



Review

Food allergomics based on high-throughput and bioinformatics technologies

Chong Wang^a, Yanbo Wang^a, Guangming Liu^b, Linglin Fu^{a,*}^a Food Safety Key Laboratory of Zhejiang Province, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, China^b College of Food and Biological Engineering, Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Jimei University, Xiamen, China

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ABSTRACT

Food allergy is a serious food safety problem worldwide, and the investigation of food allergens is the foundation of preventing and treating them, but relevant knowledge is far from sufficient. With the advent of the “big data era”, it has been possible to investigate food allergens by high-throughput methods, proposing the concept of allergomics. Allergomics is the discipline studying the repertoire of allergens, which has relatively higher throughput and is faster and more sensitive than conventional methods. This review introduces the basis of allergomics and summarizes its major strategies and applications. Particularly, strategies based on immunoblotting, phage display, allergen microarray, and bioinformatics are reviewed in detail, and the advantages and limitations of each strategy are discussed. Finally, further development of allergomics is predicted. This provides basic theories and recent advances in food allergomics research, which could be insightful for both food allergy research and practical applications.

1. Introduction

Allergy is an adverse immune reaction to specific environmental factors and has been recognized as a public health problem. Food allergies describe allergic reactions induced by food ingredients; they affect 240–550 million people (especially children) worldwide, and the prevalence is increasing (WAO, 2013). Food allergies can induce symptoms such as coughing, itching, vomiting, abdominal pain, and swelling of the pharynx, therefore significantly impairing the quality of life of sufferers. In particular cases, food allergies result in anaphylaxis, which can be life-threatening (Fu, Wang, & Wang, 2018). Consequently, prevention and treatment methods for food allergies are urgently needed.

An allergen is an ingredient of an allergenic source that can induce the allergic reactions of sufferers; the chemical nature of an allergen is usually a protein (Chardin & Peltre, 2005). Due to the lack of effective allergen-independent therapeutic approaches, oral immunotherapy, which is based on the identification of the predominant allergen, is the most efficient method for treating food allergies (Nurmatov et al., 2017). Moreover, compared with the developing therapeutic methods, allergen avoidance is the most effective method currently available, which is also based on the identification of allergens (Tang, Lozinsky, & Loke, 2020; Zuidmeer-Jonghejan et al., 2012). In general, there are

various allergens in a single allergenic source, and an allergic individual can respond to multiple allergens (Matricardi et al., 2016). Therefore, the ideal determination of food allergens should achieve two goals: first, completely identifying all the allergenic ingredients to avoid the risk of allergy; and second, excluding any of the non-allergenic ingredients to minimize the reduction of quality of life. However, traditional methods can only identify very limited kinds of allergens each time, which is insufficient for food allergy therapy and cannot provide adequate support for intensive allergy studies.

In recent years, with the development of advanced technologies such as biochips, high-throughput sequencing, and protein mass spectrometry (MS), biological research has entered a new era of big data, resulting in booming areas including genetics, proteomics, metabolomics, and so on (Gu & Taylor, 2014; Karczewski & Snyder, 2018; Zhang, Zhu, & Liu, 2015). Consequently, it is now possible to systematically study allergens with high-throughput technologies, proposing the concept of allergomes (or allergenomes) and allergomics (or allergenomics) (Chardin & Peltre, 2005; Ciardiello et al., 2013; Di Girolamo, Muraca, Mazzina, Lante, & Dahdah, 2015; Yagami et al., 2004). Depending on the research objects, allergomes can refer to the total repertoire of allergens in a particular food or the total repertoire of allergens to which a particular individual may be sensitized. Identifying the former can facilitate the production, management, and

Abbreviations: ABA, allergenic molecule based micro-bead array system; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; ISAC, immuno solid-phase allergen chip; RAST, radioallergosorbent testing

* Corresponding author.

E-mail address: fulinglin@mail.zjgsu.edu.cn (L. Fu).

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consumption of potentially allergenic food, and identifying the latter can facilitate the diagnosis, prevention, and therapy of food allergies. On the other hand, allergomics refers to the application of omics strategies to allergens in particular (Yagami et al., 2004) and is an emerging discipline focusing on allergome research.

Although the concept of allergomes has been proposed for more than 15 years (Yagami et al., 2004), due to the limitation of theories and technologies, it is inferior to recent omics concepts in various aspects, especially the completeness of identified targets, the depth of relevant research, as well as the sensitivity, throughput and efficiency of detection methods. As a concept, the allergome is frequently mentioned in allergen studies; however, most of these works have only applied traditional low-throughput methods to perform conventional investigations, and “real” allergomics studies from the perspectives of omics are rare.

Overall, to facilitate the further development and application of allergomics, this review focuses on the advances in research on food allergomics. We first introduce the brief mechanisms of food allergies and the basic allergen identification methods. Based on this, we enumerate the common research strategies for allergomics and review the recent progress and applications of allergomics. Finally, we discuss the limitations and defects of the current allergomics research and provide an outlook of future developments and applications. Further intensive allergomics investigations can improve the efficiency of preventing and treating food allergies, as well as promote the production, management, and consumption of potentially allergenic food. With all these efforts, the profit of the relevant industries can be increased, and the life-quality of allergy sufferers can be improved.

