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Review

Applications of CRISPR-Cas9 Gene Editing Technology in Food Allergy Therapy: A Comprehensive Review

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Abstract

Worldwide, the incidence of allergenicity is rising rapidly, making it an ongoing clinical challenge and public health concern. The current treatment options for allergies include allergen avoidance, medications to improve symptoms, and immunotherapy to desensitize affected individuals to specific allergens. However, these approaches have limitations, and there is an urgent need for novel and more effective therapies. Allergies are a compelling candidate for gene editing (GE) given their prevalence and the inadequacies of existing treatments. Repurposing present allergy medications and emerging novel therapies may be possible with the aid of genomics-guided determination of prospective therapeutic targets for the illness. The emergence of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-GE technology, which uses RNA to guide DNA targeting, has allowed the generation of customized organisms for specific traits. The novel genome-editing tools have shown promising potential to transform allergy research and treatment, offering new hope for patients with severe allergies. By allowing accurate alterations of the genome, GE can be used to delete, correct specific allergen gene (s) that make a person susceptible to allergies, or interfere with the transcription of those genes. Gene editing may be used to engineer immune cells to become more tolerant of particular allergens. This review goes beyond traditional disease therapies by highlighting the latest breakthroughs in this revolutionary field. The applications exemplified in this review reveal how CRISPR can be used to identify the function of allergen proteins and engineer allergen-free plants to develop hypoallergenic foods. But

some allergens play vital roles in physiological processes, such as ameliorating biotic and abiotic stress in plants and disease in animals. Just targeting their genes with CRISPR to abolish expression is not always feasible. The benefits and limitations of CRISPR-Cas9-based GE technology are compared with current treatment options.

Keywords
Allergen-free food; conventional allergy therapy; CRISPR/Cas9 system; CRISPR-based allergy therapy; CRISPR food; food allergy; gene editing therapy; immunotherapy

1. Introduction

1.1 Allergy: An Overview

Globally, with more and more industrialization, the prevalence of allergic diseases is escalating, affecting millions of people [1]. Analysis of data collected in 2021 by the National Health Interview Survey (NHIS) and the National Center for Health Statistics (NCHS) found that approximately 6% of children in the USA aged 17 or younger have a food allergy [2]. Analysis of the same 2021 NHIS data showed a similar incidence (6.2%) of food allergy among adults [3]. The report also showed the prevalence and severity of food allergies among adults.

In 2023, the Food and Drug Administration (FDA) formally published a list of nine major allergy-triggering foods: shellfish, eggs, fish, milk, peanuts, sesame, soybeans, tree nuts, and wheat [4, 5]. Outside these "Big 9", the frequency and severity of allergy symptoms to about 200 food types among 1085 adult patients were surveyed in 2026 [6]. Forty-five foods identified as allergy-inducing were further investigated [6]. The findings indicated that 66.9% had a probable food allergy [symptoms with sensitization], and 33.1% had only a possible food allergy [symptoms independent of sensitization]. Among those grouped as possibly food-allergic, fruit was most frequently reported (68.8%), (63.0%), (39.5%), vegetables (34.0%), and seeds or pits (14.4%). Regarding symptom severity, the order of decreasing severity was: seeds, fish, legumes, nuts, crustaceans, and fruits, with percentages of 39.8%, 39.2%, 34.8%, 31.9%, 31.3%, and 16.5%, respectively. In the same study, the most common severe symptoms among participants with probable food allergy were: sunflower seeds (80.0%), pine nuts (66.7%), Brazil nuts (60.0%), cashews (57.1%), and pistachios (51.7%).

The allergic disease state can be linked to uncomfortable life, increased morbidity, and possible mortality from anaphylaxis [7, 8]. The economic cost of allergic diseases for patients and their caretakers is high and rising.

The following paragraphs provide an overview of some allergy-causing foods, particularly peanuts, eggs, milk, wheat, soybeans, mustard, and pollens, which are the main topic of this review.

Allergies to peanuts are among the most common and adverse food allergies, especially in Western countries, with about 1% to 2% of the population affected [7, 9], and the prevalence has been rising, especially among children. These allergies often persist into adulthood and can be deadly if not managed promptly [10]. Initial peanut marketing has been adopted in various westernized nations as a preventive measure to reduce the risk of developing peanut allergy, though implementation faces barriers [11].

Egg allergy is the second most common food allergy among children, after peanuts, and may persist into adulthood [12]. Still, the course of the disease varies among individuals, and several factors, including geographical region, culture, and dietary patterns, have been suggested to influence its persistence [13].

Another prominent food allergen is milk. Allergy to cow's milk proteins is the most common food allergy in children, affecting 2% to 3% of infants in developed countries [14-16]. This type of allergy involves the inability of affected individuals to absorb and utilize components of cow's milk, leading to severe digestive symptoms such as diarrhea, flatulence, and even death [15]. The Cow's milk allergy may resolve intuitively during adolescence [14, 16].

In contrast to data on eggs, cow's milk, and peanuts, data on wheat allergy are limited [17]. Wheat has become one of the most prevalent food allergens in China, following egg and milk [18]. The global prevalence of wheat allergy has been gradually increasing, and more people are raising awareness about it [19]. Wheat allergy poses a diagnostic challenge owing to its clinical manifestations, including anaphylaxis, shock, and death [20, 21].

Regarding soybeans, 5% to 8% of babies and 2% of adults were reported to be allergic in the United States and Europe. In several countries, soy-based ingredients are designated as high-priority allergenic foods that should always be declared on food package labels [22]. Some children may also experience oral allergy, i.e., symptoms limited to the mouth, such as itching, a rash, or swelling. In more severe cases, some children may develop anaphylaxis. Fruits such as apples, melons, and pineapples, as well as some vegetables, are among the foods that typically trigger oral allergies [23].

Another type of food allergy is allergy to mustard, specifically seeds. In general, studies on seed allergies are limited [8]. Although mustard allergy is rare overall, it is sometimes considered among the most frequent spice allergies. It usually begins before age 3. On average, mustard allergy accounts for 6-7% of total food allergy cases, with a lower incidence in children (1.1% of allergies) [24]. In Canada, mustard is listed as a priority allergen [25]. Approximately 50% of patients with mustard allergy are also sensitized to Mugwort pollen (the common name for various species within the genus *Artemisia*) [26]. In hypersensitive people, mistakenly eating small quantities of mustard products can trigger life-threatening anaphylactic reactions [27, 28].

Pollen Food Syndrome (PFS) is a common food allergy in children and adults, with increasing prevalence, and onset typically occurs after sensitization to pollen [29]. Air pollution has been indicated to elevate the concentration of a key birch tree pollen allergen [30]. A person is primarily sensitized to pollen before increases in PFS, either in childhood or adulthood, and is more susceptible in childhood than previously believed [31]. There is growing concern about systemic reactions associated with the increasing severity of pollen allergy and dietary alterations, including the more common adoption of plant-based foods, such as soya in protein shakes, soy/nut milks, and nutritional products [32]. For reference, the pollen-food allergy has been recently reviewed [33].

1.2 Biological Origin of Allergy

Understanding the mechanisms of allergic reactions provides compelling insight into how our bodies can mistake non-dangerous substances for dangerous threats. The exact etiology of allergic diseases, which can cause a wide range of symptoms, from a runny nose to a life-threatening emergency, is complex and remains poorly understood, with research still in its early stages [34].

Next, a brief overview of the interplay among environmental, genetic, and immunological factors in allergic reactions is provided.

1.2.1 Immunological Origin

Allergy cross-hypersensitivity reactions occur when the body's immune system mistakenly identifies harmless substances (allergens) as similar to one another, triggering an immune response [35]. Figure 1 illustrates Immunoglobulin E (Inge)- mediated molecular allergic reactions that occur after accidental exposure to certain foods. Macrophages and dendritic cells are particularly important antigen-presenting immune cells that phagocytose foreign food antigens and present antigens to T lymphocytes via Major histocompatibility complex class II (MHC II) molecules [36, 37]. T cells that recognize and respond to food antigens differentiate into T helper (Th2) cells and secrete interleukins (IL), which contribute to allergies. These cytokines, which are released by Th2 T cells in response to an antigen, incite B cells to produce Inge. Allergen-bound Igga interacts with its receptors on mast cells, ultimately releasing large amounts of mediators such as histamine. Mast cell histamine causes allergic symptoms such as edema, swelling, itching around the mouth, diarrhea, breathing difficulty, coronary artery spasm, and anaphylaxis, sometimes leading to death.

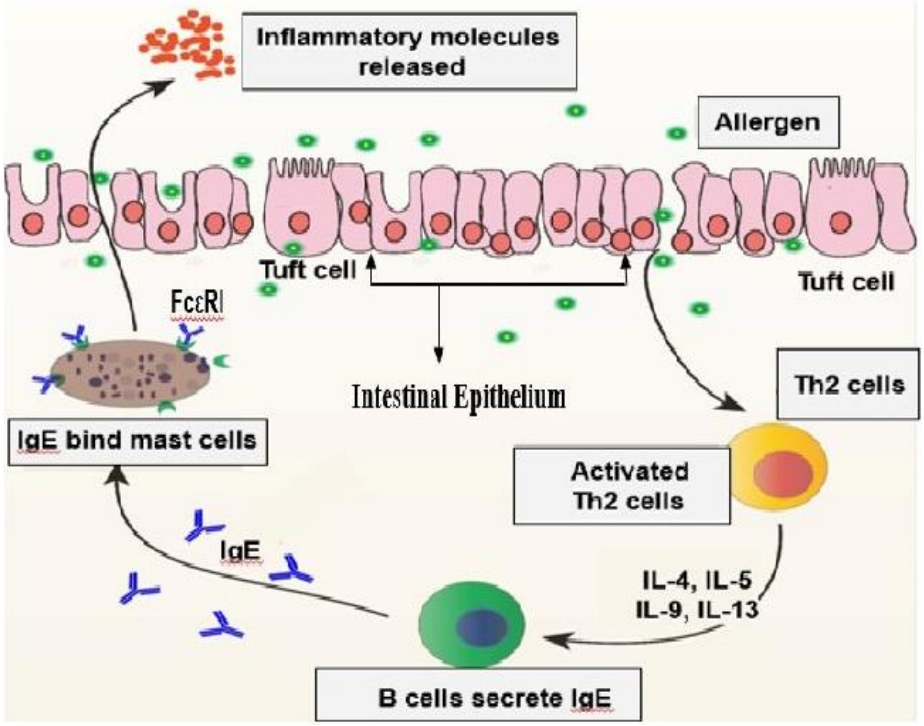

Figure 1 Simplified diagrammatic illustration of the Igga-mediated allergy mechanism. The secreted allergen is carried to allergen-specific immune cells, which recognize it as foreign material and eventually lead to allergic symptoms. Activated T helper 2 (Th2) cells secrete key cytokines, interleukins (e.g., IL-4 and IL-13), which drive the assembly of Immunoglobulin E (IgE) molecules by B cells and mast cell degranulation. The resulting cascade stimulates sustained skin inflammation and chronic itch, typical of Th2-dominant conditions. Abbreviations: FcƐRI: Fragment crystallizable epsilon receptor. (Adapted from https://med.stanford.edu/allergyandasthma/news/news-from-our-center/crispr).

