Benefits and concerns associated with biotechnology-derived foods: Can additional research reduce children health risks?

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Abstract. – The development of techniques devised for the genetic manipulation of foods poses new risks for children with food allergy (FA). The introduction of foreign allergenic proteins from different foods into previously tolerated foods may trigger allergic reactions, often complicating with anaphylactic shock in a subset of allergic babies. Children with FA, even if subjected to preventative diets, always challenge the risk of developing allergic manifestations after unintentional intake of a non tolerated food in restaurant settings, with relatives or schoolmates, etc, where product labelling is necessarily lacking. The introduction of potentially allergenic proteins into foods generally considered safe for allergic children can be done deliberately, by either substantially altering the food ingredients, or by genetic manipulation which change the composition or transfer allergens, or unintentionally by quality control failures, due to contaminations in the production process, or to genetic mismanipulation. There is a controversy between multinationalis often favored by governments and consumer association resistance, thus an equidistant analysis poses some unprecedented impediments. The importance of FA and the potential of transgenic plants to bring food allergens into the food supply should not be disregarded. The expression in soybeans of a Brazil nut protein resulted in a food allergen ex-pressed in widely used infant formulas, so paving the way to an often reported multinational debacle. Genetic engineering poses innovative ethical and social concerns, as well as serious challenges to the environment, human health, animal welfare, and the future of agriculture. In this paper will be emphasized practical concepts more crucial for pediatricians.

Key Words: Transgenic foods, Genetic manipulations, Food allergy, Frankenstein food, Multinational companies, Consumers’ apprehension, Romulus and Remus.

Introduction

Transgeneric foods have gained in 1999 a world role when Doctor Pusztai, referring to his experiments on genetically modified (GM) potatoes, has defined them, without formality, “Frankenstein food”. For this reason, Doctor Pusztai was stripped of his post, publicly humiliated as a person whose mind was badly confused, and openly accused of being incapable to carry out well designed works. His fate was similar to that of the JAMA Editor1-3. Food allergies are caused by abnormal immunological responses to substances in foods, usually naturally occurring proteins. Allergic reactions can be manifested by symptoms ranging from mild cutaneous or gastrointestinal problems to life-threatening anaphylactic shock reactions. Virtually all food allergens are proteins, but only a small fraction of the many proteins found in foods are allergenic. Since genetic modification results in the introduction of a segment of DNA containing one or more genes from one organism into a chromosome of another organism, the potential allergenicity of the newly introduced protein (NIP) should be a major component of the safety assessment process. An assessment of its allergenicity can be accomplished by evaluating the source of the gene, the NIP sequence homology to known allergens, the NIP immunoreactivity with immunoglobulin E (IgE) antibodies from the blood serum of individuals with known allergies to the source from which the genetic material was obtained, and the NIP physicochemical properties. The importance of FA and the potential of transgenic plants to bring food allergens into the food supply should not be minimized. Clearly, the determination of allergenicity of transgenic proteins by analogy to other food allergens is inadequate, and that tests must be developed that in-
volve the interaction of the transgenic protein in question with the immune system. Given the extensive recent increases in our knowledge of this important system, the development of such tests would appear to be well within the capabilities of the scientific community. Multinational companies producers of GM organisms (GMO) have gained a world fame.

**The Pros and Cons: Which Prevail?**

For the first time in history, human beings are becoming the architects of life. The variety of traits introduced into crops is astonishing, including insect protection, delayed ripening, herbicide tolerance, modified oils, disease resistance and genetically altered foods. GMOs pay for a kind of “original sin”: the allergenicity increase, such as the introduction of allergens from different sources via genetic manipulations. Such an approach was used recently to assess the possible allergenicity of a transgenic soybean with an inserted gene from Brazil nuts that expressed a high-methionine protein. Brazil nuts are known to be allergic, and it was demonstrated that the high-methionine protein was indeed a major allergen from Brazil nuts. As a result of this assessment, commercial interest in this transgenic soybean variety was abandoned. However, we stress that such experiments in the hands of not experts may pave the way to new mishaps.