2. Basics of food allergy research

2.1. Mechanisms of food allergies

Hypersensitivity describes harmful immune responses triggered by specific antigens. According to the different effector mechanisms, hypersensitivity can be classified into four types (type I/II/III/IV hypersensitivity) (Gell & Coombs, 1963; Thomas & Cunningham, 2015). Food allergies are mainly type I hypersensitivities that are mediated by immunoglobulin E (IgE) (Wu & Zarrin, 2014). Type I hypersensitivity includes two stages, the sensitization stage and the effector stage. The sensitization stage is an asymptomatic primary immune response; when the mucosal immune system is exposed to a particular allergen for the first time, a series of immune responses are triggered. With the complex cooperation of specific immune cells, especially dendritic cells, T cells, and B cells, finally an allergen-specific IgE is produced. Then, IgE binds to the specific receptor FcεRI on the surface of mast cells and basophils, leading to a sensitized status. The effector stage is a harmful secondary response; when the body is re-exposed to the same allergen, the allergen binds to the corresponding IgE on the surface of the sensitized mast cells and basophils. Then, these cells are triggered to perform degranulation, therefore releasing inflammatory mediators such as histamine, leukotriene, and prostaglandin. These mediators finally stimulate the effector organs and lead to apparent allergic responses such as vomiting, pruritus, urticarial, vascular collapse, and even life-threatening anaphylaxis (Fu et al., 2018; Mak, Saunders, & Jett, 2014).

2.2. Basic allergen detection methods

Based on the mechanisms of IgE-mediated type I hypersensitivity, two classes of allergen detection methods have been developed, which detect the allergic symptoms and the allergen-specific IgE, respectively.

Detecting allergic symptoms is the most straightforward and reliable method for allergen determination. Oral food challenges (OFC) and the skin prick test (SPT) evaluate the allergenicity directly on the human body (Sicherer & Sampson, 2018). Oral food challenges stimulate the subjects with allergens through the mouth and assess the subsequent

allergic symptoms. This procedure mimics the intake of allergenic food and therefore the results are highly reliable, but also could result in severe reactions and even fatality (Sicherer & Sampson, 2018). As an alternation, skin prick test is more widely used in clinical diagnosis, which is safer and can detect multiple allergens in a single test, but the reliability is worse than oral food challenges and the potential risk still exists. Therefore, because of the high cost, limited sources of human subjects, ethical limitations, and potential clinical risks, the applications of these methods are largely restricted. In contrast, the food allergy animal model is an ideal alternative and is suitable for intensive mechanistic research, and therefore has received extensive attention and is experiencing rapid development. Dozens of food allergy animal models have been established to date, involving allergens from egg, milk, peanut, fish, shrimp, etc. Based on these models, numerous studies on the identification of novel allergens, the elucidation of allergy mechanisms, and preventive and therapeutic methods for food allergies have been performed (Liu, Navarro, & Lopata, 2016). However, considering the large time and economic costs, these methods are not suitable for high-throughput allergomics investigations.

On the contrary, IgE-based detection methods (or serological methods) require relatively small amounts of time, have a low economic cost and are more suitable for high-throughput investigations. Relying on the basic principle of antibody-antigen interaction, various detection methods have been developed, including conventional methods such as immunoblotting, radioallergosorbent testing (RAST) and enzyme-linked immunosorbent assay (ELISA), as well as emerging methods such as the protein microarray and phage display (Ciardiello et al., 2013). It should be noted that these serological methods are more likely to produce false-positive results, and further validation by symptom-based methods is required to get more reliable results (Clark & Ewan, 2003; Ebo et al., 2008). Additionally, two-dimensional gel electrophoresis, liquid chromatograms, and protein mass spectrometry are often used to assist these serological methods.

With the development of bioinformatics and the accumulation of allergen knowledge, it is now possible to identify novel allergens by DNA or protein sequence-based prediction. This *in silico* approach is extremely high-throughput and has a relatively low cost, and therefore, is attracting increasing interest for allergomics studies.

The recent applications of allergomics strategies in the identification and quantification of novel food allergens are enumerated in Table 1, and the advantages and disadvantages of recent allergomics strategies are listed in Table 2.

3. The strategies and application of allergomics

3.1. Immunoblotting-based strategy

Immunoblotting is a classical method to determine the interaction between the antigen and antibody, and it is widely used in both detecting allergens in food as well as the allergic sera for particular allergens. It has been demonstrated that immunoblotting and skin prick test have comparable sensitivity and specificity in allergen detection (Celakovska, Krcmova, Bukac, & Vaneckova, 2017). Western blot (combined with SDS-PAGE) and ELISA are traditional allergen evaluation methods in allergy researches, which can only detect a few allergens in one test. On the other hand, immunoblotting is also the first method applied for allergome detection. In allergomics studies, immunoblotting is often combined with two-dimensional gel electrophoresis and protein mass spectrometry (Fig. 1). The conventional procedure commonly contains five steps:

1. Separate the sample protein mixture by two-dimensional gel electrophoresis.
2. Transfer the separated proteins from the gel matrix to the blotting membrane.
3. Probe the membrane with serum from the allergic individuals, or the

Table 1
Representative allergomics studies on food allergens.

