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NOTE

Model development for prediction of the allergic response to the wheat proteins ω-5 gliadin and HMW-glutenin

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Abstract As one of the eight foods that account for 90 % of food allergies, wheat must be excluded from the diet in patients suffering from wheat allergies. From studies of wheat-dependent exercise-induced anaphylaxis (WDEIA), which has been used as a model to develop hypoallergenic wheat, we now know that the gluten fraction of wheat protein, particularly ω-5 gliadin and high-molecular-weight (HMW)-glutenin, is responsible for the allergic response. However, studies of allergic responses with WDEIA have been performed with a single wheat cultivar. Thus, in an effort to provide more information for the development of hypoallergenic wheat, we compared various cultivars with different countries of origin and characteristics. For the first step, we compared the allergen contents (ω-5 gliadin and HMW-glutenin) in each cultivar and the allergic response caused by each cultivar. Domestic wheat cultivars had lower contents of ω-5 gliadin and HMW-glutenin than those of imported wheat cultivars. Additionally, some cultivars caused varying allergic responses due to their allergen components. From regression analysis of allergen contents and allergic responses in vivo, we suggest a prediction model to estimate the extent of allergic response based on the ω-5 gliadin and HMW-glutenin contents. Further studies are needed to analyze the biological interactions between allergens from various cultivars and allergic response factors.

Keywords High-molecular-weight glutenin · ω-5 Gliadin · Prediction model · Wheat allergy · Wheat-dependent exercise-induced anaphylaxis

Introduction

As a major crop worldwide, wheat has been consumed by the global population for centuries. Wheat is also used as a raw food material and is the cause of allergic responses. It is estimated that about 15 million people in the United States of America have food allergies, and wheat is one of the eight foods that account for 90 % of food allergies (Branum and Lukacs 2008). Allergy-causing wheat proteins are composed of four classes depending on their solubility: water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadin, and dilute alkaline- or acid-soluble glutenin (Nicolas et al. 1998; Bodinier et al. 2009).

Different types of wheat proteins are involved in various allergic reactions, such as baker's asthma, celiac disease, atopic eczema/dermatitis syndrome (AEDS), and wheat-dependent exercise-induced anaphylaxis (WDEIA). These types of food allergies are observed in both children and adults (Bodinier et al. 2009; Kasarda 2013; Volta et al. 2013). AEDS is primarily observed in children, whereas WDEIA occurs mainly in adults (Hischenhuber et al. 2006). Due to extensive research efforts, ω-5 gliadin and high-molecular-weight (HMW)-glutenin have been identified as major components inducing WDEIA. Patients with

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WDEIA have reactions mostly to ω-5 gliadin and HMW-glutenin (Matsuo et al. 2004). Unfortunately, the only reliable therapy is strict elimination of wheat products from dietary foods. Due to the serious problems associated with WDEIA, many studies have aimed to develop forms of hypoallergenic wheat.

As wheat consumption in Korea has increased in recent years, more patients have been diagnosed with wheat allergies (Jang et al. 2016). The genetic locus of ω-5 gliadin (Gli-B1) has been reported in 32 Korean wheat cultivars in an effort to identify hypoallergenic wheat. In a previous study, we compared allergy-inducible wheat protein contents among imported and domestic wheat flours in Korea (Kim et al. 2016). However, no reports have provided comparisons of allergy-inducing capacity among wheat proteins from imported and domestic wheat. In the present study, we aimed to correlate the content of allergy-inducing wheat protein components with the allergy-inducing capacity of imported and domestic wheat cultivars.

Materials and methods

Various cultivars of wheat

Various wheat cultivars were harvested in 2015 and provided by the National Institute of Crop Science, Rural Development Administration of Korea. Information for each cultivar is described in Table 1.

Wheat protein extraction

Wheat proteins were extracted from 100 mg flour according to the method of Wallace et al. (1990). After adding 1 mL borate extraction buffer [12.5 mM sodium borate, 1 % SDS, 2 % β-mercaptoethanol (added just before use)] to 100 mg of each type of flour, samples were vortexed and incubated with shaking at 37 °C for 2 h. After incubation, samples were centrifuged at 17,000 × *g* at room temperature for collection of supernatants, and supernatants were then used as wheat protein samples in this study.