1.2.2 Genetic Origin

Food allergies are powered by a complex interplay between environmental factors and genetic predisposition, with heritability estimates ranging from 15% to over 80%. Previous studies [38, 39] confirmed that family history is a crucial risk factor for food allergy. For example, a child whose parent or sibling has a peanut allergy has a higher likelihood of being peanut allergic than a child of parents without a history of this type of allergy. Furthermore, a twin study of peanut allergy found that concordance was substantially higher among identical twins than among dizygotic twins (64.3% versus 6.8%, respectively) [12]. No single specific “food allergy” gene is known; likely, an interplay between genes and the environment is involved. Meaning that allergy is not inherited as a single, Mendelian trait, but often linked to genes that regulate immune functions (e.g., HLA, IL4) and epithelial barrier integrity (e.g., FLG, SERPINB7). To date, only a few allergy-initiating genes have been recognized and edited (Table 1).

Table 1 Examples of the candidate genes reported to be susceptible to food allergy, and those genes have already been explored or applied in gene editing.

Genes susceptible to food allergy	**Type of food**	**Function**	**Genes explored or applied in GE**	**Type of food**	**Function**
CD14	Peanut	Crucial for immune defense	*Ara h*	Peanut	*Ara h* genes encode an array of seed storage proteins and defense-associated proteins,
FLG	Cow's milk, fish, hazelnut, hen's eggs, Peanut, sesame, and soy	Encodes profilaggrin, an important protein for preserving the skin's barrier, hydration, and pH	*ATIs*	Wheat	Encode albumins, which principally defend against pests and pathogens
FOXP3	Cow's milk and hen's	Master regulator for the development and function of Tregs	*BLG*	Milk	Encodes the main whey protein in ruminant milk
HLA	Peanut	Presents antigens to T cells, triggering immune responses, preventing autoimmune attacks	*Bra j*	Brown mustard seeds	To serve as a nitrogen and sulfur reserve for the growing embryo
IL-10	Cow's milk and hen's	Down-regulates the expression of Th1 cytokines, MHC class II antigens, and costimulatory molecules on macrophages	*CYP11A1*	Peanut	a key regulator of the expansion of food allergies by driving a specific immune response that generates Type 2 cytokines.
IL-13	Cow's milk and hen's eggs	A Key immunoregulatory cytokine generated mainly by activated Th2 cells	*Gal d1* (*Ovomucoid*)	Hen's eggs	Primarily functions as a trypsin inhibitor
KLK7	Peanut and milk	Encodes Kallikrein-related peptidase 7, a chymotrypsin-like serine protease essential for skin homeostasis	*Gly m*	Soybean	The *Glym* gene family plays varied key roles, primarily in seed composition, plant defense, and growth control.
SPINK5	Eggs, milk, and peanuts	Expressed in the thymus and codes for a protease inhibitor protein	*Sin a*	White mustard seeds	A key nutrient storage constituent in the seed

STAT6	Cashew, cow's milk, peanut, and walnut	The key molecule in the signal transduction pathway regulated by IL-4 and IL-13 in IgE isotype switching and the generation of Th2 cytokines	*VPS*	Pollen, particularly in *Arabidopsis*	The genes act as tethering factors to control vesicular trafficking, vacuole biogenesis, and, consequently, pollen tube.

Abbreviations: *Ara h: Arachis hypogea; ATI: Amylase/trypsin inhibitor; BLG: Beta-lactoglobulin; Bra j: Brassica juncea; Cluster of differentiation; CYP11A1: Cytochrome P450 family 11 subfamily A member 1; Gal: Gallus; Gly m: Glycine max; FLG: Filaggrin; FOXP3: Forkhead box P3; HLA: Human leukocyte antigen; IgE: Immunoglobulin E; IL: Interleukin; MHC-II Ags: Major histocompatibility complex class II Atypical glandular cells; Tregs: Regulatory T cells; SPINK: Serine protease inhibitor Karzal type; Sin a: Sinapis alba; STAT: Signal transducer and activator of transcription; Th: T helper; VPS: Vacuolar Protein Sorting.*

1.3 Food Allergy Therapy

As outlined before, genetics is a critical element in the pathophysiology of allergic disease. Examining molecular genetic aspects of allergy in relation to clinical trial participation offers an opportunity to gain insight into the adaptive responses occurring in the body during immunotherapy. The complete spectrum of allergic diseases, in part because of their shared pathophysiology, is ripe for studies with various technologies. Despite widespread food allergies worldwide, there is no specific treatment. To date, no direct method for determining the effectiveness or ineffectiveness of the medical intervention has been provided.

Various therapeutic approaches have been used to treat allergies (Figure 2). The current medical standard of care for sensitized patients is largely physical (strict avoidance of the allergen sources) [40-42]. However, even allergen avoidance has become challenging amid the rapid expansion of the food industry. Neither restaurants nor food manufacturers are willing to guarantee the availability of allergy-free products, leaving individuals with allergies in an uncomfortable situation [42]. For individuals with food allergies, avoiding certain foods can be problematic. In some cases, even with avoiding them, an inadvertent response to a specific allergen may result in social stress, disease, hospitalization, or sometimes death [43].

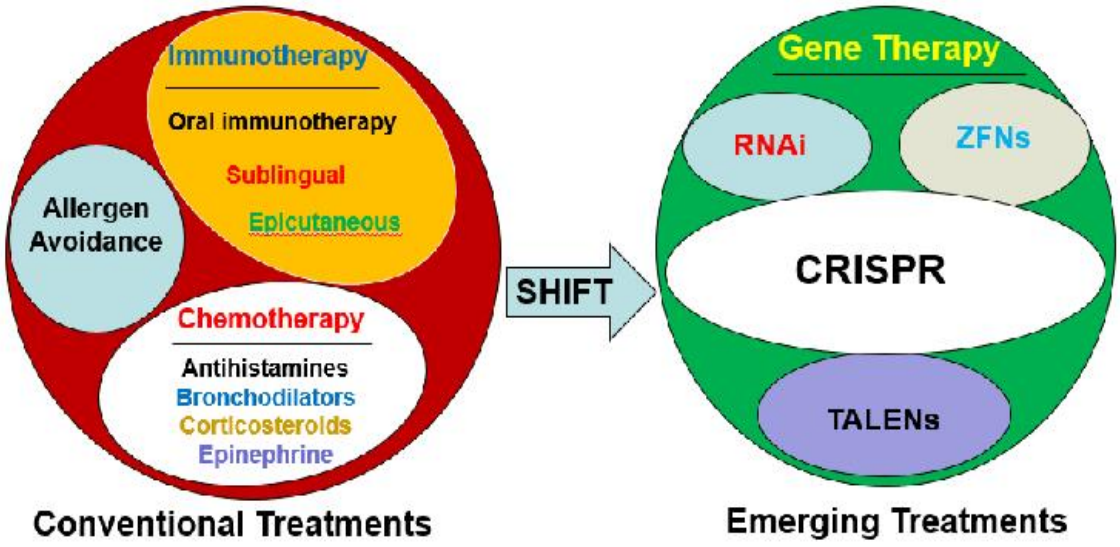

Figure 2 The shift from the conventional strategies to the emerging strategies in allergy therapy and management. Currently, novel gene therapy approaches are more successful in producing non-allergenic or hypoallergenic crop varieties. Abbreviations: CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; RNAi: Interference RNA; TALENs: Transcription activator-like effector nucleases; ZFNs: Zinc finger nucleases.

Chemical approaches (e.g., antihistamines, bronchodilators, or corticosteroids) and immunotherapy approaches, such as Epicutaneous Immunotherapy, Oral Immunotherapy (OIT), and Sublingual Immunotherapy [44], have been attempted. Oral Immunotherapy is the only FDA-approved immunotherapy [40]. In August 2024, the FDA approved the first intranasal form of epinephrine for the treatment of anaphylaxis in individuals weighing over 30 kg, which has been shown to have systemic equivalent to intramuscular epinephrine absorption in clinical trials [45, 46].

Overall, conventional therapies for allergic diseases primarily focus on managing symptoms rather than addressing the underlying immunologic causes [47]. For a more detailed comparison of conventional and modern breeding technologies, the reader is directed to reference [48]. Table 2 provides a brief comparison of the conventional, established, and emerging technologies applied in food allergy therapy.

Table 2 Comparative summary of selected conventional, established, and emerging therapeutic strategies for food allergies‡.

