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# Possibilities of Interlinking the Genomic Data and Allergenic Potential of Apples

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### Abstract

Apple allergy belongs to the most prevalent fruit allergies which in North and Central Europe is mainly attributed to cross-reaction between Bet v 1 allergen from birch pollen and Mal d 1 major apple allergen. For a long time, patients observed symptoms of unequal severity after consumption of different apple cultivars. This led scientific community to search for the basis of the cultivar-specific allergenicity. According to several studies, the amount of Mal d 1 allergen plays an important role. Currently, notable attention is mainly concentrated on genetic variability as the primary source of different allergenic potential. Mal d 1 gene family is a large family of gene isoforms and their variants differing in the primary sequence. These sequence alternations may cause changes in protein structure and potentially affect the binding capacity to IgE and thus the allergenic potential. Among many methods available to analyze genetic variability, restriction fragment length polymorphism is simple technique suitable to analyze variability of Mal d 1 allergen. This paper aims to provide a brief overview of a possible approach of interlinking genomic data (e.g. as by RFLP profiles) and clinically proven apple allergenicity.

## 1. Food allergy with focus on *Rosaceae* family

The tendency of rising relevance of food allergies is becoming more and more pronounced. The highest prevalence of food allergy (FA) has Australia (10% of infants and 6-10 % for all citizens), followed by Europe (from 3% to more than 10%) and the United States (from 6% to more than 10%) in past 10 years and a lot of data for many countries are unknown (Renz *et al.*, 2018). The prevalence of food allergy has a rapidly increasing tendency, especially for infants and children, mainly in regions with high industrial activity (Cochrane *et al.*, 2009).

Syndrom of oral allergy is invoked by various plant proteins, especially PR-protein superfamily cross-reacting with aero-allergens. Cross-reaction is caused by structural similarities in epitopes of aero-allergen and its homologue in food. Although FA reaction is initiated by the immune system, it is rarely life-threatening. It is estimated that the production food-specific IgE affects 50-70% of adults suffering from pollinosis (mainly birch, ragweed and mugwort) (Nowak-Węgrzyn *et al.*, 2017).

*Rosaceae* is a botanical family involving many kinds of fruit common for Central and Northern Europeans or Americans. Some of them (as peach, apple, cherry etc.) are known to be allergenic. Worldwide data showed that 2.2%–11.5% of children (0–6) and 0.4%–6.6% of adults perceived a fruit allergy (Zuidmeer *et al.*, 2008). The most remarkable prevalence among *Rosaceae* fruit is represented by the allergy to apples or peaches and therefore research is widely focused on the species. Apple allergy can be

associated with grass pollinosis (i.e. central Spain) or it can be provoked directly (Fernandez-Rivas *et al.*, 1997), but the later type of allergy is less frequent. Mostly, apple allergy is cross-reacting with birch-pollen pollinosis (Eriksson, 1978). Symptoms of the type of apple allergy are strongly subjective in both kind and intensity. Moreover, immune system may react with various symptoms or can "only" produce specific IgE without symptoms recognizable to a patient. On the other hand, there are different isoforms of gene's product exposed to specific IgE. Unanswered questions create a wide range of issues, but a variety of fruit is considered as one of the possible answers.

## 2. Cultivar-specific allergenicity

For a long time patients' experiences evidence that the severity of allergic reaction to apple consumption is not only dependent on individual sensitivity of a patient but is also related to apple cultivar. The proposed cultivar dependent allergenicity is highly supported by scientific literature (Bolhaar *et al.*, 2007; Kootstra *et al.*, 2007; Gao *et al.*, 2008; Ricci *et al.*, 2008; Vlieg-Boerstra *et al.*, 2011; Vergo *et al.*, 2016). Bolhaar *et al.* (2007) divided apple cultivars into groups with low, moderate and high allergenicity. For example, Santana, Topaz, Braeburn and Elise were identified as low allergenic cultivars by the authors (Bolhaar *et al.*, 2007). The new cultivar Santana is generally regarded as a cultivar with reduced allergenic properties, supported by clinical trials from numerous research

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teams (Bolhaar *et al.*, 2005; Kootstra *et al.*, 2007; van der Maas *et al.*, 2009). In Netherlands the cultivar has even been recently marketed as a cultivar suitable for mild apple allergy patients. On the contrary, results of prick-to-prick method determined Belinda, Jonagold, Golden Delicious, Gala and Pinova as high-allergenic cultivars (Bolhaar *et al.*, 2007).

These differences between cultivars drew the question to the basis of cultivar-specific degree of allergenic potential. Allergenicity may be according to some researchers attributed to the amount of Mal d 1 proteins (Son *et al.*, 1999). Considerable variations among apple cultivars have been indicated by determination of Mal d 1 content using ELISA-tests (Vieths *et al.*, 1994; Matthes and Schmitz-Eiberger, 2009), one-dimensional electrophoresis and immunoblotting (Son *et al.*, 1999). Son *et al.* (1999) observed ten-fold difference in Mal d 1 content between high-allergenic Golden Delicious and low-allergenic Gloster. This hypothesis is, however, poorly supported due to the few cultivars studied.