Table I shows the plant species transferred by genetic engineering or GMO, and Figure 1 those cultivated in the US and imported in Europe. We understand that such plants are genetically manipulated to obtain products with prolonged average life and better aspect and taste; however allergic patients can run the risk of anaphylaxis due to the introduction of new allergens even into wholly common foods?

Table I shows the plant species transferred by genetic engineering or GMO, and Figure 1 those cultivated in the US and imported in Europe. Tomato is not included. From references.

<table>
<thead>
<tr>
<th>Introduced protein</th>
<th>Crop products and targets</th>
</tr>
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<tbody>
<tr>
<td>ACC deaminase, antiseNSE PG, antiseNSE ACC synthase</td>
<td>Delays without impairing the tomatonatural ripening and softening, to obtain a more concentrated juice</td>
</tr>
<tr>
<td>Phosphinotrinic acetyltransferase</td>
<td>Renders corn tolerant to herbicides</td>
</tr>
<tr>
<td>Neomycin phosphotransferase II</td>
<td>Protects from insects potato and delays tomato natural ripening and softening</td>
</tr>
<tr>
<td>Glyphosate oxidoreductase</td>
<td>Renders corn tolerant to herbicides</td>
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<tr>
<td>Btt-HD1 insecticidal protein</td>
<td>Protects from insects corn and tomato</td>
</tr>
<tr>
<td>Btt-HD 73 insecticidal protein</td>
<td>Protects from insects potato</td>
</tr>
<tr>
<td>CP4 EPSPS synthetase</td>
<td>Renders canola, corn, cotton, soy and sugarbeet tolerant to herbicides</td>
</tr>
<tr>
<td>β-D-glucuronidase</td>
<td>Renders soy tolerant to herbicides</td>
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Adapted from references. Abbreviations: ACC = 1-amino-1-cyclopropane-carboxylic acid, Btt = Bacillus thuringiensis subsr pes tenebrionis, Btk = Bacillus thuringiensis subsp. kurstaki, from strains HD-1, CP4 EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium strain CP4, PG = poligalatturonas. Notes: Recently there were controversies regarding transgenic soy and corn, some types of modified maize have been prohibited in Austria, France, Greece, Luxembourg. Denmark has interrupted both farming and selling of such crops, in response to public opinion and in the United Kingdom the cultivations were limited for 3 years, but large supermarkets were forced to stop stocking GMFs. However, the UE has authorized the import and selling of some varieties of transgenic soy and maize, experimentally produced in US and Canada. The Italian government, resuming two laws issued from UE on 16/2/1996 and 2/6/1998, has prohibited the use of such foods for the infantile alimentation (Decr PR 7/4/1999). Adapted from FoE Groups and Biotech Campaigning. Link 2000; 93: 21-23.
present instead in the sleeves, to combat malnutrition\textsuperscript{11}, but also rice allergy in the Chinese and Japanese populations, eating a high daily quantity\textsuperscript{12,13}, not to mention the transgenic fishes deriving from monosexualization or doubling maternal DNA\textsuperscript{14}. Similarly, the introduction of peanut genes into tomatoes and of fish proteins into potatoes, to enable storage of the vegetable below 0°C, may cause serious anaphylactic reactions in children allergic to these foods\textsuperscript{15,16}. Not only the toxin \textit{Bacillus thuringiensis} (Bt) can kill the monarch butterfly, but also Bt toxin can bind to soil particles and persist in the soil for over 200 days, harming soil health. However, testing the effect of Bt insecticidal preparations on a number of human cell types denotes that spore-containing Bt products have an inherent capacity to lyse human cells in free and interactive forms and may also act as immune sensitizers\textsuperscript{17}.