Strategy (type of drug)	Mechanism	Aspect of Comparison			References
		Application Scope	Advantages	Disadvantages	
CONVENTIONAL * Avoidance and Symptomatic Meds (Antihistamines, Steroids	Non-specific lessening of inflammation and histamine response.	All allergies (Food, environmental).	* Fast relief * Readily available * Low cost.	* No long-term cure (Temporary) * Side effects with long-lasting use.	[40, 41]
CONVENTIONAL * Herbal medicine (Use of the entire plant or its parts)	* Decrease in histamine and IgE plasma levels * Decline in B cells in the spleen * Modify gut microbiota	Food allergy	* Reduce anaphylactic symptoms * May have fewer side effects * Cost-effective	* Limited clinical data (More investigations are needed to decipher the mechanistic insights) * Variability in herbal product potency * Risk of self-treatment without professional guidance * Needs standardization and regulation	[49, 50]
CONVENTIONAL * OIT (Allergen- e.g., Palforzia is taken orally)	Allergen exposure promotes the expansion of allergen-specific $CD4^+FOXP^+$ Tregs and immune fluctuations.	Food allergies (Baked cow's milk, cashew, cow's milk, egg, peanut, sesame, shrimp, wheat)	* Allows consumption of small amounts *Larger maintenance doses (g) than SLIT/EPIT (mg). * More effective than SLIT * FDA-approved product available for peanut allergy * Multi-food OIT may treat multiple food allergies concurrently * Prevents fatal reactions.	* Highest side effects (allergic reactions/anaphylaxis) * Not tolerated in 15%-20% of suffers * Possibility for eosinophilic esophagitis * Needs long-term treatment (Daily compliance needed). For example, to date, it is not clear how long wheat OIT should be continued.	[17, 40, 51]

ESTABLISHED * SLIT (Allergen-{Drops/Tablets} is taken sublingually)	* Similar to SCIT, it induces tolerance via the mucosa. * Potentially functions via increasing allergen-specific $CD4^+FOXP3^+$ Tregs population	Aeroallergens (Dust mite, grass).	* High safety profile * Fewer side effects than OIT tolerated in 5% of patients * Home administration.	* Less effective than SCIT. * Only capable of reaching small maintenance doses (mg) * Not as effective as OIT * Needs long-term therapy (Daily adherence) * Theoretical risk of Eosinophilic esophagitis.	[52]
ESTABLISHED * Subcutaneous Immunotherapy (SCIT - Allergen is administered via injections)	Induces tolerance by promoting IgG4 Antibodies and Treg cells. Increase in allergen-specific IgE along with an increase	* Aeroallergens * Some food allergies * Venom (Not FDA approved for food).	* Long-lasting relief * M durable long-term immune tolerance * Disease modification * Allows for multi-allergen formulations * Generally, it needs less frequent dosing during the maintenance phase	* Needs frequent office visits * High risk of anaphylactic shock * Systemic hypersensitivity * Treatment usually involves a longer build-up phase	[53]
EMERGING * Biologics (e.g., Omalizumab/Xolair)	Humanized mouse monoclonal antibodies blocking key immune pathways involved in allergic responses (IgE or cytokines {IL-4/5/13})	* Designed to target IgE to treat moderate-to-severe allergic cases * Food Allergies (as of 2024/2025).	* High efficiency for severe cases (Enhanced efficiency in disruption of IgE: FcεRI complexes) * Fast action * Potentially desensitize to multiple food allergens * May have the least side effects * The FDA approved some monoclonal antibodies * Specificity minimizes off-target effects and systemic immunosuppression * Can be used alone or alongside OIT to improve	* Expensive * Need continual injections often over long periods * Typically manage symptoms rather than treating the allergy * Long-term safety data are still early	[54]

			desensitization success rates		
FEMERGING * Epicutaneous Immunotherapy (EPIT - Patch)	* Delivers antigens to skin dendritic cells to induce tolerance * Potentially functions via increasing allergen-specific $CD4^+FOXP3^+$ Treg cell population	Food allergies (Peanut)	* High safety (no systemic absorption) * High compliance * May have fewer side effects than SLIT and OIT * May be safer in broader clinical settings	* Lower efficacy compared to other allergen immunotherapy modalities (OIT/SCIT) * Only able to reach small maintenance doses (mg) * May not be safe in patients with AD * Prolonged duration of treatment	[55, 56]
EMERGING * LAMP-DNA vaccines Lysosome-Associated Membrane Protein (LAMP) chimeric DNA vaccines)	* Bacterial plasmids containing DNA encoding the target allergen and lysosomal-associated membrane protein 1 (LAMP-1) for increasing immunogenicity * Engineered to enhance immune responses by fusing an antigen with LAMP-1, directing it to the MHC class II pathway.	Food allergies	* Promotes antigen presentation * Can generate potent immune responses without the requirement for traditional therapy * Stable and safe * Generally inexpensive	* Limited human data * May need additional enhancers to improve immunogenicity * Administration via injection may result in injection site reactogenicity	[51, 57]
FUTURE/RESEARCH * Microbiome Modulation and transplantation (Probiotics/FMT)	* Transferring stool from a healthy donor into the gastrointestinal tract of a recipient restores gut balance, reduces systemic inflammation	* Food allergy * AD	* Potentially curative by treating the root cause	* Lacks conclusive clinical trial data * Limited large-scale clinical data * Variability in patient responses * Need for standardized protocols * Regulatory hurdles	[49, 51, 58]

	and modulates immune responses				
FUTURE/RESEARCH * Engineered Immunotherapy for vaccination (mRNA/Nanoparticles)	* Deliver genetically-modified allergens to cells for producing therapeutic proteins or antigens using nanoparticles	All allergic diseases	* High targeting efficiency * Fewer adverse effects	* Needs chemical modifications to decrease immunostimulatory impacts and nanotechnology to inhibit degradation and guarantee intracellular delivery * Early stages (preclinical/early clinical)	[58]
FUTURE/RESEARCH * CRISPR therapy	Based on the editing of allergen genes	* Generating hypoallergenic food sources (peanuts, soy, wheat, etc.) * Evolving allergen-free livestock (buffaloes, cows, goats, sheep) * Engineering therapeutic immune cells to produce tolerance	* CRISPR has established success in several clinical applications, such as preliminary KO research on allergenic proteins * High editing efficiency (>90%)	* Potential for "off-target" editing * Challenges in delivering CRISPR reagents in vivo to target tissues.	[9, 35, 59-63]

‡ Abbreviations: AD: Atopic Dermatitis; CD4: Cluster of Differentiation 4; CSU: Chronic Spontaneous Urticaria; EPIT: Epicutaneous immunotherapy; FcεRI: High-affinity IgE receptor; FMT: Fecal microbial transplantation; FOXP3^{+}: Forkhead box P3-positive; IgE: Immunoglobulin E; IgG: Immunoglobulin G; IL: interleukin; MHC: Major histocompatibility complex; OIT: Oral Immunotherapy; SCIT: Subcutaneous Immunotherapy; SLIT: sublingual immunotherapy; Treg cell: regulatory T cell.

In addition to the previously addressed drawbacks associated with traditional therapies, many of them couldn't obtain approval for use due to toxicity or inefficacy. Additionally, the medications for allergies are expensive, and there is a burden of self-sustaining epinephrine [64], and the drug or desensitization dose has to be prescribed lifelong. Furthermore, only a few clinical trials have been conducted to verify the efficacy of targeted immunotherapies for food [64] or pollen [65] allergens.

Moreover, exogenous mRNA has been transferred into cells to enhance the translation of the encoded genetic information into therapeutic proteins [55]. However, using mRNA as a drug faces several challenges, including its inability to cross cellular membranes due to its large size and negatively charged phosphate backbone. Additionally, exogenous mRNA has immunostimulatory

effects and is inherently unstable due to rapid degradation by cellular ribonucleases [56]. A key feature of engineering therapeutic mRNA is the removal of immunogenic double-stranded RNA during construction, which significantly increases the immunogenicity of in vitro-transcribed mRNA [57]. Lipid Nanoparticle (LNP) Platform Technology for mRNA Delivery has emerged as a promising approach for mRNA therapeutics due to its ability to protect mRNA from degradation and facilitate its cellular uptake [58].

Given the limited success or failure of efforts to select low-allergen food varieties using traditional methods [9], new gene-editing (GE) technologies have emerged. These technologies have diverse potential applications, including improving understanding of allergens and allergic diseases, generating hypoallergenic or allergen-free foods, and engineering immune responses to allergens.

As GE methods, the traditional interference RNA (RNAi), as well as the first generation (Zinc finger nucleases, ZFNs) and the second generation (Transcription activator-like effector nucleases, TALENs), can shift the course of allergic diseases, offer long-term relief, and restore quality of life by reducing dependence on emergency healthcare. However, these technologies are limited by their complex design and risk of off-target effects (OTEs).

In contrast, the third generation (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9)) boasts significant improvements in editing efficacy and breakthroughs in GE efficacy, offering unique throughput, simplicity, rapidity, accuracy, reproducibility, specificity, and cost-efficiency [63, 64]. Due to its unique and superior features, CRISPR-based GE has become commonplace in the scientific community for targeting specific genes responsible for particular diseases.

Using CRISPR tools, researchers can cut DNA to create gene knockouts (KOs) and modify allergy-causing genes. Figure 3 shows the basic structure and function of the CRISPR/Cas9 system.

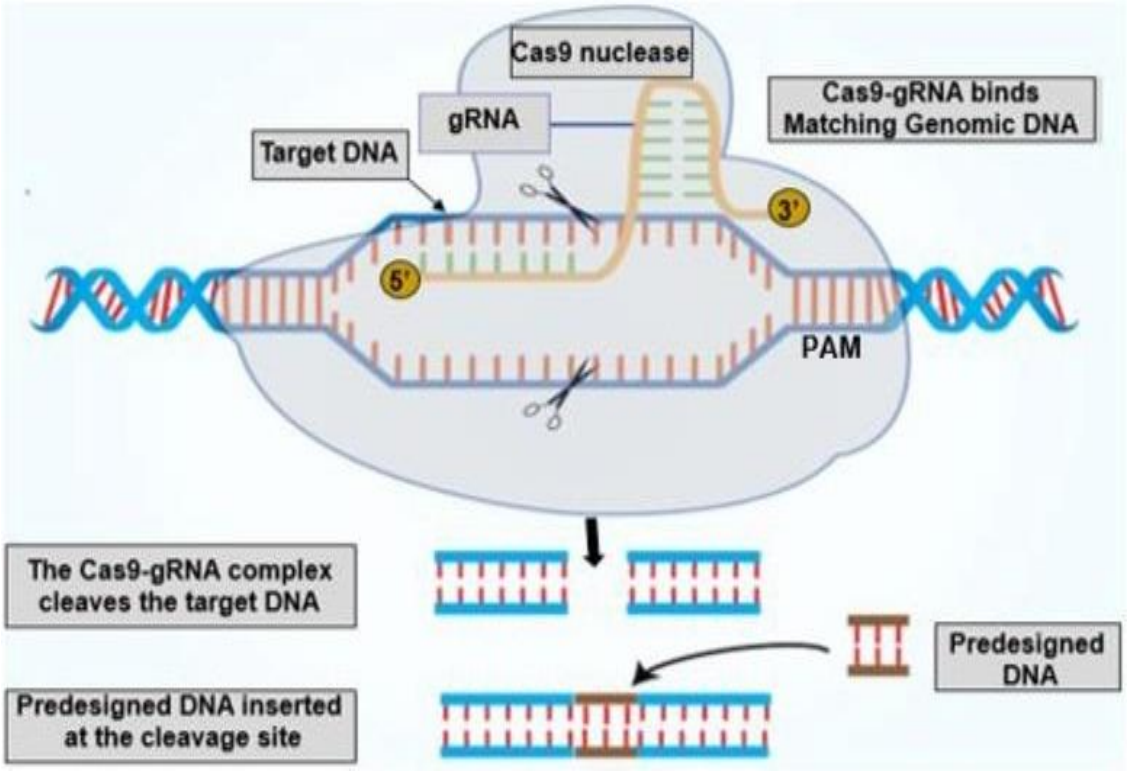

Figure 3 CRISPR/Cas9 structure and function. Cas9 is a single protein that has both DNA-targeting and cutting activity. Cas9, loaded with an sgRNA, identifies the PAM and hybridizes with it at a particular genomic site. The sgRNA/Cas9 complex initiates a DSB upstream of the PAM. The intrinsic cellular mechanisms that repair the target gene disrupted by DSBs through either NHEJ or HDR, by integrating a donor DNA template carrying the correct sequence. Abbreviations: Cas9: CRISPR-associated protein 9; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; DSB: Double-strand breaks; sgRNA: small guide RNA; HDR: Homology-directed repair; NHEJ: Nonhomologous end joining; PAM: Protospacer-adjacent motif. (Adapted from Figure 4 in reference [48] and Figure 1 in reference [66]).