Quantification of specific protein content

To quantify the specific protein contents of various wheat cultivars, densitometric analysis was performed. After quantifying wheat protein samples by the Bradford method, wheat proteins were mixed with sample buffer and heated for 10 min at 99 °C (Cho et al. 2015). Equal amounts of wheat proteins were loaded for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 12 and 10 % polyacrylamide gels to analyze the ω-5 gliadin and HMW-glutenin contents, respectively. The gels were then analyzed using ImageJ and Photoshop software programs after Coomassie blue staining.

In vivo allergic response tests

For in vivo allergic response tests, C3H/HeJ mice were used based on a study by Matsuo et al. (2005). C3H/HeJ mice were purchased from Nara Biotech (Seoul, Korea) and divided into nine groups ($n = 4$) consisting of a negative control group and eight groups treated with different cultivars. Incomplete Freund's adjuvant was used as this adjuvant caused less pain to the animals. For injection, 100 μg wheat protein extracted from different cultivars was injected intraperitoneally with incomplete Freund's adjuvant at a 1:1 ratio (v/v). Injections were performed three times at 2-week intervals. At 24 h after the final injection, C3H/HeJ mice were sacrificed, and serum was collected. The histamine concentrations in the serum were analyzed to determine allergic responses (Enzo Life Science, New York, NY, USA). This experiment was approved by the Institutional Review Board of the College of Medicine of Korea University (IACUC#: KUIACUC-20151013-2).

Statistical analysis

Differences in the contents of ω-5 gliadin and HMW-glutenin between cultivars were analyzed by one-way analysis of variance using SAS 9.4 (SAS Institute, Cary, NC, USA). When ANOVA indicated significance, means were

Table 1 Various cultivars of wheat

No.	Abbreviation	Cultivar	Country	Characteristics
1	Jokyung	Jokyung (Gyeongsangnam-do/Hapcheon)	Korea	Hard wheat
2	NS	Northern spring wheat	USA	
3	HRW	Hard red winter wheat	USA	
4	AH	Australian hard wheat	Australia	
5	Goso	Goso (Jeollabuk-do/Jeonju)	Korea	Soft wheat
6	SW	Soft wheat	USA	
7	Keumkang	Keumkang (Jeollanam-do/Gwangju)	Korea	Medium wheat
8	ASW	Australian standard white wheat	Australia	