Several methods exist to manipulate FA, but one side is to achieve a selection of strains with reduced allergenic content, and the other is to reduce the allergenic content by changing the relative ratio of the normal constituents of a food. However there is a great need for standardization of the methods employed for testing potential allergens, but such controlled program to assess allergenicity in manipulated foods should be settled within an international framework\textsuperscript{15}. The greater problem is the high number of foods potentially interested by genetic engineering (Table II)\textsuperscript{9} and the first place of France and the second of Italy among the European countries, regarding the number of fields where transgenic cultivations are experimented (Figure 2)\textsuperscript{18}.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|}
\hline
Apricot & Melon \\
Asparagus & Mustard \\
Barley & Oats \\
Bilberry & Oil Seed Rape \\
Black currant & Orange \\
Broccoli & Papaya \\
Buckwheat & Pea \\
Cabbage & Peach \\
Carrot & Plum \\
Cauliflower & Potato \\
Celery & Raspberry \\
Chicory & Rice \\
Colza & Rye \\
Corn & Soybean \\
Eggplant & Strawberry \\
Fennel & Sugarbeet \\
Grape & Sweet potato \\
Horseradish & Tomato \\
Kiwi & Walnut \\
Lemon & Wheat \\
Lettuce & \\
Licorice & \\
Lotus & \\
\hline
\end{tabular}
\caption{Additional foods transformed with techniques for genetic manipulations.}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Number of fields where transgenic cultivations are experimented. From NIH data.}
\end{figure}
A recent debate on GMOs has triggered controversial, but not unfounded discussions. The European Union (EU) has decided that all GMOs totally or partly introduced into marked foods should be detailed on the label, but excluding such a guarantee for the consumers when the GMO level is $\leq 1\%$. A pediatrician can easily maintain that the core of the problem does not regard the 1% present in foods, but the missing prerequisite of precisely clarifying it on the label stating that they have been genetically engineered. A very strange procedure: who shall evaluate or check the exactness of this 1%? Who has so sophisticated weighing-machines to precisely measure 1%, or has money enough to buy such precision instruments? We all remember that a similar exception was provided for chocolate bars containing instead of the usual cocoa butter, one deriving from inferior vegetable oils, hence denaturing the habitual taste. We have demonstrated that only one drop of cow’s milk (CM) can trigger an anaphylactic shock in a baby. When the hydrolysate formulas (HFs) were first commercialized, certain studies have claimed that reactions to HFs were a rare occurrence, however we have documented in two papers that HFs have provoked about 240 reactions until anaphylaxis, probably because of minimal traces of $\beta$-lactoglobulin, one of the most immunogenic CM proteins. A drop is a big dose, the sensitizing substances are measured in $\mu$g, one $\mu$g is $= 0.000001$ g. The Monsanto supporters claim that GMOs provoke no harm. Often the truth has been found only after several years, as it was the case of DDT, of thalidomide, of HFs: nobody could have foreseen such consequences.

Diagnosis

Diagnosis is the appropriate means to ascertain whether parts of GMOs are present, with possible noxious implications for children’s health. Thus is the more delicate moment, but also the more critical. Thereby, several organizations have tried to protect the consumers and to comply with the desiderata of the public opinion. In particular the Food and Agriculture Organization (FAO) of the United Nations has suggested new labelling procedures of foods of potentially allergenic nature. However, their recommendations resulted even more liberal than those above mentioned of the EU, proposing that GMO present in concentrations less than 5% to 25% of the food needed not to be declared. Consequently, HFs should be “exempted”, whereas very sensitive children may react to even low amounts of residual epitopes in these HFs. Therefore, new guidelines specific for GMOs have been selected. There it is stated that “the transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the transferred gene does encode the pertinent allergen”. Basically, three categories of GM crops can be considered:

1. GM crops which have the same composition as the parent crop,
2. GM crops which have the same composition as the parent crop with the exception of a well-defined trait,
3. GM crops which are different from the parent crop.

However, such guidelines denote a more marked interest for both allergens and databases than for GMOs and it is significant that other authors found it beneficial to compile lists of allergens, more useful for immunology textbooks.