On the contrary, qualitative characteristics are of raising interest and they represent different insight into cultivar specific allergenicity. Mal d 1 is encoded by a big gene family containing 18 members described so far. Mal d 1 genes are mapped on three linkage groups of the apple genome (Atkinson *et al.*, 1996; Gao *et al.*, 2005). Since some of the Mal d 1 genes have alleles coding for different isoforms, apple cultivars can besides quantity differ also in the composition of Mal d 1 protein (Son *et al.*, 1999; Gao *et al.*, 2008). Unsurprisingly, Mal d 1 proteins can therefore possess different binding ability to birch pollen-specific IgE (Son *et al.*, 1999; Ferreira *et al.*, 1996; Ma *et al.*, 2006). The study from Gao *et al.* (2008) precisely deals with the association between apple allergenicity and genetic diversity of Mal d 1 genes and suggests that quantitative factors may as well contribute to apple allergenic potential.

### 3. Genetics and genomics data in allergen screening

Understanding the genetic variability is important for breeding programs but can be also useful to study the genetic basis of plant food allergenicity. Currently, there are numerous techniques suitable for analysis of the genetic variability among varieties for diverse plant species. One of the widely used and probably the most simple methods is restriction fragment length polymorphism (RFLP). Restriction fragment length polymorphism is a molecular biology method for differentiation of genotypes and observing changes in sequences at genetic level without the use of more expensive sequencing. Firstly, sequence of interest is amplified by PCR and verified by electrophoresis. Secondary, restriction enzymes are selected either by *in silico* cleavage, or randomly and used to cut amplified sequences in specific cleavage site/s. Splitting of sequences into two or more fragments demonstrates the presence of specific cleavage site/s and different numbers of fragments suggest changes in the sites of sequences. By electrophoretic separation, restriction profiles are acquired and further are translated into 0-1 matrices which are used to generate dendrograms. This technique can be used as a suitable tool to analyze genetic variability of apple allergens. A recent study evidenced the existence of the variability in the restriction profile of Mal d 1 promotor among apple varieties (Žiarovská *et al.*, 2019). Based on the existing knowledge, this study aims to provide a brief overview on different allergenic potential between apple cultivars and to associate our genomic data (as RFLP profiles) of Mal d 1 gene and gene product allergenicity from different varieties of apples. The RFLP analysis was performed on two amplicons of Mal d 1 gene in 6 apple cultivars (Gala, Fiesta, Florina, Jonagold, Raika and Topaz) with restriction enzymes AseI, NlaIII and NcoI. The cleavage generated 28 fragments which created different profiles and showed considerable range of polymorphism among studied

varieties. Only for the AseI cleavage of amplicon 2, one pattern was produced in all studied varieties. Varieties Florina and Raika shared the same profile for cleavage with all enzymes used in both amplicons. Topaz and Jonagold shared the same cleavage profile for AseI (both amplicons) cleavage, NcoI cleavage and differed in the presence/absence of only 1 fragment in NlaIII cleavage. Cultivar Gala had a unique pattern for all restriction cleavages except for AseI (amplicon 2, which was identical for all cultivars). Raika and Florina shared the identical profile and are therefore clustered together. Based on RFLP analysis, Jonagold and Topaz share 96% similarity of RFLP 0-1 matrices. Gala revealed a unique profile for all restriction cleavages except for the cleavage of amplicon 2 with AseI (which was monomorphic for all apple cultivars).

### 4. Actual interlinking of genomic and proteomic data

Current state of knowledge is insufficient to provide explicit elucidation of the basis underlying the unequal allergenic strength of apple cultivars. It was hypothesized that the amount of allergenic protein might be the cause (Son *et al.*, 1999), however, lately the existence of allergenicity-associated protein variants has been revealed (Gao *et al.*, 2008). One of the basic genomic strategies is to analyze the genetic diversity of apple cultivars by RFLP and to associated it with allergenicity data from clinical trials.