Allergomics strategy	Detailed method	Sample	Detected allergens	Ref
Immunoblotting-based strategy	combined with mass spectrometry (LC-MS/MS)	egg	YGP40	(Sogawa et al., 2018)
	combined with mass spectrometry (MALDI-TOF MS)	fish	NDPK B	(Liu et al., 2014)
	combined with mass spectrometry (LC-MS/MS)	shrimp	Hemocyanin	(Piboonpocanun, Jirapongsananuruk, Tipayanon, Boonchoo, & Goodman, 2011)
	combined with mass spectrometry (MALDI-TOF MS)	milk	8 allergens with prevalences	(Natale et al., 2004)
	combined with mass spectrometry (LC-MS/MS)	wheat	3 allergens in wheat Norin 61	(Takemoto, Asano, & Takahashi, 2015)
Phage display-based strategy	combined with mass spectrometry (LC-MS/MS)	soybean	5 novel allergens	(Gagnon, Poysa, Cober, & Gleddie, 2010)
	combined with ELISA and thin-layer isoelectric focusing	peanut	Ara h 1	(Burks et al., 1991)
	combined with N-terminal sequencing	almond	2S albumin and conglutin gamma	(Poltronieri et al., 2002)
	pluFo cloning system	peanut	6 allergens with prevalence	(Kleber-Janke, Cramer, Appenzeller, Schlaak, & Becker, 1999)
	pCTCON2 surface display system	coffee	3 allergens with prevalence	(Peters et al., 2015)
Allergen microarray-based strategy	pCTCON2 surface display system	plant	Actinidin with immunochemical characterization	(Popovic et al., 2015)
	T7Select 415-1 and T7Select 10-3b system		10 olive pollen allergens and 9 mustard seed allergens with mimotopes	(San Segundo-Acosta et al., 2018)
	ISAC	sera	Up to 5000 allergens	(Deinhofer et al., 2004)
	ABA	sera	11 allergens	(Pomponi et al., 2012)
	allergens immobilized on microarray	plant lipid transfer proteins	6 allergens with comparative analysis	(Bernardi et al., 2011)
Bioinformatics-based strategy	multiplex biochip-based immunoassay	apple	12 apple cultivars with comparative analysis	(Pasquariello et al., 2012)
	combined databases	chickpea	7 allergens	(Kulkarni et al., 2013)
	combined databases	plant lipid transfer proteins	28 putative allergenic homologs	(Garino et al., 2016)
	combined with comprehensive proteomics approach	olive pollen	"complex allergenome" (203 allergens)	(San Segundo-Acosta et al., 2019)
	combined with proteogenomic annotation	house dust mite	"allergenome" (39 allergens)	(Erban et al., 2020)

Table 2
Comparison of different allergomics strategies.

Strategy	Immunoblotting	Phage display	Allergen microarray	Bioinformatics
Sensitivity	low	-	high	-
Throughput	one each time	whole genome screening	up to 5000	unlimited
Time cost	days	weeks	hours	variable
Economic cost	low	high	low for each allergen	very low
Identification of novel allergen	yes	yes	barely	barely
Advantages	conventional equipment and technology, easy to identify novel allergens	high-throughput, unbiased process, controllable precision	high-throughput, time saving, undenatured allergen structure, quantifiable, highly reliable	low cost, unlimited throughput, no additional equipment
Disadvantages	denatured allergen, low-throughput, biased process	recombinant allergen, unrobust process, time consuming	high cost microarray, specific equipment, hard to identify novel allergens	limited by available databases, low reliability, hard to validate, hard to identify novel allergens

- serum from a non-allergic individual as a negative control. Detect with an anti-IgE secondary antibody with proper labeling.
4. Identify the protein of each positive point by protein mass spectrometry.
 5. Combine all the data to establish the allergome.

As a relatively straightforward and classical method, the immunoblotting-based strategy is widely applied, and the concept of allergomics is proposed by using this method (Chardin & Peltre, 2005; Yagami et al., 2004). Originally, immunoblotting served as a complementary method for allergy studies. For instance, it revealed some characteristics of egg allergens (Anet et al., 1985) and demonstrated that different allergic individuals may have diverse allergen profiles for the same food (Sutton, Skerritt, Baldo, & Wrigley, 1984). However, due to the limitation of technologies at that time, these studies were not able to identify the exact allergen protein.

With the improvement of protein mass spectrometry and relevant databases, identifying unknown proteins is getting easier, making immunoblotting-based allergomics possible. This strategy is still one of the dominant approaches for allergome determination nowadays (Di Girolamo et al., 2015). By combining reducing and non-reducing two dimensional electrophoresis and LC-MS/MS gel-free proteomic, Mamone and colleagues revealed the formation of peanut allergen complex with Ara(s) subunits (Mamone et al., 2019). By comparing the immunoblotting results of *Ailanthus altissima* pollen collected during two consecutive seasons with allergenic patient sera, Mousavi and colleagues assessed the allergome alternation over time (Mousavi, Shahali, Pourpak, Majd, & Ghahremaninejad, 2019).