Allergenic Sources of Genes

As it has been clearly indicated, if a gene transferred in one or more foods is obtained from a source commonly known for its allergenicity, data should establish that the gene does encode the allergen in question. Figure 3, above all critical for the identification and labelling of these foods, shows all the necessary approaches, including tests of proven validity:
In vitro or first level tests, RAST (radioallergosorbent test), ELISA (enzyme-linked immunosorbent assay) and immunoblotting,

In vivo or second level tests, SPTs (skin prick tests) and DBPCFC (double-blind, placebo-controlled food challenge).

The diagnostic protocol shall conclude for the complete negativity if it can be established that the gene transferred did not encode, as previously alluded to, any foreign allergen and the product could be freely marketed, even if we cannot exclude reactions in children allergic to that food. Instead, if whatever analysis results positive, the label should specify that the product contains allergenic proteins from food and should not be eaten by patients allergic to food.

To assure a correct execution of the procedure, it is crucial that:

1. Any GMO should be investigated following the method shown in Figure 3 before becoming commercially available,

2. Any test should be performed in independent laboratories (for example from multinationals) of recognized standard having access to sufficient numbers of patients previous diagnosed as allergic to the food(s) in question (“reference laboratories”),

3. Patients should be diagnosed with testing strictly adhering to EAACI guidelines.

Additional techniques, including studies on animal models and systems suited for determination of DNA or RNA in foods may soon be developed and subjected to adequate procedures and may be included in the standard diagnostic program after sufficient testing.

Taylor et al suggest a somewhat different procedure:

- The combination of tests involving allergic human subjects or blood serum from such subjects should provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small rate of the population allergic to the source material.
- Any positive results obtained in tests involving allergic human subjects or their blood serum as before would provide a high level of confidence that the novel protein was a potential allergen. Foods containing such novel proteins would need to be labelled to protect allergic children.
- A novel protein with either no sequence similarity to known allergens or derived from a less commonly allergenic source with no evidence of binding to IgE from the blood serum of a few allergic individuals (n < 5) but that is stable to digestion and processing should be considered a potential allergen. Further assessment would be necessary to address this uncertainty. The nature of the tests would be determined on a case-by-case basis.
- A novel protein with no sequence similarity to known allergens and that was not stable to digestion and processing would have no evidence of allergenicity. Similarly, a novel protein expressed by a gene obtained from a less commonly allergenic source and demon-

Figure 3. Allergenicity assessment of potential transgenic foods. Flow chart for investigation of genetically modified foods for potential allergenicity before their release on market, with suggestions about labelling of the pertinent foods. Modified from reference 15,16.
Nonallergenic Sources of Genes

We follow a clear assessment to verify the nonallergenic sources of candidate genes: as yet there exists no single predictive assay to insure the identification of the allergenic potential of food proteins deriving from nonallergenic food sources. Viewing Figure 3, it is possible to compare the typical biological and physicochemical properties of a transferred protein with known allergenic proteins. Although it is known that the analysis is very complex, it seems an urgent priority in this field to focus on two approaches, either searching the amino acid sequence homology of the transferred protein, or analyzing the physicochemical properties of the above protein.

Amino Acid Sequence Studies

As a first point, a comparison of the amino acid sequence homology between the introduced protein and allergenic protein is performed based on the reported sequences of several allergens, including food allergens. Even if many T-cell, B-cell and IgE-binding allergenic epitopes have been mapped, the distinction between allergenic and nonallergenic epitopes remains to-date concealed. Considering previous studies on the number of contiguous amino acids necessary to the binding of peptide fragments to T-cell epitopes of allergenic proteins, a sequence homology, to be immunologically relevant, requires at least 8 contiguous identical amino acids. However, further tests in this field yielded no results, since the genes introduced into the tested proteins do not encode known allergens or their homologues, and no such protein shares linear epitopes with known allergens, so they are T-cell epitopes to be better analyzed.