The amount of existing apple varieties reaches 7 000 and majority of research focus is aimed at few of the most commercially important apple cultivars. For that reason data on majority of the cultivars are unknown and for the set of cultivars used in our study, there is only a limited portion of publications analyzing their allergenicity (Ricci *et al.*, 2010, Bolhaar *et al.*, 2005, Vlieg-Boerstra *et al.*, 2011). Based on prick-to-prick method, apple varieties were previously divided into low-allergenic, moderate-allergenic and high-allergenic (Bolhaar *et al.*, 2005). Among the cultivars tested in the study, Topaz was identified as a low-allergenic, Fiesta and Raika as moderate-allergenic and Golden Delicious as a high-allergenic cultivar. In a SPT testing in 33 Dutch adults with OAS before and during the birch pollen season in fall and spring, the percentage of negative SPT responses was determined as follows: Gala (19,4 / 3,3 for fall and spring, respectively), Raika (13,3 / 3,3), Fiesta (12,9 / 6,7), Topaz (n.a. / 3,3), and Jonagold (3,0 / 10,0) (Vlieg-Boerstra *et al.*, 2011). On the other hand, Topaz seemed to have high allergenic potential similarly to Golden Delicious as seen in another study by Kootstra *et al.* (2007). The general view is that over the *Rosacea* family, the peel of fruit is more allergenic than the pulp (Fernández-Rivas *et al.*, 1999). However, based on PTP-SPT results, Ricci *et al.* (2010) suggested than for Golden Delicious and Jonagold, the pulp was more allergenic than the peel. Contrastly, the peel of Florina, Fiesta and Gala was more allergenic than the pulp (Ricci *et al.*, 2010). The authors ranked the cultivars according to their pulp and peel allergenicity as follows. The allergenicity of the pulp increased from Gala to Florina, Topaz, Fiesta, Golden Delicious and Jonagold whereas in the case on peel the allergenicity rised from Topaz to Jonagold, Florina, Golden Delicious, Fiesta and Gala. As for the pulp, the cultivars Jonagold, and Golden Delicious appeared to be the most allergenic cultivars (Ricci *et al.*, 2010). In the case of the peel, higher allergenicity was shown for Gala, Fiesta and Golden Delicious.

In our study, Raika and Florina shared the same cleavage pattern for the used restriction enzymes and could be hypothesized to have similar allergenic potential. As mentioned above, Raika was identified as a moderately allergenic cultivar (Bolhaar *et al.*, 2005). For both the pulp and the peel, also Florina belonged to the less allergenic among tested cultivars (Ricci *et al.*, 2010).

According to the results of our study, cleavage patterns of Jonagold and Topaz are similar and the cultivars create another cluster in the dendrogram. Therefore, we anticipated a comparable degree of allergenicity for the two varieties. **Kootstra et al. (2007)** showed that Topaz had similar visual analogue scale scores (VAS) as Golden Delicious (GD) and similarly to GD caused significantly more allergic symptoms than Santana. These results suggest that Topaz has a comparable allergenicity as a high-allergenic GD. In another study, Jonagold appeared to be one of the most allergenic cultivars (when considering the pulp) but this was not the case of Topaz (**Ricci et al., 2010**). On the contrary, **Bolhaar et al. (2005)** assigned Topaz as a low-allergenic cultivar.

Cleavage of Mal d 1 from Gala and Fiesta showed unique profiles with a relatively low degree of similarity with other cultivars analyzed. Gala demonstrated the highest percentage of negative SPT responses among the six cultivars analyzed in our study (**Vlieg-Boerstra et al., 2011**) and in the ranking of the pulp allergenicity Gala belonged to the less allergenic among tested apple cultivars (**Ricci et al., 2010**). Fiesta was also classified as a moderately-allergenic cultivar (**Bolhaar et al., 2005**). On the contrary, the peel of Gala fruit and also of Fiesta apple appeared to be among the most allergenic (**Ricci et al., 2010**). Moreover, the pulp of Fiesta fruit also caused larger wheals than Topaz, Florina or Gala.

In many cases different research approaches gave inconsistent results in the assessment of the cultivar-specific allergenicity. These discrepancies can be attributed to several factors. **Asero et al. (2006)** found a large inter-patient, inter-apple and intra-apple variability. The variability can considerably distort results and might be caused by large variations in Mal d 1 content of the cultivars (**Marzban et al., 2005; Rur, 2007**), the small study population but also different storage conditions and seasonal influences (**Sancho et al., 2006; Matthes and Schmitz-Eiberger, 2009**). Another important aspect is the selection of a suitable method for apple allergenicity assessment. **Vlieg-Boerstra et al. (2011)** found no correlation between VAS scores and SPTs. This phenomenon is indeed largely supported by some studies evidencing poor correlation between the size of the skin test and the intensity of symptoms induced by oral challenge (**Vieths et al., 1994; Asero et al., 2006; Kootstra et al., 2007**). Moreover, a nonstandardized way of pricking location of the apple can considerably affect outcomes of the study as seen in a recent work from **Vlieg-Boerstra et al. (2013)**. The team examined whether and how the location of pricking in the apple influences results of the PTP test. They found that pricking the apple near the stalk gave greater PTP responses than pricking in the middle region of the apple (**Vlieg-Boerstra et al., 2013**).

## 5. Conclusion

The source of cultivar-specific allergenic potential was hypothesized to emanate from the amount and the composition of the allergenic compound. The precise causes of the specific apple allergenicity have not been elucidated to date. Current efforts are directed to determine the particular gene isoforms and variants associated with increased allergenicity. The allergenic potential of the fruit is clinically analyzed using several methods and approaches, which sometimes give inconsistent results. The allergenic potential is, furthermore, affected by a number of factors such as growing and storage conditions, biotic and abiotic stress or differences in spatial distribution of allergen in fruit. These factors make the issue more complex and call for standardized process in assessment of apple allergenicity. Further investigation of the genetic variation of Mal d 1, its expression pattern in different varieties and comparison of the data with allergenicity information are

required to unveil the mechanisms underlying apple allergenicity.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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