The system consists of 2 major components [48, 66, 67]. The first is the Cas9 nuclease (Cas9 Protein), which acts as a “molecular scissors” or “snipping tool” to cut DNA at a specific location. The second is guide RNA (gRNA) [or small guide RNA (sgRNA)], a specifically designed, short synthetic RNA sequence that guides the Cas9 enzyme to the precise target sequence within a gene in a cell. The Cas9-sgRNA complex recognizes the Protospacer Adjacent Motif (PAM), which is about 100 nucleotides (nts) long and consists of a 17- to 20-nt-long guide sequence that must be located near the target site for Cas9 to bind and cleave the DNA efficiently [48, 66, 67]. The protospacer is complementary to the target DNA sequence, while the remaining sequences bind to Cas9, positioning the enzyme precisely at the target site.

The attachment of the sgRNA to its target DNA stimulates Cas9 to cut the exact DNA sequence corresponding to the sgRNA, producing site-specific double-stranded DNA breaks (DSBs) in the genome. The newly formed DSBs must be repaired appropriately; otherwise, they are lethal to cells. A DSB in DNA can be repaired by two highly conserved DNA repair pathways [66, 67]. The first mechanism, which is more often used, directly ligates broken strands without the necessity for a

homologous template (Non-Homologous End-Joining; NHEJ). This pathway is imprecise and error-prone, causing gene disruption and enabling scientists to create insertions or deletions (indels) in the target DNA, which can lead to frameshift mutations in the corresponding protein sequence. The resulting mutations could be a compelling means of understanding the role of particular genes. The second method for repairing DSBs is to insert a precise short DNA strand at the spliced region. Therefore, the target gene can be modified at the break site. By designing and supplying special DNA templates to the cell, scientists can change a gene as needed or correct a mutation. This frontier technology has far-reaching consequences. The second method to fix is homology-directed repair (HDR), which requires a donor DNA template with flanking homology arms to direct accurate, indel-free repair [66, 67].

The success of CRISPR editing of the desired DNA sequence can be demonstrated using several methods, such as Sanger sequencing, Next-Generation Sequencing (NGS), Tracking of indels by Decomposition (TIDE) assay, indel Detection by Amplicon Analysis (IDAA), and mismatch cleavage assays (T7E1/Surveyor) [66, 68-70]. Using this technology, scientists can easily cleave DNA with unmatched accuracy to generate gene KO, alter genes, and identify genes that initiate disease. The applications of CRISPR/Cas9 for genome-wide, large-scale screening are now well established across various fields. However, a major concern in practical CRISPR/Cas9 screening is that Cas9-Induced DNA DSBs activate a p53-dependent DNA damage response (DDR). This response can lead to cell-cycle arrest, apoptosis, or p53-dependent toxicity, thereby considerably reducing editing efficiency and creating a selective growth disadvantage for successfully edited cells with functional p53 [71, 72]. Consequently, there is a strong interest in investigating DDR-related biomarkers to develop revolutionary therapeutic regimens and in advancing very sensitive tools for DDR diagnosis [73].

CRISPR/Cas9 technology has primarily been employed in research to study diseases using cell and animal models. However, it often faces critical challenge in therapeutic applications due to the hidden risks of generating random large-scale chromosomal deletions [74, 75] and chromosome fragmentation [76], as well as other genomic rearrangements [75-77], the CRISPR/Cas9 system is prone to OTEs, in which unintended mutations may occur in the gene being edited, because Cas9 functions as a monomer that recognizes a shorter target region, and sgRNAs can tolerate some mismatches. Therefore, different versions of efficient Cas9 enzymes: Cas 12, Cas13, Cas9n (Cas9 nickase), CRISPRa, and CRISPRi (CRISPR interference) have been designed to deal with the complex genomic alterations that occur during diseases [66, 67, 78]. In contrast, CRISPRi, as an epigenetic control tool, allows modification of gene expression without constantly altering the genome or introducing breaks by combining a repressive domain with dCas9. Consequently, evades concerns about genotoxicity [64]. Recent optimization of the CRISPR-based GE sensitivity and specificity through automation and artificial intelligence (AI) [79, 80] may enable more effective use of functional assessments across a wide range of screening contexts. However, ongoing research led to easier and more precise discoveries, such as Base Editing (BE).

The innovation (EB) has been launched as a possible alternative to empower the aforementioned generations of the CRISPR system [48, 81, 82]. This platform has two forms: cytidine base editors, which enable C-to-T conversions; and adenine base editors, which permit A-to-G conversions. This technology is tailored to overcome the limitations of conventional CRISPR-Cas9, particularly by providing a more site-specific and accurate “pencil and eraser” apparatus for single-letter DNA edits without inducing DSBs. As of 2025-2026, this technology has progressed beyond the early “genetic

scissors" era into an advanced stage, offering enhanced accuracy in treating genetic diseases and permitting novel agricultural applications.

Prime editing (PE) technology is evolving rapidly in 2025 and early 2026, shifting from a basic research tool to a high-efficacy "search-and-replace" phase for therapeutic applications and complex GE [83, 84]. It can introduce the desired base-to-base conversions as well as small insertions and deletions. Major advancements focus on improving editing efficiency, expanding the editing window, and enabling large-fragment insertions without DSBs. Plant PE, especially codon optimized for plant cells, is evolving into a groundbreaking mechanism for plant genome engineering, enabling precise genetic amendments across diverse crop species [83] A more detailed representation of the basic structure and mechanism of action of the CRISPR/Cas systems, as well as the base and primer editors, can be found in [84] (Figure 3 and Figure 4 in reference [84]).

Advances in GE technologies, applied to the treatment of various human diseases, have been the best solution for addressing treatment gaps in the therapy of allergic diseases. The objective of this review was to describe how the CRISPR/Cas9system has been "successfully" applied as a powerful strategy for enabling precise editing of specific genes involved in allergic diseases. Because the CRISPR system surpasses other tools, this review focuses on CRISPR as the most advanced technology for GE.

Several existing or potential uses of CRISPR-based GE technology in the treatment of allergy are presented. Following authentic methodology, the review concludes that, despite promises to address unmet needs in allergy care, limitations remain.

2. Method

2.1 Information Sources and Search Strategy

The search was limited to literature published between 03 February 2016 and 12 Apr 2026. To find credible sources on this trending topic, three major academic databases were used independently: Google Scholar, PubMed, and Web of Science. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [85] were followed to ensure transparency and reproducibility (Figure 4). The search keywords included "Allergy", "Allergic diseases", Allergen-Free", "allergen-reduced", and "CRISPR", "CRISPR-Cas", "CRISPR therapy". The AND/OR Boolean operators were used to refine the demand; the study selection process involved screening titles and abstracts, followed by full-text screening.

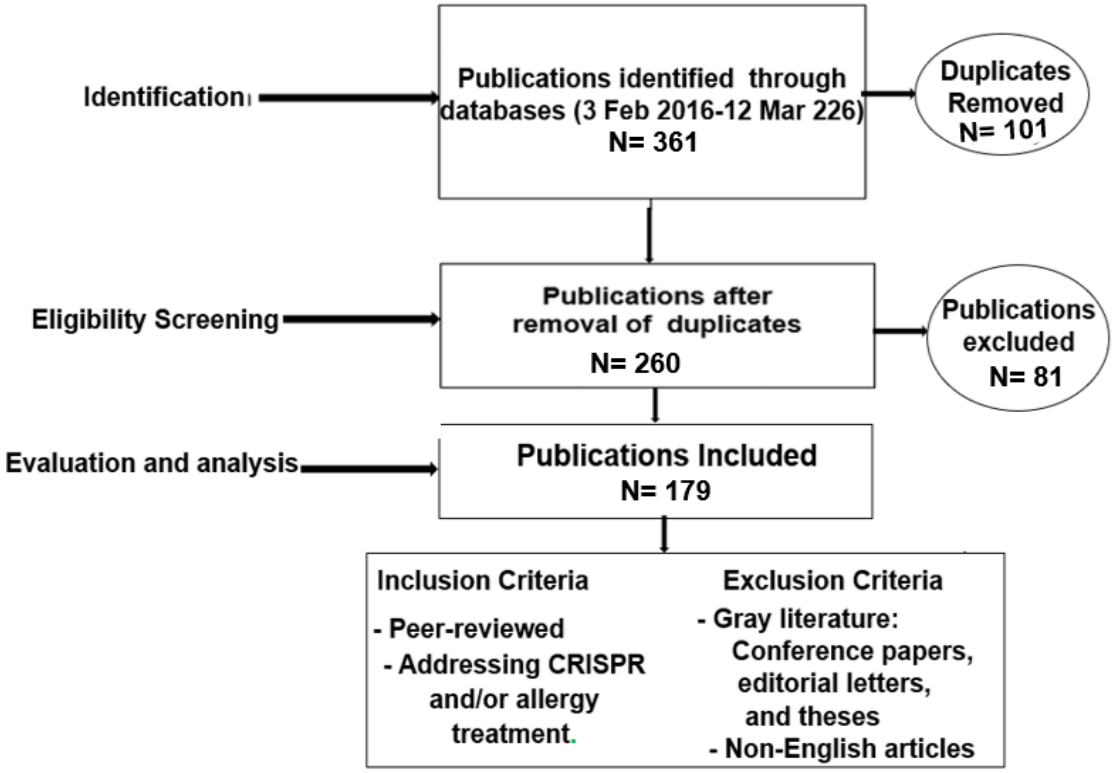

Figure 4 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart. The diagram outlines the systematic search and screening process, including the identification of records from databases (361), removal of duplicates (81), full-text eligibility screening, and reasons for exclusion, culminating in the final set of included studies (179) [85].