In recent years, several more advanced technologies have been incorporated into immunoblotting-based strategies for allergomics research. By strengthening the downstream detection methods with multidimensional liquid chromatography-ion trap mass spectrometry (MDLC-ESI-LTQ-MS/MS), Hu and colleagues identified nine potential allergens in snake (*Probothrops mucrosquamatus*) venom that can hardly be separated by normal methods (Hu, Yang, Yang, He, & Wei, 2017). On the other hand, by improving the upstream sample preparation methods with ammonium sulfate precipitation and ion-exchange chromatography, Wagner and colleagues remarkably increased the number of detectable allergens in *Hevea brasiliensis* latex, and the results can be verified by the skin prick test (Wagner et al., 2007). Similarly, after being enriched by hexapeptide ligand libraries, low-abundance allergens can be identified by immunoblotting (Shahali, Sénéchal, & Poncet, 2019). Moreover, Smiljanic and colleagues identified the allergome of ragweed (*Ambrosia artemisiifolia*) pollen and determined the allergenicity by ELISA, resulting in quantitative allergomics (Smiljanic et al., 2016). Combined with the transcriptome, Bordas-Le Floch and colleagues performed a more comprehensive allergomics study and identified several novel allergens from house dust mites (Bordas-Le Floch et al., 2017). Combined with cell biology methods, Oseroff and colleagues identified house dust mite allergens that can activate T cells but cannot bind IgE, facilitating future diagnostics and immunotherapeutics for house dust mite allergy (Oseroff et al., 2016).

Compared with other allergomics strategies, the immunoblotting-based strategy can directly identify allergen proteins in a de novo way, so it is still widely used in identifying novel allergens, especially those totally different from any known allergens that can hardly be detected by other methods. Based on this strategy, plenty of data regarding allergens have been accumulated, and the basic theories on allergomics have been established. All these achievements are indispensable for further allergomics studies.

3.2. Phage display-based strategy

Phage display is a high-throughput method for investigating protein interactions and is widely used in life science (Fig. 2) (Sioud, 2019; Tan, Tian, Liu, Zhu, & Yang, 2016; Wu, Liu, Lu, & Wu, 2016). The

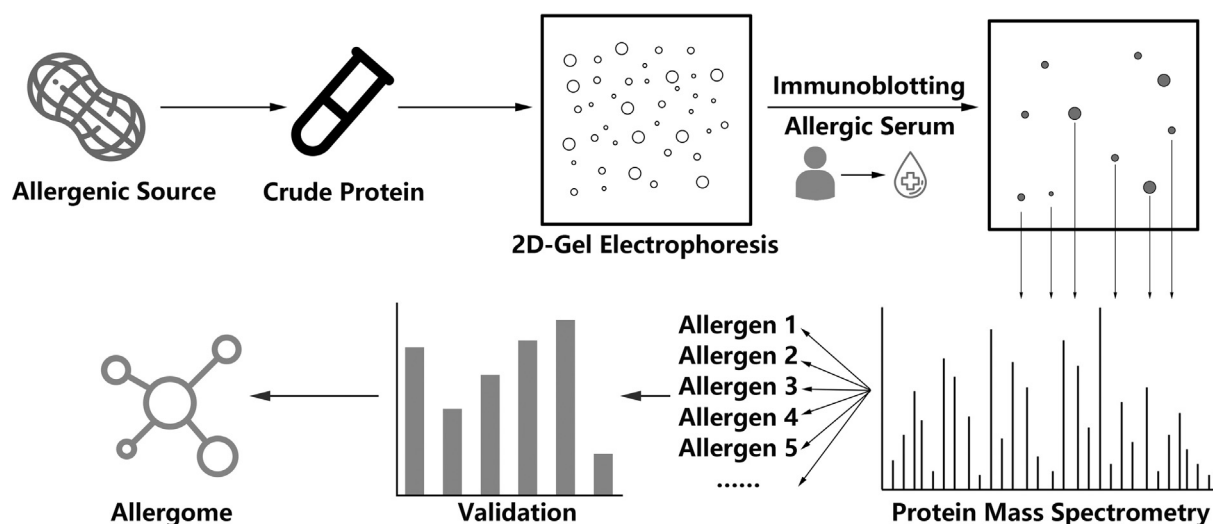


Fig. 1. Schematic diagram of the immunoblotting-based allergomics strategy.

conventional procedure commonly contains five steps:

1. Set up the relevant phage DNA library and express it in bacteria, thus obtaining the mixed phage library with surface-expressed proteins or peptides.
2. Enrich the specific phages with immobilized bait molecules.
3. Amplify the enriched phage library in bacteria.
4. Repeat steps 3 and 4 for several rounds to reach an ideal specificity.
5. Sequence the finally enriched phages to identify the target proteins or peptides.

When the cDNA library of a particular food allergen source is used to set up the phage DNA library and allergic serum is used as the bait molecule, the phage display can be used to determine all the IgE-binding proteins in the allergenic source; i.e., the allergome (Rhyner

et al., 2004). In addition, when the cDNA fragment collection of a particular allergen is used to set up the phage DNA library, the method can be used to identify the allergen epitopes (Havenith et al., 2017; Liu et al., 2018).