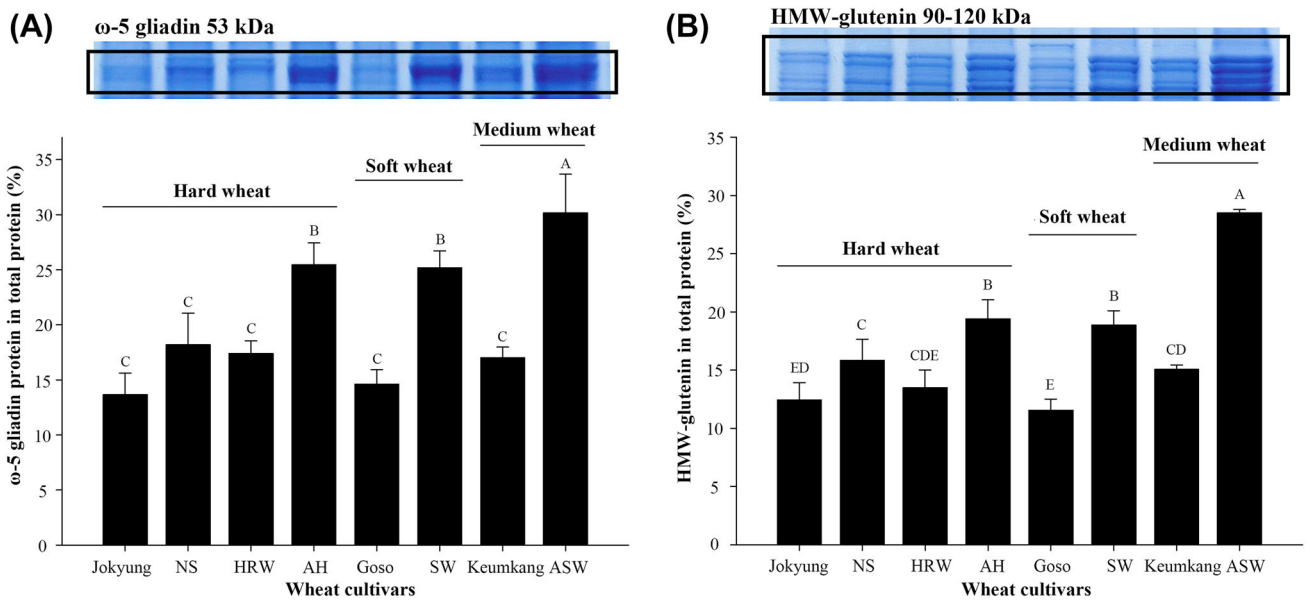

Fig. 1 ω-5 gliadin and HMW-glutenin contents of various wheat cultivars. Both the contents of ω-5 gliadin (53 kDa) (**A**) and HMW-glutenin (90–120 kDa) (**B**) were measured with domestic and imported wheat cultivars

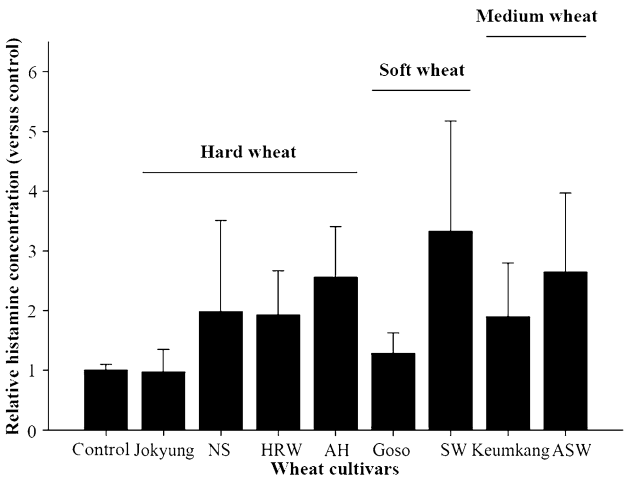

Fig. 2 Allergic response, as determined by measuring histamine concentrations in mouse serum. Mice were injected with wheat proteins from various wheat cultivars

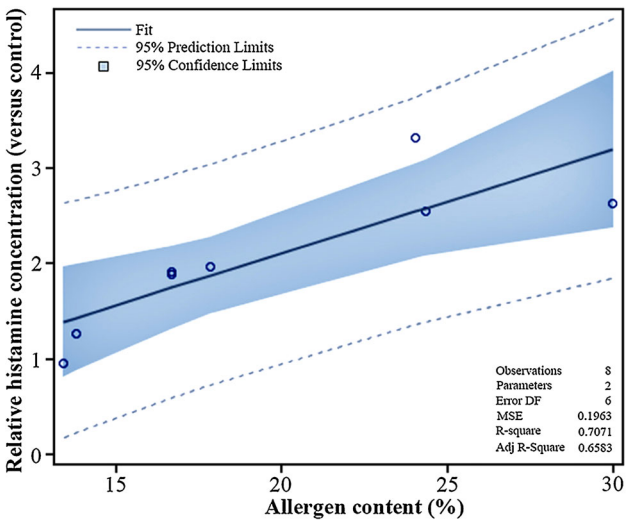

Fig. 3 Regression analysis between wheat allergen content and histamine concentration as an allergic response. The allergen content was calculated using the equation: $0.8 \times$ (ω-5 gliadin content) $+ 0.2 \times$ (HMW-glutenin content). The R^2 value was 0.7071

separated using Duncan's multiple range test at $\alpha = 0.05$. Regression analysis was also performed using SAS 9.4.