Studies on Stability to Digestion

The controversies related to food allergens can be overcome by testing for example their stability to digestion. The ability of such allergens of reaching and crossing the mucosal membranes of the bowel is increased if the allergens succeed in maintaining their stability in the gut, characterized by the acidity and proteolysis there prevailing. Since different allergens exhibit proteolytic activity, the physicochemical properties favoring such stability can uncover the allergenic potential. To evaluate the potential digestive stability of a number of common food allergens an experimental model was prepared with the objective of simulating the mammalian gastrointestinal fluids. Test protein were so incubated in a solution of pepsin at acid pH with the results that food allergens were stable for at least 2 minutes, and the major allergens were stable for >60 minutes. We easily contend that only one μg is able to trigger an anaphylactic shock.

Is GMO Overestimated?

What we have hitherto discussed has an only meaning: the high interests of the multinationals. The Monsanto supporters claim that GMOs provoke no harm. Often the truth has been found only after several years, as were the discussed earlier cases: nobody could have anticipated such outcome. Once again the unaware consumer and parents of infants and young children must make a leap in the dark and/or eat boiled crow. We deem it very urgent that it is clearly specified whether foods to be sold in supermarkets are Frankenstein’s food or not? Certainly both WHO and EU look after the perfect correspondence between normal and GM foods. However the label should always indicate not only whether the food is or not genetically manipulated, but also the amount of GM food it contains. As yet the regulations fail to specify such characteristics on the product labels, a deficiency often stressed by us in other fields. As previously alluded to, among the UE directions there is even the exemption from the obligation of specifying on the label that it may contain a GMO. The confusion (and the damage to consumers) has been amplified by the recent discussion on the seven GM oils of which nobody knows whether they are to be banned and the resulting ascertainment that all processed foods potentially containing those oils should be removed from grocery shelves, without knowing, practically, which foods contain such oils as ingredients.

We pass over the damages laid on the citizens by biodiversity reduction, substituting it with a few standardized products, however the “own-goals” of the multinational industries producing GMOs are now countless: Monsanto only recently should have discovered that in the GM soybean produced seven years ago to the FDA to get the official permission to commercialize soy-
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bean, there were two more genes in addition to the three that were denounced: a 166% increase. According to Monsanto such genes remained “dormant” during seven years, were completely inactive: not only we object, which analysis the Monsanto has done to affirm this truth, but also whether they have evaluated the world consequences, being soybean a natural ingredient of a myriad of GMOs. Which credit can be given, from now on, to the Monsanto, to all multinational companies interested in GMOs? The second own-goal of the multinational is the discovery in April 2001 that the laboratories that should control the GMOs can identify ONLY SIX GMO SEEDS OUT OF 24 that are diffused throughout the world, a 433.3% reduction! Thus, the tests for GMOs may not be accurate. The third own-goal was reached with 550% of honey cans containing pollen traces, that were GM pollen\(^4\), therefore contraindicated for children with respiratory allergy and/or oral allergic syndrome\(^5\), and with GM canola transported by the wind on fields with biological farming\(^6\): the GMO supporters are served.

The zenith (of own-goals) was reached when the Royal Society of Canada has issued on 20.1. 2001 a document stating that 53 new procedures should be fulfilled before a new permission to cultivate GMOs could be released\(^7\). The Society stresses that lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that living modified organism intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects. The safety assessment process, as it stands, is not adequate to pick out every GM crop harmful to human or animal health. The Royal Society also reported that “the use of substantial equivalence as a decision threshold by regulatory agencies is, in the Panel’s view, scientifically unjustifiable when used to exempt new products from full scientific scrutiny”. Substantial equivalence is the concept that underpins the safety assessment of GM crops around the world. The basic premise is that if a GM food is shown by composition analysis to be the same as a non-GM food then it should be considered to be as safe as the non-GM food. However, GM foods cannot be exactly the same as non-GM foods, by the very fact of the novel proteins they contain, and so it was determined that they would be considered as safe as normal foods if they were substantially equivalent to them\(^8\).

But the risks have reached the climax when the GM corn destined to animal feeding was mistakenly mixed with maize prepared for human alimentation: The global marketing of GM food has been dealt a blow following reports of allergic reactions to Starlink corn, which was detected in corn food products. The case of allergic reactions reported after the consumption of products containing the GM maize Starlink was unusual simply because consumers were made aware that they were eating it. Starlink corn was not approved for human food, thus any food found to contain evidence of Starlink was recalled from the marketplace by the manufacturers. Unfortunately, the news media often portrayed Starlink as an allergen or a potential allergen, causing consumer concerns\(^9\).