Similar to the immunoblotting-based strategy, profiting from the development of advanced biological technologies, especially in molecular biology, as well as the improvement of genomics and proteomics databases, the phage display-based strategy has acquired increasing application in allergomics research. Cramer and colleagues first applied the phage display into allergomics study. They displayed the cDNA library from *Aspergillus fumigatus* on the surface of filamentous phage M13. These phages were screened by human serum IgE, and several allergens were identified, which could be verified by skin prick test (Cramer & Blaser, 1996). Similarly, the allergomes of *Cladosporium herbarum*, *Coprinus comatus*, *Malassezia furfur*, peanut (Cramer et al.,

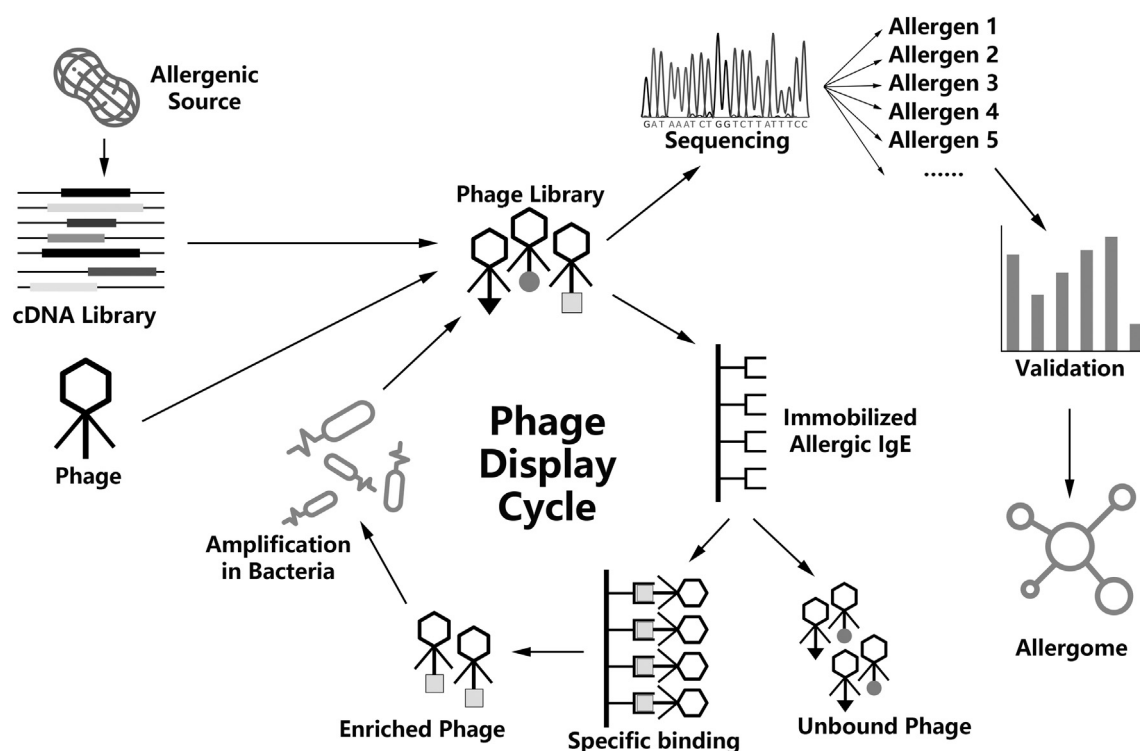


Fig. 2. Schematic diagram of the phage display-based allergomics strategy.

2001), coffee (Peters, Frenzel, Brettschneider, Oldenburg, & Bittner, 2015) and the fish parasite *Anisakis simplex* (Lopez & Pardo, 2011) were also identified by phage display-based strategies.

In addition to express the whole proteome, phage library can also be used to express the collection of potential allergenic peptides, and therefore can be applied not only for identifying allergens, but also for revealing allergenic epitopes. By applying an optimized peptide phage display library, Kern and colleagues identified the repertoire of soy bean epitopes that can bind with either IgE or IgG in allergic sera, which can be regarded as a more detailed allergome and immunome (Kern et al., 2019). By using the similar strategy, the IgE-reactive components in olive pollen and yellow mustard seed were also identified (San Segundo-Acosta et al., 2018). In addition to linear epitopes, phage display-based strategy can also reveal the IgE-binding mimotopes (mimics of natural epitopes) that mimic conformational epitopes, therefore provided more information on the in vivo allergenicity of foods in a high-throughput way (Chen & Dreskin, 2016).

Because a large portion of the eukaryotic proteins cannot be correctly and efficiently expressed in prokaryotic cells, several eukaryotic cell-based methods have been developed to displace the traditional phage display. These novel methods include yeast surface display (Popovic et al., 2015) and mammalian cell display (Tomimatsu et al., 2013). The yeast surface display was proven to be a potent technique to characterize plant-derived food allergens (Popovic et al., 2015). On the other hand, the mammalian cell display has not yet been applied in determining food allergomes, which might be due to the rarity of mammal-derived allergens.

Similar to immunoblotting-based strategy, phage display is also a de novo identification method (though it is DNA-based) and thus can be used for identifying novel allergens. Moreover, this method has almost unlimited throughput and the procedure is unbiased. Therefore, phage display-based strategy is an ideal allergomics method, and it would be increasingly powerful if accompanied by the improvement of relevant technologies.