Results and discussion

Wheat is the major component of the human diet in western countries and is consumed in various foods for centuries. Consumption of wheat in Korea has increased steadily in recent years, and the number of patients with wheat allergies, such as WDEIA, celiac disease, and baker's asthma, have also increased accordingly (Jang et al. 2016). The gluten protein contents in wheat may play a key role in the determination of dough formation properties. Gluten provides strong viscoelastic and cohesive properties (Gourbeyre et al. 2012). The rheological properties of dough correlate with the size of glutenin polymers and ratio of gliandin and glutenin (Payne et al. 1987; Cornec et al. 1994). Wheat proteins can be categorized into four classes depending on their solubilities, and dramatically imbalanced amino acid compositions have been identified, including gliadins composed of high proportions of glutamine and proline (32–53 and 11–29 mol%, respectively)

and glutenins composed of low contents (<2 mol%) of lysine, methionine, and tryptophan (Scherf et al. 2016). Gliadins, which are soluble in alcohol, are monomeric proteins classified as α-, β-, γ-, and ω-gliadins; these proteins make up 44–60, 30–45, and 6–20 % of total gliadins, respectively (molecular weight: 30–50 kDa) (Matsuo et al. 2004). ω-gliadins are composed of ω-1, -2, and -5 gliadins (Bodinier et al. 2009). Glutenins, which are soluble in dilute alkaline or acid, are polymeric proteins linked by interchain disulfide bonds and composed of subunits of low-molecular-weight (LMW)-glutenin (molecular weight: 30–45 kDa) and HMW-glutenin (molecular weight: 900–120 kDa) (Nicolas et al. 1998).

WDEIA is an IgE-mediated allergic response affected by at least two factors, including physical activity and wheat ingestion. Because of extensive studies on WDEIA, we now understand that the gluten fraction of wheat protein, particularly ω-5 gliadin and HMW-glutenin, is responsible for WDEIA (Matsuo et al. 2005). This present experiment was designed to compare the allergic activities of the gluten fraction among different wheat cultivars with the aim of developing convenient methods for evaluating the extent of allergic activity of various wheat cultivars. As a first step, we analyzed the contents of allergens of various wheat cultivars. Wheat proteins extracted from each wheat cultivars were separated by SDS-PAGE. Densitometric analysis was performed with 12 and 10 % acrylamide gels to measure the contents of ω-5 gliadin (Fig. 1A) and HMW-glutenin (Fig. 1B), respectively. Both the contents of ω-5 gliadin and HMW-glutenin were the highest in cultivar ASW ($p < 0.0001$) among all cultivars. The lowest ω-5 gliadin content was recorded with cultivar Jokyung, the value of which was less than half the value of ASW. Cultivar Goso had significantly lower HMW-glutenin content ($p < 0.0001$) among the other cultivars, measured at only about two-fifths the value of ASW (Fig. 1). Thus, the results suggested that cultivar ASW had the highest contents of ω-5 gliadin and HMW-glutenin. However, when the in vivo allergic response was measured with the serum histamine concentration, the extent of the in vivo allergic response was not well matched with the contents of ω-5 gliadin and HMW-glutenin. For example, even though the contents of ω-5 gliadin and HMW-glutenin were the highest in cultivar ASW, the extent of the in vivo allergic response was the highest with cultivar SW (Fig. 2). In contrast, cultivars Jokyung and Goso had lower allergic responses and ω-5 gliadin and HMW-glutenin contents than the other tested wheat cultivars.

Matsuo et al. (2005) reported that about 80 % of patients with WDEIA react to ω-5 gliadin, whereas the other 20 % react to HMW-glutenin. Accordingly, regression analysis was performed between allergen content and mouse serum histamine concentrations as a readout of the allergic response (Fig. 3). The allergen content was calculated using the following equation: 0.8 × (ω-5 gliadin content) + 0.2 × (HMW-glutenin content). The R^2 value was 0.7071, indicating that the suggested model explained 71 % of the variability of the obtained data around the mean value. With further development, the suggested model could explain more variability between wheat allergen content and allergic responses using serum histamine concentration. The results of Fig. 2 also suggested that the ω-5 gliadin and HMW-glutenin contents of imported wheat cultivars were generally higher than those of domestic wheat cultivars. This finding indicated that domestic wheat cultivars might be associated with a lower incidence of wheat allergies than imported wheat cultivars, as supported by the results presented in Fig. 3.

Overall, we concluded that the extent of the allergenic response could be predicted partially by measuring the content of wheat allergenic proteins using the model suggested above. We suspect that some parts of the allergic response may have originated from nonprotein allergenic factors and/or variable amino acid compositions among wheat allergenic proteins. Future studies are needed to perform sequential analyses of more cultivars and allergic responses and to analyze the biological interactions between allergens of various cultivars and allergic response factors.

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