MILK ALLERGY IN HLA-DRB1*14:19/14:21 PAEDIATRIC PATIENTS: A BIOINFORMATICS APPROACH

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Abstract

Allergens are processed in specialized antigen-presenting cells located at mucosal surfaces by the machinery of human leukocyte antigen (HLA) proteins. The susceptibility and protection against some allergies are associated mainly with the polymorphism in HLA class II. The alleles that bind to allergen peptides could be considered as susceptible to particular allergy and the nonbinding alleles – as protective. In the present study, we report the cases of two Caucasian males, 6 and 14 month-old, diagnosed with allergic reactions to β-lactoglobulin (Bos d 5) and BSA (Bos d 6) and genotyped as HLA-DRB1*14:19/14:21. The presence/absence of association between milk allergens and HLA specificity is analysed using bioinformatics tools. The protein sequences were cleaved *in silico* by pepsin, trypsin and chymotrypsin and the binding affinities to HLA-DRB1*14:19/14:21 of the peptide fragments survived after the digestion were predicted. Practically, after the digestion, no binders to HLA-DRB1*14:19 and *14:21 remain. Both alleles are close in structure to HLA-DRB1*03:01 which is protective against cow's milk allergy. The bioinformatics analysis showed that the observed milk allergy in these young patients rather is due to age-connected low concentration and activity of chymotrypsin than to HLA specificity.

Allergy to cow's milk is the most common food allergy in infants and young children.[1]Approximately 2.5 % of children younger than three years of age are allergic to milk. Nearly all infants who develop milk allergy do this in their first year of life. Most children eventually outgrow the milk allergy. The allergy is most likely to persist in children who have high levels of cow's milk antibodies in their blood. Blood tests can help to determinethe milk allergy. Sensitivity to cow's milk varies from person to person. Some people have a severe reaction after ingesting a tiny amount of milk. Others have only a mild reaction after ingesting a moderate amount of milk. Reactions to milk can be severe and life-threatening.The allergy symptoms are hives, stomach upset, vomiting, bloody stools (especially in infants), anaphylaxis (rarely). The main cow's milk allergens are caseins, β-lactoglobulin, and α-lactalbumin.[2] Less common allergens are serum albumin and immunoglobulins. Allergens are processed in specialized antigen-presenting cells located at mucosal surfaces by the machinery of human leukocyte antigen (HLA) proteins.[3] HLA are extremely polymorphic and polygenic.[4] The susceptibility and protection against some allergies are associated mainly with the polymorphism in HLA class II.[3] The alleles that bind to allergen peptides could be considered as susceptible to particular allergy and the nonbinding alleles – as protective.[3,5]

Very few reports describe any presence

or absence of associations between milk proteins allergy and HLA polymorphism.[6,7]Recently, we analyzed these associations applying a bioinformatics approach.[5] It was found that the peptides survived after digestion by pepsin, trypsin and chymotrypsin bind preferentially to DRB1*01:01, but not to DRB1*03:01, DRB1*04:04, DRB1*12:01 and DRB1*15:01. Here, we report the cases of two Caucasian males, 6 and 14 month-old, diagnosed with milk allergy and genotyped as HLA-DRB1*14:19/14:21. The presence/absence of association between milk allergens and HLA specificity is analysed using bioinformatics tools.

Specific IgE were measured by EUROIMMUN enzyme immunoassay kit and analyzed by EURO lineScan software (EUROIMMUN, Germany). Genomic DNA for HLA-genotyping was prepared by the QIAampBlood kit (Qiagen, Hilden, Germany). HLA genotyping for the screening of HLA-DRB1 allele and subtyping of DR1 and DR4 was performed by the use of reverse hybridization kit (RDB2035, AID Diagnostika GmbH, Strassberg, Germany) according to the manufacturer's instructions.

The present study describes two pediatric patients with milk allergy genotyped as HLA-DRB1*14:19/14:21. This is the first report on patients with milk allergy carrying this extremely rare HLA allele. Less than 0.01% of the human population in China, Chile, Georgia, Rwanda and the Asian population in USA bears HLA-DRB1*14:19 or *14:21 allele.[11] The patients were 6- and 14-month-old Caucasian males. The 6-month-old boy showed reaction to Bos d 5 and to Bos d 6, while the 14-month-old boy – only to Bos d 6. Additionally, in the present study a 5-year-old boy of the same HLA allele was diagnosed with no milk allergy.