3.3. Allergen microarray-based strategy

The protein microarray (or protein chip) is a high-throughput technology to determine protein interactions. The microarray is a set of particular proteins immobilized to a support surface (such as a glass slide). When the labeled probe molecules are hybridized to the microarray, a specific interaction between the immobilized protein and the probe molecule happens, and then the affinity can be quantified. Therefore, a large number of proteins can be analyzed in parallel (Fig. 3) (Gupta, Manubhai, Kulkarni, & Srivastava, 2016). This method is often used to determine the allergome of a particular allergic individual, where various allergens are immobilized to the microarray and the serum sample of the individual serves as the probe. The most widely used allergen microarray technologies are RAST, ImmunoCAP (Axcn, Drcvin, Kober, & Yman, 1988), Allergy Explorer (ALEX) (Heffler et al., 2018) and the allergenic molecule-based micro-bead array system (ABA) (Pomponi et al., 2012).

RAST and ImmunoCAP are conventional methods to detect specific IgE in human sera. In RAST, specific allergens are immobilized on beads to absorb the corresponding IgE in the sample, and the latter is detected by radioactively labeled anti-IgE secondary antibody. In ImmunoCAP, the allergens are covalently coupled to the solid phase enclosed in a capsule; then, the corresponding IgE in the sample is enriched and detected by labeled anti-IgE secondary antibody. Both methods are based on the same mechanism and procedure, but ImmunoCAP improves on the basis of RAST and therefore is more sensitive and has higher throughput, and the obtained result can better reflect physiological conditions (Ewan & Coote, 1990; Jeep, Kirchhof, O'Connor, & Kunkel, 1992; Schulman & Pohlig, 2015). Therefore, ImmunoCAP has been considered as a gold-standard for allergen detection (Al-Mughales, 2016). The recent application of ImmunoCAP, the immuno solid-phase

allergen chip (ISAC), can evaluate the affinity of sera and hundreds of common allergens in several hours with a small amount of serum (30 µL), therefore establishing the allergome of the allergic individual (Buzzulini et al., 2019; Oksanen et al., 2019). Similarly, Allergy Explorer (ALEX) is a recently developed microarray-based allergen detection method. This method allows the acquisition of an allergome comprising 282 reagents (including 157 extractive allergens and 125 molecular components) in seconds. In addition, this method can also exclude the non-specific binding of cross-reactive carbohydrate determinants (CCDs) (Heffler et al., 2018). However, the quantitative results from ISAC and ALEX are not always comparable, therefore the analytical performance needs to be further investigated on a larger scale (Buzzulini et al., 2019; Heffler et al., 2018). On the other hand, ABA is a promising allergomics method that combines allergen microarray and flow cytometry. In ABA, different allergens are immobilized to beads with various fluorescence labels; then, the beads are used to absorb IgE in samples. After further being tracked by fluorescence-labeled anti-IgE secondary antibody, the signals are detected by flow cytometry. The kind of allergen is indicated by the fluorescence of the beads, and the IgE affinity is quantified by the fluorescence of the secondary antibody. This strategy was established by Pomponi and colleagues, and the reactivity between 11 allergens and allergic patient sera was determined. They also demonstrated that this method has similar sensitivity to ISAC but the background is lower (Pomponi et al., 2012).

Although these methods are mainly used to detect the allergome of particular individuals, they can also be applied to measure the allergome of particular allergenic sources after proper modifications. Bernardi and colleagues immobilized the allergen candidates to a microarray and characterized two novel allergens of kiwi fruit (Bernardi et al., 2011). Based on the competitive interaction between allergen samples and immobilized known allergens on allergic sera, Pasquariello and colleagues revealed the discrepancy of allergens between different apple cultivars (Pasquariello et al., 2012). Unlike the other three strategies, allergen microarray can use undenatured allergen proteins to actually represent and quantify the interaction between IgE and allergens. Furthermore, as the high-throughput method to determine the allergome of a particular individual, the allergen microarray-based methods (ISAC and ALEX) is acceptable for clinical diagnosis, and have been authoritative approved in many countries (including China, the USA and the European Union) (Heffler et al., 2018; Westwood et al., 2016). Overall, because of the high throughput, rapid speed, and quantifiability, allergen microarray-based strategies have great potential and value in allergomics investigations.

3.4. Bioinformatics-based strategy

Bioinformatics is an interdisciplinary of biology, mathematics and computer science and has greatly promoted the development of omics studies. In fact, bioinformatics is widely used in most of the allergomics strategies described above, especially in the processes involving sequence analysis. On the other hand, bioinformatics can be even more important and play a predominant role in some specific strategies.