2.2 Inclusion Criteria

Only authoritative peer-reviewed journal publications and organizations with full text were included. Studies were complete comprehensive texts, original research articles, or reviews addressing CRISPR and/or allergy treatment.

2.3 Exclusion Criteria

Excluded full-text articles were gray literature, such as conference papers, editorial letters, meeting abstracts, and theses, that did not meet the inclusion criteria. Articles with insufficient data or in a non-English language were excluded.

2.4 Data Synthesis

The outcomes were organized thematically, addressing CRISPR therapy for allergic diseases, allergen-targeted genes, food species, the CRISPR approach, outcomes, and limitations. A meta-analysis was considered but not undertaken owing to heterogeneity of the results and study designs.

2.5 Risk of Bias Assessment

The primary criterion for minimizing bias in the review process was that each study be "blindly" assessed at least twice. The "blind" collection/analysis of the data was the second criterion used to avoid "bias" and ensure quality. The risk of bias was assessed by assigning each study a quality grade/level of 'High', 'Low', or 'Unclear' across several key domains, such as methodological limitations, indirectness of the evidence to the question at hand, inaccuracy of estimates, inconsistency, and the likelihood of publication bias. In general, if the needed information to rationally assess the risk of bias was not clearly stated, a score of 'Unclear' was given. Otherwise, it was assessed as either 'High' or 'Low' according to the reported methods. A strong evidence certainty score indicates that the researchers are quite confident the effect they observed across investigations is close to the true effect, whereas a very low score indicates they have very little confidence in the effect.

3. Results

3.1 Study Selection

The initial search identified a large number of articles from the three online databases (361). The titles and abstracts were first screened to select those eligible for full-text review. After removing (101) duplicates and (81) full-text records that did not meet the inclusion criteria, only the most relevant works (179) were retained for evaluation and data extraction (Figure 4). Of the deemed eligible articles, (3) were categorized as book chapters. The publications that met the inclusion criteria were classified into four categories: pure allergic studies (75), pure CRISPR research (60), hybrid allergy/CRISPR investigation (41), and others (3). About 63.7% (114 out of 179) of them appeared between 1 Jan 2023 and 12 Apr 2026.

The following sections briefly review recent advances in CRISPR-based editing technologies applied to genes relevant to the treatment of selected food allergies.

3.2 CRISPR Therapy for Allergy Diseases

CRISPR GE screening technology is now being tested to identify new therapeutic targets for treating allergy and ultimately achieve significant improvements in the treatment of allergic disease. Preliminary KO research on allergenic proteins using CRISPR editing presents promise. Given the advantages of CRISPR and its ability to target specific DNA sequences in allergen genes, CRISPR GE is a viable strategy for addressing allergy, potentially resulting in substantial disease improvement.

Table 3 summarizes successful case studies of CRISPR-based editing of allergen genes from various sources.

Table 3 A summary comparing allergen targets, species, CRISPR approach, outcomes, and limitations.

Primary Target Gene	Food	Species	CRISPR-GE Approach	Outcomes	Limitations	References
Gal d1-2	Eggs	*Gallus domestics*	CRISPR/Cas9 was used to establish chickens with *Gal d1* and *Gal d2* mutations	* Ovalbumen and ovomucoid genes were efficiently knocked out * No off-target at predicted potential loci * Ovalbumen eliminated without effect on fertility	* Technical problems in accessing and maneuvering chicken embryos * Mosaicism in offspring * Regulatory/safety	[86, 87]
		Jinhua chicken (*Gallus gallus*)	CRISPR/Cas9/CRISPRi - gene KO	CRISPRi avoids concerns about genotoxicity	CRISPRi is less efficient in chicken cells than in typical mammalian systems.	[64]
BLG	Milk	Dairy animals (cows, goats, sheep, and buffalo).	CRISPR/Cas9 *BLG* bi-allelic KO	* Production of no allergen or hypoallergenic milk * Milk nutritional values are retained	* Low cloning efficiency * Ethical and safety regulatory issues	[88-90]
ATIs	Bread	*Triticum aestivum*	CRISPR/Cas9-mediated gene KO	* Reduction in α-amylase and trypsin inhibition activity to 50.7% and 44.9%, respectively.	* High Genome Complexity (High sequence redundancy) * Possible effects of drastically reducing certain proteins on dough elasticity and baking quality. * Need regulatory approval for consumer consumption.	[91]
Gliadins				* Elimination or reduction of gliadin epitopes * Up to 85% decrease in gliadin toxicity achieved		[92]
Ara h 1-3, 6	Oil	*Arachis hypogaea*	CRISPR-Cas9 and CRISPR-Cas12a KO or genes' modifications	* Production of reduced levels of major allergenic proteins * Preserved agronomic viability, flavor, and nutritional quality * Cost-effective	Complex, polyploid genome structures with numerous homologous copies make it difficult to remove all allergenic proteins simultaneously.	[9, 35, 93-95]
Gly m 5, 6	Oil	*Glycine max*	CRISPR/Cas9-mediated KOs of the genes	* Successful generation of lines that do not produce IgE and allergens * Upgrading of food safety	* Multiple homologs for allergens, such as *Gly m4* (eight homologs), make complete removal hard.	[96, 97]

				* Edited lines displayed stable, inherited decline of allergenicity in T2/T3 generations.	* Potential OTEs	
Bra j and Sin a	Oilseeds	*Brassica juncea* and *Sinapis alba*	CRISPR/Cas9-based KOs of the genes	Successful production of inheritable insertions/deletions in the gene's alleles	* Some edited lines revealed decreased seed formation and viability * High variability in sgRNA effectiveness necessitates extensive screening	[98]
VPS	Pollens	Primarily *Arabidopsis*	CRISPR/Cas9-mediated KO to cause mutations in multiple genes simultaneously	* Efficient production of defective (sterile) pollen, which inhibits pollen tube germination and prevents the release of cytoplasmic allergens.	* Homozygous KO results in embryonic lethality, making it difficult to maintain the plant line. * Designing sg RNAs that target only the allergenic homologs without affecting necessary vegetative functions is difficult. * OTEs	[99]
*Lyc e1.*and *Lyc e1.02*	Tomato	*Solanum lycopersicum*	CRISPR/Cas9-mediated editing of the allergenic profilin-encoding gene	* Successful editing decreased profilin accumulation in the mutant tomato fruits. * Potential development of hypoallergenic other related crop species.	Nothing reported	[100]

Abbreviations: *Ara h: Arachis hypogaea*; *ATIs:* α-amylase/trypsin inhibitors; *BLG:* β-lactoglobulin; *Bra j: Brassica juncea;* CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; CRISPR/Cas9: CRISPR-associated protein 9; CRISPRi: CRISPR interference; *Gal d*: *Gallus domesticus*. GE: Gene editing; *Gly m*: *Glycine max;* KO: Knockout; *Lyc e*: *Lycopersicon esculentum;* OTEs: Off-target effects; sgRNA: Small-guide RNA; *sin a: Sinapis alba; VPS*: Vacuolar Protein Sorting.

3.2.1 Editing Gal d Genes in Chicken

Total egg white protein accounts for approximately 11% of the egg's weight. The ovomucoid (*Gal d1*) and ovalbumen (*Gal d2*), conalbumin (*Gal d3*), and lysozyme (*Gal d4*) account for most of the allergenic proteins in the egg white [99]. While *Gal d2* is the most abundant allergenic protein, *Gal d1* is the dominant egg white allergen and is responsible for most allergic reactions. Unlike other egg allergens, *Gal d1* retains its allergenicity even after extensive heating. Nevertheless, protein allergenicity varies with factors such as the protein's biochemical properties, the matrix, application conditions, processing techniques, and individual patient sensitivity. It has been found that the IgE/IgG binding ability of *Gal d1* was reduced after the Maillard reaction with maltose (a non-enzymatic browning that occurs when amino acids and reducing sugars in food are heated), typically at 140-165°C [101]. Therefore, it is essential to select appropriate food processing procedures to reduce the allergenicity of *Gal d1*.

In the cultured chicken primordial germ cells, *Gal d1* and *Gal d2* were knocked out using the CRISPR/Cas9 system [86]. The achieved sgRNA editing efficiency exceeded 90%. In this study, primordial germ cells (PGCs) lacking *Gal d1* were transferred into chicken embryos. Homozygous *Gal d1* KOs were observed among the offspring of the second generation. However, the allergenicity of eggs yielded by ovomucoid-deleted chickens was not examined. The results provide proof of principle for applying CRISPR/Cas9 to eliminate the main egg allergen proteins and, eventually, to create hypoallergenic eggs.

In a more recent study [64], the achievements and genomic safety of CRISPR/Cas9 and CRISPRi were systematically evaluated in PGCs from fertilized Jinhua chicken (*Gallus gallus*). While CRISPR/Cas9 accomplished high editing efficacy, it also caused significant DNA damage, apoptosis, and sex-specific cell-cycle arrest, demonstrating the marked genotoxic sensitivity of PGCs. In comparison, CRISPRi was satisfactorily tolerated but failed to execute efficient gene repression in chicken cells. Comparative tests indicated that CRISPRi was efficient in human 293T cells but not in chicken PGCs or somatic DF-1 cells, indicating species-dependent limitations of mammalian-optimized repression systems.

3.2.2 Editing *BLG* Gene in Milk

Milk mainly contains two casein proteins (α-s and κ-casein), and two whey proteins (α-lactalbumin and β-lactoglobulin) [88]. Beta-lactoglobulin (*BLG*), the principal protein in milk whey, is a significant allergen because it is absent from human milk [102]. The traditional milk treatment methods: fermentation, pasteurization, or ultra-high temperature, not only proved costly, but also couldn't decrease or withdraw *BLG* from milk [103]. In addition, these procedures affect nutritional quality and may sustain allergenicity by generating new epitopes [104]. Alternatively, recently developed GE techniques provide a precise and targeted approach to modify gene(s) responsible for milk allergenicity without compromising milk qualities.