In the wide and variegated field of Frankensteins foods something appears to move. Suppliers of soy formulas to prevent atopy in at-risk neonates, and to cure atopic infants and children (Abbott, Dieterba, Milupa) have announced that their production shall utilize no GM soy. However, two producers, Nestlé and the aforesaid Die terba were subjected to an inquiry, having introduced into their SPFs GM soy in the 1% proportion. The marketing of 4 types of GM corn has been discontinued (however excluding 3 types of GM rape oil). Moreover the Novartis multinational has stopped the production of GM foods (but not of the pertinent seeds) and the French government has decided to destroy the GM soy illegally introduced into the country.

**Stimulating Challenges**

The recent polemics related to the mad-cow hysteria has somewhat obscured another wilderness linked with GM foods: they have no taste. A recent presse-prononciation of the great French “chefs” declares that these GMO represent the “nothing” in the gastronomic specialties at the point that the “chefs” are not able to find out pure ingredients anywhere in this field.

An additional presse-release regards not genetically but DNA-selected tomatoes which were given a gene to delay their ripening, so they may remain in a refrigerator without losing their characteristics, except the natural ta-
ste. When scientists tried to feed tomatoes to rodents, however, the animals wouldn’t eat them. So it is all at the expense of our taste: we usually eat what we like, but the highest choice is for food palatability\textsuperscript{41}.

An estimated $3.5 \times 10^{12}$ transgenic plants have been grown in the US. in the past 12 years, with over two trillion being grown in 1999 and 2000 alone (Figure 4)\textsuperscript{42}. In a press-inquiry done in Italy on a sample of 1200 citizens, 67% virtually refused GMO, 75% judged less adequate or fully inadequate the laws in force in Italy, and 60% blamed that nobody warrants the consumers’ food safety. Of those polled, 98% said yes, and 2% said no\textsuperscript{43}. In fact, not only Europeans but also Americans have called for a recall of GM-foods on the market. GM food is still selling briskly on US stores, but probably only because GM foods are not labelled, so consumers have no idea what they are. GMO soybean growers in the US claim savings of $5 to $20 per acre (0.447 hectares) from reduced fuel and herbicide costs. However, the Americans are now becoming worried about GM foods, and the US secretary of agriculture has suggested the need for unbiased research on the safety of GM crops\textsuperscript{44}. Problems for GM foods are arising in Australia, Austria, EU, Thailand; and in Canada. Should all GM foods be labelled? Can additional research reduce uncertainties and increase parent confidence? Certainly, Americans would continue their efforts to convince the Europeans to change their policies. Therefore, it is hoped that a collaboration between the EU and the US would give a rational basis for protection of children of both countries\textsuperscript{45}.

\textbf{Future Frontiers}

According to the legend of Romulus and Remus, the twins were abandoned by their mother on the river-bed of the Tiber river of Rome. A wolf took care of the babies and breastfed them. The twins survived in such a hostile environment as the Tiber river being fed with such different milk as the wolf’s milk. However, they grew so strong as to enable them to build Rome. This fascinating legend teaches us that human newborns are able to overcome many difficulties. However, there is no doubt that the twins would fail to react in this way if they would have been fed GM foods in our times. US citizens blame that borrowing genes from various creatures and implanting them in others, scientists are creating super-fast growing GM salmon, trout and carfish, oysters that can withstand viruses\textsuperscript{46}.

Investigations of any potential FA risks associated with GM food are vital for consumer protection.

In conclusion, the safety assessment of GM crops should be subjected to full review in light of the suggestions of Table 3\textsuperscript{47}; We ask therefore, who has established for sure the perfect correspondence of GMO and natural foods considering the resources of vitamins and minerals, especially of trace elements, as regards both nutrition and growth of children?

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