As a low-cost and high-throughput method, bioinformatics usually serves as a screening method in the initial stages of allergomics studies. Screening by homologous sequences in a database is commonly used in allergomics study, and this method identifies potential allergenic proteins in a novel source according to the amino acid sequences homologous to known allergens; therefore, a complete allergen database is particularly important to this strategy. Allergome (www.allergome.org) is the most comprehensive allergen database, which currently includes more than 3000 allergenic molecules (Mari & Riccioli, 2004; Mari & Scala, 2006). In addition, common databases such as NCBI and UniProt provide essential information for allergic sources such as genomes, proteomes, and transcriptomes, as well as alignment tools such as BLAST. Moreover, the allergen search engine AllergenOnline, the

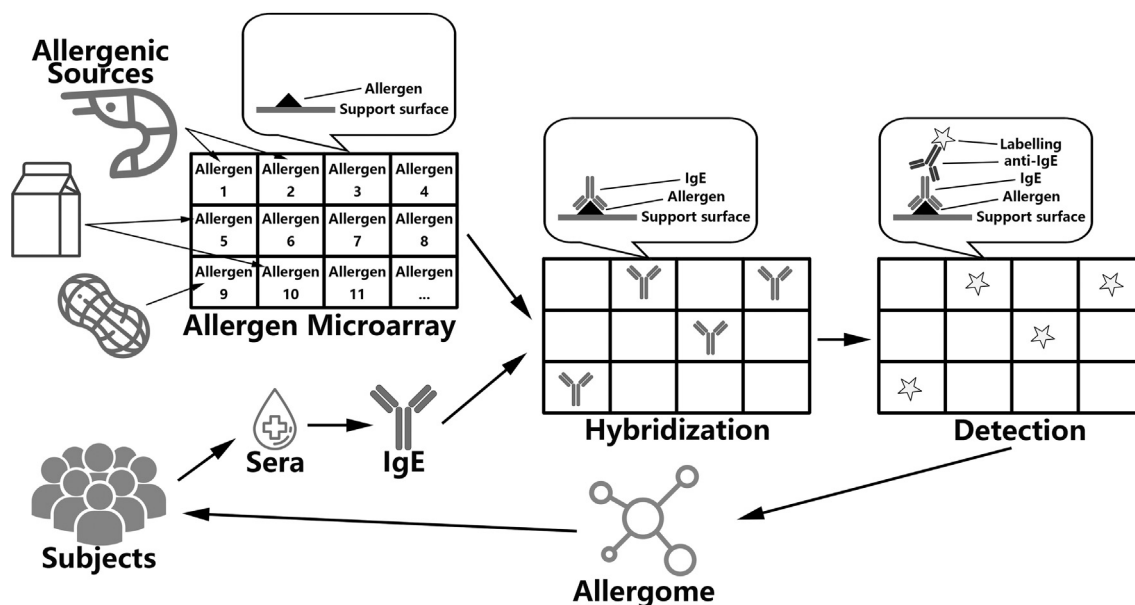


Fig. 3. Schematic diagram of the allergen microarray-based allergomics strategy.

allergen nomenclature database IUIS, the allergen protein family database AllFam, and the allergen structure database SDAP can also be utilized in allergomics studies (Sircar, Sarkar, Bhattacharya, & Saha, 2014). A comprehensive introduction and comparison of these databases have been reviewed in a recent paper and therefore will not be detailed here (Radauer & Breiteneder, 2019). These resources can largely facilitate traditional allergomics studies. For instance, by comparing the proteome and transcriptome data with the Allergome database, Campbell and colleagues established a relatively complete allergome of Johnson grass pollen (Campbell et al., 2015). Relying on the mass spectrometry-based proteomics and bioinformatics identification, the olive pollen allergome was established and Ole e 15 was identified as a novel allergen (San Segundo-Acosta et al., 2019). Similarly, by performing a proteogenomic annotation of the house dust mite, and aligning the sequence data in IUIS with modified algorithm, Erban and colleagues identified the relatively complete allergen repertoire of house dust mite, and reveal the differences in proteomes between male and female mites (Erban, Klimov, Talacko, Harant, & Hubert, 2020).

In recent years, with the intensive investigations on allergens and the accumulation of relevant data, it has become possible to predict novel allergens by allergenic epitopes or domains. For instance, FUGUE recognizes distant homologs by sequence-structure comparison (<http://www-cryst.bioc.cam.ac.uk/fugue/>) (Shi, Blundell, & Mizuguchi, 2001), Allermatch (<http://allermatch.org>) predicts the potential allergenicity of proteins and peptides according to the current FAO/WHO Expert Consultation data (Fiers et al., 2004), AlgPred provides a high-accuracy prediction tool for allergenic proteins by integrating various approaches (Saha & Raghava, 2006), and AllerCatPro predicts the allergenic potential based on the similarity of amino acid sequences and protein structures (Maurer-Stroh et al., 2019). Kulkarni and colleagues collected known allergen sequences in *Fabaceae* family from IUIS and performed protein BLAST to retrieve similar sequences from chickpea (*Cicer arietinum*), and therefore identified seven potential allergens in chickpea. Furthermore, the allergenicity of these putative allergens was assessed by multiple in silico methods such as AllerMatch, Allergen-Online, FUGUE and AlgPred based on the sequence, structural and physicochemical similarities (Kulkarni, Ananthanarayan, & Raman, 2013). Similarly, Garino and colleagues identified 28 putative allergenic lipid transfer protein homologs from allergome databases by using seven publicly available web tools (Garino, Coisson, & Arlorio,

2016). To validate the bioinformatics results, Herman and colleagues compared the results of different approaches on known allergens, assessed the sensitivity and selectivity of these methods, and found that sequence similarity rather than identity is better for identifying true allergens (Herman & Song, 2019).