Knocking out the *BLG* gene was attained in goat fibroblasts by co-injecting three *BLG*-specific CRISPR sgRNAs with Cas9 mRNA into goat embryos [105]. This protocol not only significantly reduced *BLG* expression in the mammary glands of the engineered goats but also lowered *BLG* levels in their milk, laying the groundwork for improving the composition of goat milk. Among the progeny of *BLG*-KO goats, genome-targeting efficacies ranged from 12.5% to 28.6% after injection of 1 or 2 sgRNAs, respectively.

As for cow milk, the *BLG* gene was KO using CRISPR/Cas9 to produce hypoallergenic cow milk [90]. These hypoallergenic milk studies underscore the importance and therapeutic potential of applying the GE strategy to livestock for human health.

CRISPR/Cas9 technology was used to edit the primary allergen gene, *BLG*, in buffalo milk [88]. These researchers cloned lines of *BLG*-edited fibroblast cells. Three sgRNAs were constructed and evaluated for electroporation-mediated CRISPR editing of the *BLG* gene. Then, the somatic cell nuclear transfer technique was used to generate blastocyst-stage embryos that were developmentally similar to wild-type embryos. They reported an overall success rate of approximately 50% in CRISPR-based editing of the *BLG* gene in buffalo. This is the first report that established the generation of *BLG*-modified embryos in buffalo. Thus, the described CRISPR strategy opens the door to producing *BLG*-free milk in buffaloes.

3.2.3 Editing ATIs Genes in Wheat

Wheat (*Triticum aestivum*) allergies are principally triggered by gluten proteins. These proteins are mainly responsible for the development of gluten sensitivity and celiac disease [106]. The α-gliadin genes harbor several conserved activating peptides, including an immunodominant 33-mer peptide. Because of the large number of gluten genes and the complexity of the wheat genome, traditional breeding alone cannot produce coeliac-safe wheat that retains its baking quality. Researchers [92, 107] successfully targeted and knocked out the α-gliadin-encoding genes using CRISPR/Cas9 with gliadin-specific gRNAs in polyploid wheat cultivars (bread and durum [pasta]). In these studies, the content of gluten proteins in the grains of the edited wheat lines was significantly reduced by up to 85%, thereby lowering the allergenicity of the wheat by reducing the presence of immunogenic gluten epitopes [107]. No OTEs were detected at predicted likely off-target sites. This opens the door to developing wheat varieties that are safer for individuals with celiac disease or wheat allergies.

Another example of successful application of CRISPR in durum wheat cultivar Svevo to simultaneously knock down two of the α-amylase/trypsin inhibitors (ATI) subunits, leading to decreased expression of both subunits [9]. These subunits, particularly the 0.28, CM3, and CM16 subunits, are implicated in Celiac disease [108] and baker's asthma [9] because they elicit a strong IgE response and contribute to the progression of wheat allergies [109]. The sgRNA targets were tailored to the coding region of the CM16 and CM3 genes. These sgRNAs were constructed and cloned into several vectors. Then, the plasmid vectors were co-bombed with the durum wheat cultivar. The regenerating plantlets were transferred to a regeneration medium and allowed to mature. Evaluation by sequencing and biochemical analyses showed that 14 of 97 regenerated plants carried CRISPR edits [108]. No off-targets were identified by in silico analysis, and the ELISA revealed no reactivity to ATI CM3, demonstrating that the mutations caused a gene KO.

Taken together, the outcomes of these studies confirm the importance of high-efficiency CRISPR editing for advancing the development of new hypoallergenic wheat varieties with reduced immunogenicity. Although the polyploid nature of wheat poses an additional challenge to targeting multiple gene copies or alleles simultaneously to achieve a functional KO, the enhanced efficacy and versatility of CRISPR technology will no doubt advance targeted modification of polyploid genomes compared with conventional breeding approaches.

3.2.4 Editing *Ara h* Genes in Peanut

In Peanut (*Arachis hypogea*) seeds, 16 allergenic glycoproteins can induce IgE antibody production in sensitized individuals. Of these allergens, *Ara h1*, *Ara h2*, and *Ara h3* are the best known for triggering allergic reactions in individuals with peanut allergy [110]. Despite the intense hypersensitivity response, there was no specific cure for peanut allergy until CRISPR was developed. This occurred when researchers [111] suggested using CRISPR to deactivate specific genes encoding the major peanut allergens. Alternatively, knocking down gene expression with RNAi to efficiently delete *Ara h2* and other key peanut allergens (*Ara h1*, *Ara h3*, *Ara h6*) using CRISPR. Later, multiplex CRISPR/Cas9 genome modification was applied to KO the *Ara h2* gene in peanut protoplasts [112]. These studies provided evidence that these strategies could cultivate peanut varieties that maintain their nutritional value while reducing or eliminating allergenic risk [34].

On the other hand, the Cytochrome P450 family 11 subfamily A member 1 (*CYP11A1*) gene was directly modified using the CRISPR/Cas9 tool in the human $CD4^+$ T-cell line SUP-T1 [113]. Targeting *CYP11A1* decreased the expression of the *CYP11A1* gene by >50%, and IL-13 generation was considerably reduced. These data indicate that the *CYP11A1*-$CD4^+$ T cell-IL-13 axis might be linked to the development of peanut allergy in children. Consequently, the GE of *CYP11A1* may represent a new therapeutic target in children with peanut allergy.

Using tools such as CRISPR-dCas9 systems, researchers epigenetically modified DNA by altering histone marks at specific loci to regulate allergen expression in peanut [114], thereby reducing peanut allergenicity. Epigenetic modification of the expression or silencing of the *Ara h6* and *Ara h8* genes, without altering the peanut genome, may produce allergen-low peanuts that are safer for patients with specific sensitivities. Although research on the epigenetic control of *Ara h6* and *Ara h8* is in its infancy, initial results are promising. One of the main challenges in generating allergen-free peanuts through epigenetic approaches is achieving stable, consistent modifications [115].

3.2.5 Editing *Gly m* Genes in Soybeans

Soybean (*Glycine max)* has high plant-based nutritional value because it is rich in protein, which is increasingly used in food processing. The glycoproteins: *Gly m* Bd 28 k, *Gly m5*, *Gly m6*, and *Gly m8* are the main allergenic seed storage proteins [116]. Research has focused on targeting the *Gly m5* and *Gly m6* genes, which encode known soy allergens. The genes that encode these were simultaneously knocked out in two soybean varieties using two sgRNAs [116]. The soybean seeds from both the second and third generations showed indels at both target loci, as well as several deletions that induced frame-shift mutations. Subsequently, these changes decreased protein expression and storage in the seeds [117]. The outcomes of this study confirmed that GE using CRISPR/Cas9, coupled with *Agrobacterium tumefaciens*-mediated transformation, provides a direct solution for producing hypoallergenic soybean products. Thus, CRISPR-based down-regulation of such allergen genes may pave the way for hypoallergenic soy varieties [61]. Today, genome editing is not restricted to a few model cultivars. Researchers [118] targeted the two subunit genes of β-conglycinin (*Glyma. 20G146200, Glyma. 20G148200* using the CRISPR/Cas9 technique. The generated soybean lines have stable, inherited seed protein phenotypes characterized by reduced recognition of β-conglycinin-specific IgE. These results underscore the potential of targeted genome editing to enable accurate genome modification in crop development, leading to safer soy-based foods. Another group of scientists [119] targeted *GsDELLA* and successfully generated homozygous

mutants in wild soybean (*Glycine soja*), with reduced plant height and branch number. An adaptable transformation platform may accelerate genetic improvement in both wild and cultivated soybeans.

3.2.6 Editing *Bra j* and *Sin a* Genes in Mustard Seeds

Gene editing of the main brown mustard (*Brassica juncea*) allergen has emerged as a key agricultural technique, with recent developments emphasizing improvements in seed quality (particularly taste) and modifications to pollen development to enable effective hybrid generation. Whole-genome sequencing technology has enabled the identification of crucial structural variants, contributing to allelic diversity in breeding populations. CRISPR's potential to reduce allergenicity in brown mustard involves modifying genes encoding the main allergenic proteins, such as *Bra j1*. Research shows that CRISPR could decrease allergenic potential without affecting agronomic traits [98]. Significant biotechnological advancements, including the application of CRISPR/Cas9, have enabled the production of high-quality mustard hybrids [120-122]. These researchers demonstrated the feasibility and potential of CRISPR/Cas9 for genetic transformation in *Brassica* spp.

The *Bra j* protein belongs to a family of seed storage proteins known as 2S albumins. In addition, *Sin a1* and *Sin a2* (11S globulins) have been found and identified in the seeds of two Canadian mustard varieties (the white and the brown mustard; *Sinapis alba* and *B. juncea*, respectively) [25]. These proteins have been found to bind IgE, initiating the allergic cascade responses in patients' sera [123]. cDNAs encoding *Sin a3* and *Sin a4* have been amplified by polymerase chain reaction, cloned, and sequenced [26]. Knocking out the *Bra j1* gene using CRISPR/Cas9 resulted in hypoallergenic or nonallergenic proteins in mustard-derived products [98]. The generation of allergen-free mustard plants is now possible, potentially advancing safety for people with mustard allergy.

3.2.7 Editing of VPS Genes in Pollens

Description and functional analysis of genes preferentially expressed in late pollen may help clarify the processes vital to pollen germination and tube growth. However, examining the biological functions of prospective candidate genes using mutant studies may be both expensive and time-consuming [60]. Developing a strategy for direct genome editing in pollen would overcome the problem of targeted mutagenesis in plants, which is useful for determining gene functions and rapidly generating new crop varieties.

In plants, the *vacuolar protein sorting* (*VPS*) genes control vacuolar transport and vesicle fusion, which are required for pollen wall development and nutrient transport during seed germination. CRISPR-based GE of pollen is an advanced technology for introducing intended genetic changes, such as modifying traits or preventing the dispersal of transgenic pollen [69, 124]. It enables accurate, transgene-free adjustments in plants by modifying reproductive cells, using haploid cells to modify plant genomes without continually transferring undesired transgenic material. Genome editing has several valuable uses for which the Cas transgene needs to be retained in the plants, including RNA-guided Cas9 as an in vivo desired-target mutator [125] and haploid induction-coupled editing [126] through the paternal haploid, with a null mutant as the female gametophyte. Partial sterility indicates that, although mutant pollen grains are less competitive than wild-type pollen, they preserve their fertilization capacity [60].