The milk proteins undergo an extensive digestion in GIT that leads to significant reduction of the number of potential HLA-DRB1 binders (Table 1). Practically, after digestion by pepsin, trypsin and chymotrypsin, no binders to HLA-DRB1*14:19 and *14:21 remain. Pepsin activity is likely to be low early in life but by 6 months of age it becomes similar to that of adults.[12] Trypsin concentration and activity are similar to those of adults by one month postpartum,[13] while the chymotrypsin concentration reaches adult levels in 3-year-old children.[14]

Table 1. *Number of peptide fragments and peptide binders/non-binders to HLA-DRB1*14:19 and *14:21before and after digestion.*

Digestion	Bos d 5	Bos d 6
Before digestion	340 (48 binders)	1198 (159 binders)
After digestion by pepsin	5 (3 binders)	21 (3 binders)
After digestion by pepsin + trypsin	1 (1 non-binder)	8 (non-binders)
After digestion by pepsin + chymotrypsin	3 (1 binder)	13 (non-binders)
After digestion by pepsin + trypsin + chymotrypsin	1 (non-binder)	5 (non-binders)

The allele HLA-DRB1*03:01is the closest in structure to HLA-DRB1*14:19 and *14:21 (Figure 1). There are four mutations in HLA-DRB1*14:21, three of them are located in the peptide binding site.[15]The mutations affect the following positions: 26(Y→F), 74(R→A), and 77(N→T). Three additional mutations exist in the binding site of DRB1*14:19: 28(D→E), 47(F→Y), and 86(V→G). At most positions, the mutations lead to residues with similar physicochemical properties (size, lipophilicity, electronic properties), only the mutation 74(R→A) leads to significant change in size and lipophilicity. Position 74 is part of pocket 4 in DRB1 binding site[16] and the mutation R→A makes the pocket deeper and more lipophilic in HLA-DRB1*14:19 and *14:21 than it is in HLA-DRB1*03:01.

Figure 1. *Sequence alignment of HLA-DRB1*03:01, *14:19 and *14:21.*

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AA Pos.                 10        20        30        40        50        60        70        80        90        100
DRB1*03:01:01:01  GDTRPRFLEY STSECHFFNG TERVRYLDRY FHNQEENVRF DSDVGEFRAV TELGRPDAEY WNSQKDLLEQ KRGRVDNYCR HNYGVVESFT VQRRVHPKVT
DRB1*14:19        ---------- ---------- -----F-E-- ---------- ------Y--- ---------- ---------- --AA--T--- -----G---- **********
DRB1*14:21        ---------- ---------- -----F---- ---------- ---------- ---------- ---------- --AA--T--- ---------- ----------
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We predicted the binding affinities to HLA-DRB1*03:01 (Table 2) and compared them with those to HLA-DRB1*14:19 and *14:21 (Table 1). Differences were found neither in the number of binders/non-binders, nor in the peptide sequences.

Table 2. *Number of peptide fragments and peptide binders/non-binders to HLA-DRB1*03:01 before and after digestion.*

Digestion	Bos d 5	Bos d 6
Before digestion	170 (1 binder)	599 (5 binders)
After digestion by pepsin	5 (non-binders)	21 (1 binder)
After digestion by pepsin + trypsin	1 (non-binder)	8 (non-binders)
After digestion by pepsin + chymotrypsin	3 (non-binder)	13 (non-binders)
After digestion by pepsin + trypsin + chymotrypsin	1 (non-binder)	5 (non-binders)

As DRB1*03:01 is found to be protective against cow's milk allergy,[5] we can conclude, by analogy, that HLA-DRB1*14:19 and *14:21 are protective as well. The observed milk allergy in these young patients from the present study rather is due to age-connected low concentration and activity of chymotrypsin than to HLA specificity. Even more, the presence of a healthy control of the same HLA allele further confirms this conclusion.In these cases, substitution of cow's milk with extensively hydrolyzed milk formulas, preliminary prepared by enzymatic hydrolysis, and elemental formulas, prepared from synthesized free amino acids, is prescribed.[17] Indeed, both patients were successfully treated with diet.

Consent

An informed consent was obtained from the parents according to the requirements of the ethics committee.

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