The bioinformatics-based strategy has been applied in allergomics studies in several aspects and thus has greatly furthered research progress. First, as the basic sequence analysis tool, bioinformatics is the foundation of all the high-throughput biological methods such as proteomics and genomics, which is the premise of allergomics researches. Second, as an initial screening method, it can pick out the potential allergens from massive amounts of data with relatively low time and economic costs for further validation. Third, bioinformatics can predict some special information of allergens (such as the protein structure) which traditional methods can hardly obtain, providing threads for further investigations. And last, bioinformatics can validate the results of traditional strategies, therefore guaranteeing the reliability of the conclusions.

4. The limitation of current allergomics methods

Despite the great significance of allergomics in facilitating the treatment and prevention of food allergies, allergomics is still in its infancy. It is considered that only a small proportion of allergens have been identified according to the recent data, and the complete allergome has not been described to date for any allergenic source (Ciardiello et al., 2013). These defects are mainly due to the limitations of current technologies and insufficient data (Table 2).

Although providing the most direct and reliable results, clinical symptom-based methods such as skin prick test and oral food challenges are limited in throughput, and therefore cannot play a crucial role in allergomics studies. Additionally, roughly detecting the final effect of allergic responses is not helpful for further detailed mechanistic investigations.

Serological methods based on allergen-specific IgE detection have become the dominant method to study allergomes. Despite many advantages, the immunoblotting-based strategy also has certain disadvantages, such as low accuracy and an inability to detect extremely hydrophobic, acidic, alkaline, or low-abundance proteins (Faeste, Ronning, Christians, & Granum, 2011). In addition, the process of manually picking and individually identifying the spots does not

conform to the concept of high-throughput omics research. Consequently, the immunoblotting-based strategy normally can only identify individual allergens and cannot acquire all allergome information. On the other hand, the allergen microarray-based strategy requires the preparation of a huge allergen library, which is a long and costly process and can hardly be applied to all allergic sources. Moreover, in most cases, recombinant proteins rather than native allergens are used in these methods, resulting in the possibility of misfolding or the loss of post-translational modifications, thus impairing the reliability of the results. Furthermore, the non-specific IgE binding, especially to some common modifications such as the cross-reactive carbohydrate determinants (CCD), may lead to the results being more doubtful (Mari, 2002; Mari et al., 1999; van Ree, 2002). And the discrepant results got by microarrays from different sources indicated the unrobust and inferior reliability of the methods (Buzzulini et al., 2019). Bioinformatics-based strategies are the quickest method with the highest throughput but are also the most unreliable. Therefore, further validation by more direct methods is always required for bioinformatics-based strategies. In addition, bioinformatics methods are based on the known allergen sequences or structures, making the methods difficult to identify totally novel allergens. For instance, although some studies asserted the identification of the complete allergome of a specific food, their prediction results were based on the allergen databases, so the identified novel allergens were all similar to known allergens (in the same protein superfamily or even transcribed from the same gene homologs in different species). Moreover, although bioinformatics methods are fast and high-throughput, the subsequential validating procedures still require traditional methods and thus limited efficiency. Overall, any of the allergomics methods have their own limitations; however, by combining several methods, these limitations might be overcome, and more ideal results might be obtained.

It has been demonstrated that the protein structure changes brought about by food processing may significantly alter allergenicity. However, due to the denaturing process of the immunoblotting-based strategy, it cannot be directly used in studying protein structure changes. On the other hand, because of the difficulty in immobilizing processed proteins for phages or microarrays, phage display-based or allergen microarray-based strategies are also incapable of investigating processed food allergens. By contrast, in principle, the bioinformatics-based strategy can reveal the structural epitopes of the allergens and even simulate the structural changes during food processing, but the method is far from complete yet and requires further improvements before practical application. Therefore, although traditional low-throughput methods such as skin prick test, ELISA, and the degranulation assay can be used to investigate the relationship between protein structural change and allergenicity variation, the application of allergomics methods in this area is rare.

5. Prospect of allergomics

In recent years, with the development of relevant technologies, life science has entered the big data era, promoting the development of allergy investigations and creating the field of allergomics. Allergomics makes it possible to identify the repertoire of allergens in a particular food, which is the basis of developing targeted detection and elimination methods and producing hypoallergenic food. The identification of allergomes also provides allergen databases that can facilitate further food allergy investigations. On the other hand, demonstrating the allergomes of particular individuals will promote the personalized prevention and therapy of food allergies, which will maximize treatment efficiency and minimize the impairment of quality of life. Therefore, allergomics research not only deepens our understanding of allergy mechanisms but also has vital clinical significance. However, the current allergomics technologies are far from sufficient to obtain that goal. One of the most significant drawbacks of allergomics is the relatively low reliability, which limited the application of allergomics, especially

in clinical and industrial fields. Consequently, improving the methods as well as developing validating methods are required, and the authorities' approval of allergomics approaches application in various fields should be promoted. Moreover, novel allergomics technologies with higher throughput, more robust output, stronger detection capacity, and lower time and economic costs are urgently needed. Improving the present technologies, combining the available methods, and developing novel strategies may push through the current limitations of allergomics and could be an important direction for future development, which will not only facilitate the current applications but also expand the novel application fields of the methods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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