In allergy therapy, CRISPR/Cas9-engineered dendritic cells were used to treat allergic rhinitis [127]. Targeted KO of the VPS-associated protein 37A (*VPS37A*) and *VPS37B* genes in human blood dendritic cells lessened Th2 cytokine assembly after being co-cultured with allergic rhinitis patients-derived $CD4^+$ T cells. This innovative modality, which uses genetically engineered dendritic cells, can provide an efficient therapeutic and preventive approach for allergic diseases.

3.2.8 Editing *Lyc e* Genes in Tomato

Despite their widespread consumption, tomatoes (*Lycopersicon esculentum*) are a known source of food allergens, particularly for people sensitized to birch pollen due to cross-reactivity (100]. Therefore, decreasing the allergenic potential of these crops is a primary objective for food safety and customer health. At a minimum, seven allergenic proteins have been identified in tomato, including Bet v1-homologous PR-10 proteins, β-fructofuranosidase, cyclophilin, nonspecific lipid transfer proteins 1 and 2, and profilin [128]. Among these, profilin is a key allergen encoded by two closely related genes, *Lyc e1.01* (*Solyc08g066110.3.1*) and *Lyc e1.02* (*Solyc11g070130.2.1*). Earlier investigations using RNAi to silence *Lyc e1* and *Lyc e3* reported a partial decrease in profilin expression and lessened allergic responses [100], indicating the potential for genetic interference targeting these loci.

Despite the identification of profilin as a major allergen in tomato fruits [100], no efficient methods have been established to abolish its expression through precise genome editing. generated transgenic plants via Agrobacterium-mediated transformation, in which homozygous Cas9-free lines without profilin proteins were generated in post-generations. A framework using the CRISPR/Cas9 system to KO *Lyc e1.01* and *Lyc e1.02*, and eventually to evolve hypoallergenic tomato cultivars, has been successfully applied to Solanaceae crop improvement [100]. More recently, innovative procedures, such as [ultra-efficient prime editing (UtPE)], are being utilized to simultaneously edit multiple targets for better nutrition and allergen reduction [129]. The mutant lines retained their reproductive ability, and although plant height was reduced, fruit growth was unaffected. Future research should focus on comprehensive allergenicity testing and on assessing the field performance and safety of the edited lines.

Previous sections highlighted the value of the CRISPR approach for engineering hypoallergenic food, developing allergen-free plant models, or determining the biological functions of allergen proteins. They also outlined existing clinical applications of CRISPR technology to edit genes associated with food allergies. The next sections will discuss its advantages and limitations.

4. Discussion

The incidence of food allergy varies worldwide, with increases largely driven by genetic susceptibility to environmental factors. A precise diagnosis of food allergy is extremely important. Understanding basic immune mechanisms is important for designing safe and effective allergen-specific treatments in the long term. This review focused on CRISPR-associated allergen research because this system offers several advantages over earlier gene-targeting strategies, such as TALENs and ZFNs, as well as conventional treatment strategies, including immunotherapy. Historically, avoidance and anaphylaxis precautions have been the only operational alternative for food allergies. New treatments have recently been approved, and the therapeutic landscape continues to change [130]. Conventional allergen immunotherapy strategies (OIT and anti-IgE therapies (e.g.,

omalizumab (Xolair)) have shifted the paradigm from strict avoidance toward active disease modification. In the context of food allergies, OIT is considered safer and more suitable than injectable therapies because it can be administered without clinical oversight and may decrease the risk of systemic allergic reactions [131]. Immunotherapeutic approaches have been shown to induce patient desensitization or tolerance to repeated exposure to food allergens [132, 133]. Although the outcomes were successful in numerous desensitization trials, adverse events, including anaphylaxis, frequently occurred during OIT. In addition, some patients do not desensitize after OIT, and the therapeutic response often does not persist [134-136]. Therefore, the requisite duration, safety, and maintenance of sustained immunotherapy remain to be fully demonstrated [61].

The CRISPR/based allergy treatment methodology offers significant advantages that could be transformative:

a. providing the potential, long-lasting resolution to effectively erase the allergen genes at the source, which may substantially benefit allergic people.
b. reducing the incidence of adverse allergic reactions, thus. refining the quality of life of vulnerable individuals.
c. utilizing a reverse genetics method by down-regulating suspected allergen-encoding genes to ascertain allergenicity.
d. decreasing the healthcare costs and making it less time-consuming.

Though the therapeutic potential of CRISPR GE has only recently been discovered, the technology will undoubtedly shape the evolution of food allergy disease management and guide novel approaches to tackling it. However, there are challenges to think of. Ethical concerns also persist regarding the broader use of GE in food production, and public approval remains a hurdle in some countries. Furthermore, regulatory frameworks will have a central role in shaping the future of CRISPR-edited crops [137-139]. The main challenges and limitations of applying the CRISPR/Cas modality in allergy research are briefly addressed in the following sections.

4.1 Technical Limitations

4.1.1 Selection of the Experimental Model

In this review, a few studies involving animal models were included. This may be attributed to differences between small-sized animals (mice and rats), medium-sized animals (rabbits and cats), and large-sized animals (sheep, pigs, cattle, and buffaloes). More specifically, reproduction physiology (number of eggs produced and length of the gestation period) as well as the size and the complexity of the genome. Difficulties in editing the cat genome, such as the limited availability of cat embryos and the invisibility of the pronucleus in embryos, have been reported previously [140]. Microinjection into the pronucleus is preferred for CRISPR/Cas9 GE owing to its direct access to the target DNA, thereby promoting efficacy and reducing OTEs [141]. However, zygote microinjection frequently yields mosaic embryos and small GE rates. This poses difficulties in producing homozygous animals due to the prolonged gestation time of large animals. In the case of the cat embryonic cells, the presence of dark lipids in their cytoplasm makes it extremely hard to microinject the Cas9 and sgRNA construct directly into the pronucleus [141].

With respect to specifically targeting reduced allergenicity in plants, considerable progress has been made using CRISPR for broader crop development goals. However, few studies have been reported in the published literature on plants used to produce hypoallergenic mutants. A review of

the literature on hands-on allergy management notes a significant, persistent problem: the difficulty of eliminating or modifying allergenic proteins without compromising the food's nutritional value or consumer acceptance. Another major research obstacle lies in the complexity and limited genetic variability in plants, such as peanut, soybean, and other legumes, as well as the complex profiles of allergenic proteins [142, 143]. The complex, heterogeneous, and polyploid nature of plants such as wheat poses an additional challenge: the need to simultaneously target multiple alleles or gene copies to achieve a functional KO [144]. The enhanced efficiency and versatility of CRISPR systems will certainly improve targeted genome editing in polyploid genomes compared to traditional breeding approaches [145]. As mentioned before (Page 12), plant PE technology, especially when codon-optimized for plant cells, is being developed as a cutting-edge tool for plant genome engineering, enabling genetic corrections across diverse crop species, broadening its application in plant breeding [83, 146].

4.1.2 Selection of the CRISPR Delivery Method

Delivering CRISPR elements (the Cas9 enzyme and guide RNA) directly to the specific immune cells responsible for IgE generation in humans is complicated. Current research emphasizes the use of nanoparticles (NPs) to deliver gene-editing tools. A significant challenge for CRISPR technology is the effective transfer of CRISPR components into target cells for GE. Currently, strategies that efficiently carry the CRISPR construct to diseased cells in vivo are lacking [147, 148]. However, there are three primary methods to achieve this: viral, non-viral, and physical, each with advantages and disadvantages [149]. Despite the outstanding efficacy and specificity of viral vectors, such as adenovirus-associated viruses (AAVs), the limited cargo capacity of the AAV genome restricts their applications. Nonviral vectors with target-identification roles, such as LNPs, may be a focus of future research. Yet, transferring CRISPR reagents via NPs may be limited by potential toxicity concerns or insufficient targeting specificity [150-152]. In addition, LNPs exhibit limited organ selectivity, frequently accumulating in the liver, thereby limiting their wider clinical translation [153]. Recent advances, particularly those in cationic nanocarriers, are promising means to advance this GE platform. Pathological and physiological alterations following disease onset are anticipated to act as identifying factors for selected delivery or targets for GE [151]. More recently, the use of nanosized, naturally occurring extracellular vesicles for delivering therapeutic molecules to target cells has attracted particular attention owing to their unique characteristics [147]. The combination of CRISPR technology and exosome delivery offers a remarkable prospect to develop a highly effective and individualized therapeutic strategy. Still, key obstacles to translating exosome-mediated CRISPR therapeutic strategies from bench to bedside remain [152].

4.1.3 Minimizing Mosaicism

Mosaicism is a critical technical challenge in GE, in which the two alleles of the edited gene are present in some cells but absent in others. Mosaicism occurs following errors during DNA duplication or repair during GE, specifically when applying techniques such as CRISPR/Cas9. This could result from different sources. First, the GE elements (Cas9 and guide RNA) may not be present or active in all cells; second, GE efficacy may vary among cells; and third, the timing of GE relative to cell division can affect the level of mosaicism [150, 154, 155]. Mosaicism can decrease the efficiency and safety of GE by producing a blend of edited and unedited cells. This can be particularly

problematic for treatments aimed at correcting genetic diseases, where the presence of unedited cells may lead to disease resolution or relapse. Mosaicism can result in OTEs and, consequently, generate Cas-generated mutations and pleiotropic effects [155]. Furthermore, mosaicism may lead to inconsistent expression of edited genes, potentially affecting treatment outcomes and risk assessments.

Uncovering and understanding mosaicism is critical because it is a potential source of OTEs, can affect the outcomes of GE treatments, and can hinder the development of strategies to reduce it. In addition to optimizing the editing protocols, such as selecting ideal cell kinds that are less liable to mosaicism, timing of GE, and delivery system, two strategies can be followed to reduce the occurrence and impact of mosaicism during more precise GE technologies, such as BE and PE (two forms of developed GE technologies) [154]. The BE strategy enables the direct, irreversible replacement of one DNA base with another without requiring DSBs. Prime editing extends CRISPR/Cas9 to enable more accurate editing by combining Cas9's accuracy with the efficacy of reverse transcriptase. Conventional microinjection techniques have struggled with variable delivery efficiency, often leading to mosaicism or incomplete GE, which confounds downstream analyses [155]. By systematically timing injections, this research team achieved significantly improved biallelic editing efficiency with both Cas9 and Cas12a nucleases.

