

References