In the future, the overrepresentation of allergy-associated gene mosaicism in late-onset forms should be investigated in patients with adult-onset allergy symptoms.

4.1.4 Minimizing Off-Target Editing

Although CRISPR shows great promise, there are still challenges to consider. A primary concern associated with the application of GE is verifying the desired on-target mutations and avoiding unintended OTEs, including unintended gene editing that introduces random mutations into the genome and creates new risks [88, 156]. The occurrence of OTEs is due to abundant homology between the exact sgRNA sequences and untargeted genomic sequences, leading to cuts that may result in adverse outcomes [157]. Ethical concerns also persist regarding the broader use of gene editing in food production, and public acceptance remains a hurdle in some regions.

This concern has driven ongoing attempts over the years to polish the CRISPR technique, particularly to make genetic modifications reversible. The first message to convey is that advances in CRISPR techniques have significantly reduced off-target editing, making it a safer option than earlier GE methods [158, 159], making it a safer option than earlier gene-editing methods. Furthermore, the wild-type RNA-guided CRISPR/Cas9 nuclease can be repurposed to achieve improved on-target specificity and reduced off-target potential [158, 160]. For example, Cas12 or Cas13 CRISPR systems may be used to generate staggered DNA DSBs or to directly target RNA, respectively [84]. Alternatively, adopting systems (e.g., BEs and PEs) that work independently of Cas9-mediated DSBs, which are a significant source of OTEs in CRISPR/Cas9 genome editing [83, 84, 158].

Detecting OTEs is difficult because their positions and numbers are unknown [161]. The potential off-target mutation rate must be assessed using Sanger sequencing during the preclinical development of CRISPR-based therapies [158]. Genome-wide analysis can also detect off-target mutations [155, 162], underscoring the importance of a comprehensive sequencing-based approach to off-target assessment. The planned design bioinformatics programs predict off-targets by

comparing CRISPR sgRNA sequences against the entire genome of interest. When substantial OTEs are detected, the specificity of GE can be improved by using accurate delivery of more precise sgRNAs and the Cas involved [163]. The delivery vector of Cas9, whether DNA, mRNA, or ribonucleoprotein, plays a key role in determining its exposure duration and expression, thereby impacting both editing efficacy and off-target activity [148]. It should be recalled that detecting OTEs is important for identifying their safety risks. A recent study [164] demonstrated that hybrid gRNAs can substantially reduce OTEs, including bystander edits, while preserving high on-target editing efficiency. A CHANGE-seq-BE-specific method for identifying off-target activity is being developed [165].

4.1.5 Avoiding Immunogenicity

The recognition of specific CRISPR/Cas9 components, such as Cas9, sgRNA, and viral or non-viral delivery vectors, may trigger both innate and adaptive responses, limiting its efficacy [166, 167]. The resulting complex interactions between these components and host immune reactivity play an important role in determining the safety and efficacy of CRISPR-based therapies [167]. Evidence from preclinical and clinical in vivo CRISPR trials indicates that specific immunity can fail the desired GE therapy. The challenge of immunogenicity remains a significant roadblock to the clinical utility and applications of CRISPR therapeutics [166]. Therefore, there is a need for advanced immunogenicity prediction algorithms that address current limitations to advance clinically translatable CRISPR-based therapies for universal use. Machine learning-based prediction implementations are expected to advance the development of less immunogenic CRISPR therapeutics [168].

To mitigate severe Cas9-immunogenicity-related immune reactions, researchers are developing strategies, including immunosuppressive therapies. (a) *ex vivo* GE strategies, where the immunotoxicity of CRISPR therapeutics might not be a significant concern [169]; (b) selecting a target tissue that has limited innate immune response [170]; (c) Optimizing CRISPR delivery systems [166, 167]; (d) engineering innovative CRISPR nucleases that can minimize the OTEs and escape the immune system [167]; (e) controlling the CRISPR time of activity [170]; (f) epitope engineering [167]; and (g) monitoring the immune reaction to CRISPR therapeutics [166]. Addressing these immunological obstacles requires an integrated method that combines visions from immunology with pioneering engineering solutions. As the field advances, overcoming Cas9 immunogenicity will be a major hurdle to realizing the full therapeutic potential of the CRISPR/Cas9 system across diverse clinical applications.

4.2 Safety and Ethical Concerns

In late 2023, CRISPR-mediated therapy got its first regulatory approval. Since then, the scientific community has shifted its focus from potential applications in personalized therapies to improving the safety and efficacy profiles for clinical applications. Much public concern centers on whether modifying genetic material without a comprehensive understanding could lead to unpredictable outcomes for patients and their descendants [84]. Fears of producing genetically modified (GM) crops persist even as new allergen-free plants are developed, despite the unprecedented accuracy of CRISPR and the extensive testing before release.

The opponents argue that GM biotech crops are “unnatural” and can, in the long term, lead to significant harm to humans. They warn that such modifications could cause the emergence of numerous new allergic responses [171]. The critics’ worries might stem from a reported case involving a methionine-enriched GM soybean, in which an edited soybean accidentally became more allergenic after the insertion of a Brazil nut methionine gene. This example, while ultimately prevented from marketing, underscored the necessity for precise allergenicity testing in GM crops. Clearing the way for GE techniques like CRISPR used to target specific allergens requires communicating, educating, and convincing people.

Despite the growing body of literature on GM technologies and the CRISPR/Cas system, comprehensive, objective comparisons between them remain scarce. Concerns exist, and debates extend to ethical, societal, and economic matters, stressing the importance of equitable access and public engagement. Both GM-based and CRISPR-based GE have been subjects of ongoing debate [172]. These questions make it even more vital to identify the conditions for sustainable applications. Common ground must be reached between the arguments of the disputing sides.

The key difference between GM and CRISPR technology is that the former involves the introduction of foreign DNA (Transgenesis). In contrast, the latter involves precise, targeted single-nucleotide substitutions (SNPs), insertions/deletions (indels), or gene replacements within a plant’s genome without introducing foreign genes or requiring integration [84, 172-174]. In this context, if a gene-edited plant is markedly equivalent to a conventionally bred plant. Some of the CRISPR-edited crops, such as GABA tomatoes, mustard greens, soybeans, and white button mushrooms, have already been marketed [173]. Other CRISPR-edited crops are undergoing field trials. Thus, crops engineered with CRISPR are considered safer to use than conventionally GM crops [9, 68-70]. Additionally, crops developed with CRISPR may encounter fewer regulatory and commercialization obstacles than traditionally GM crops [9, 52-54].

Furthermore, scientific reports describe the development of a programmed pollen self-elimination CRISPR-Cas (PSEC) system in which pollen is infertile when PSEC is present in haploid pollen [175]. The PSEC can be inherited through the female gametophyte, thereby preserving genome-editing activity in vivo across generations. This system can significantly reduce legislative concerns about the widespread diffusion of genetically modified elements into natural and agricultural environments through outcrossing.

In contrast to GM crops like Bt cotton and maize, which are constructed by transferring a gene from the bacterium *Bacillus thuringiensis* to confer novel resistance to certain pests [176]. In contrast, the new genomic techniques allow DNA to be altered without introducing external genetic material into the plant.

For safety assessment, the FDA guidelines require companies to label their GM foods only if the food has a nutritional or food-safety property that is markedly different from what consumers would expect of that food [43, 177-179]. For instance, if a modern GM food contains a protein that may be an allergen not likely to be present (e.g., a peanut protein expressed in a soybean). Therefore, foodstuffs modified using the CRISPR method are not considered material information that must be disclosed. Regulatory policies on packaging, GM food, and clear labeling differ across countries and regions and can be broadly grouped into mandatory or voluntary.

Because of the above clarifications, CRISPR/Cas in crop plants has gained greater public acceptance and recognition. Perhaps the next decades may witness an increasing prevalence of CRISPR-edited crops as a translation of what is so-called “the next big thing in agriculture.” Still,

some key questions regarding CRISPR-edited crops need to be settled to achieve more public and market acceptance, as the world remains divided over how to resolve unresolved issues. The integration of CRISPR with AI technologies may be of interest.

5. Conclusions and Recommendations

In this review, we first summarize the molecular architecture and mechanistic basis of CRISPR/Cas9 and then consider its latest applications in food allergy therapy. Then, we discuss current challenges facing these applications. While concerns about the potential for GM foods to create new allergens do exist despite intensive testing before release, there appears to be less public and regulatory concern about CRISPR technology. This could clear the way for its use to precisely eliminate or reduce allergens in food crops. The application of CRISPR DNA-targeting technologies to understand the full spectrum of allergic diseases is just beginning, and most allergy research has focused on identifying the function of specific genes. CRISPR has reshaped allergy treatment, transforming from management plans to potential cures. This shift might lead to integrated strategies combining GE with other therapies, thereby improving overall effectiveness. The studies reported herein provide a basis for eliminating allergenicity and for generating hypoallergenic or nonallergenic products to assist allergic individuals in the future. For example, identifying the appropriate therapy for each patient and implementing personalized treatment strategies.

Progress in preclinical and clinical models and clinical trials to treat allergic diseases may encounter certain technical hurdles; for instance, some allergens play crucial physiological roles in mitigating biotic and abiotic stress. In some cases, targeting their genes with CRISPR to silence expression is not always practical. Research is a crucial step in the correct direction and will offer society an allergy-free future! One scenario is the integration of CRISPR with immunotherapy or other treatments, as well as the creation of mathematical models that simulate the dynamics of GE in allergy treatment.

Taken together, the outcomes of the present review reveal a vital trade-off: “efficient but toxic” versus “safe but ineffective”—when applying CRISPR technology in food allergy therapy. The findings highlight the need for species-adapted, low-toxicity genome-editing platforms. Thus, while seeking to lower or eliminate allergenicity, one must also consider nutritional value. The goal is that any future progress in creating new genotypes must maintain a reasonable balance between novelty and the protection of the ecological environment and human safety, ensuring that gene-edited foods do not unintentionally pose new dangers to consumers.

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