

Do HLA genes play a role in the genetics of peanut allergy?

Hypersensitivity to foods is now recognized as a problem throughout the industrialized world.¹ Among the common causes of food allergy, peanuts are the most frequent cause of life-threatening and fatal reactions.

A number of factors must come together to result in food allergy. The most obvious are the nature of the allergen, the process whereby the allergen is presented to the immune system, and the immune response to the allergen. However, the environment (broadly defined to include maternal influences and the microbial constituents of the gastrointestinal tract), the integrity of the gastrointestinal tract, and the function of the mucosal immune system appear to be important and may affect individuals to varying degrees based on their genetic predisposition.^{2–5} In addition, the individual can react to an allergen in different ways, depending on the timing, method, and context of its presentation.^{3–5} Three recent reviews of possible ways in which genetic and environmental factors might interact to lead to the development of food allergies have recently been published.^{4,6,7}

Although genetics cannot explain the apparent increasing prevalence of peanut allergy, a recent twin study reported that the expression of peanut allergy has a substantial genetic basis (mean, 81.6%; 95% confidence interval, 41.6%–99.7%).⁸ The genetic basis for the inheritance of peanut allergy is unknown, and the number of studies that have addressed this important question are few. The study by Shreffler and colleagues⁹ reported in this issue of the *Annals* examined expression of HLA class II alleles in children with peanut allergy compared with a control group of peanut-tolerant siblings and did not demonstrate any significant associations. Because of methodologic considerations, there is still ample opportunity for HLA class II genes to play an important role in this disorder. Nonetheless, it is likely that the genetics of peanut allergy will turn out to be much more complex than previously thought.

The major histocompatibility complex (MHC) is a complex group of genes on chromosome 6p21.31 that includes the genes that code for human leukocyte antigens (HLA).^{10,11} HLA molecules are highly variant heterodimers expressed on the cell surface that function primarily to present antigen to T cells. The MHC class I protein families (HLA-A, HLA-B, and HLA-C) are composed of a variant α chain and an invariant molecule, β_2 -microglobulin. The MHC class II protein families (HLA-DR, HLA-DQ, and HLA-DP) are composed of a 34-kDa α chain, noncovalently associated with a

29-kDa β chain.¹⁰ Together there are more than 1,300 HLA class II alleles, although these can vary in frequency in any given population and are inherited in groups (haplotypes).¹² The MHC class II alleles can be distinguished by serologic testing or DNA sequencing. Low-resolution sequencing identifies groups of serologically related alleles, whereas high-resolution sequencing separates HLA alleles based on amino acid sequences.

The rationale for examining the MHC class II genes should be apparent to all who have studied immunology. Soluble exogenous antigens from proteins such as food allergens are internalized by antigen-presenting cells and, after unfolding, disulphide reduction, and proteolytic cleavage, peptides bind to well-defined binding grooves on a limited repertoire of class II proteins. The MHC class II peptide complex is then expressed on the surface of antigen-presenting cells and recognized by specific T-cell receptors (TCRs) on CD4⁺ T cells. This leads to T-cell activation, B-cell development, class switching to IgE, and secretion of IgE by plasma cells.^{12,13} Since each class II allele binds a limited repertoire of peptides, it is reasonable to hypothesize that specific HLA alleles determine the ability of the immune system to recognize allergenic peanut proteins, leading to a specific IgE response.

The first report of an association of HLA alleles with peanut allergy was by Donovan and colleagues,¹⁴ who described a family with peanut allergy and found that peanut allergy segregated with the paternal HLA-DR4 haplotype and not with the TCR- α or TCR- β alleles. Howell et al¹⁵ reported the first large study examining studied HLA class II loci in 50 peanut allergic subjects, 34 non-peanut allergic relatives, and 293 unrelated controls. They found significant differences between the peanut allergic subjects and the unrelated controls for the presence of *DRB1**08 and *DQB1**04 but no significant differences between the peanut allergic patients and their non-peanut allergic relatives.¹⁵ *DRB1**03 was the only allele to have a suggestion of increased presence in the peanut allergic subjects relative to the peanut-tolerant sibling controls, but this did not achieved statistical significance when *P* values were corrected for multiple comparisons. So it appears likely that Howell and colleagues identified HLA associations that may be important for atopy within their population but not necessarily for peanut allergy.

Shreffler and colleagues designed the current study to replicate the study by Howell et al with the intent to then perform high-resolution DNA typing of HLA class II alleles

if low-resolution typing showed evidence of an association between HLA alleles and the presence of clinically important peanut allergy.⁹ The study by Shreffler et al was powered at 80% to detect the level of association seen in the study by Howell et al. Unexpectedly, these data did not replicate the findings of Howell et al in that there was not even a hint of association of peanut allergy with the *DRB1*03* allele. Furthermore, no significant associations were discovered among 25 other HLA class II serotype equivalents examined. Possible explanations include differences in the populations studied (but both were predominantly white), the number of individuals studied, the lack of an association between susceptibility to peanut allergy and class II alleles, and the complexity of the human immune response to peanuts.

Investigators examining associations between HLA alleles and any disease fight a difficult statistical battle. First, there may be multiple T-cell epitopes (and thus multiple MHC-peptide combinations) that can cause a pathologic immune response. Second, the more HLA alleles that are examined, the greater the likelihood of a type II error, that is, the greater likelihood of finding an association by chance alone. The statistical tool used to correct for this problem is the Bonferroni correction wherein *P* values are corrected by multiplying them by the number of alleles tested. To overcome this intrinsic difficulty in HLA studies, one must examine large number of individuals and/or group the alleles into families. By limiting the number of comparisons, the chance of a type II error is less. In both the studies by Howell et al and Shreffler et al, the HLA alleles were identified by low-resolution DNA typing, which naturally groups the alleles into families that are similar to those identified by serologic typing.

An alternative approach to grouping HLA alleles is to perform high-resolution DNA typing and group alleles based on similarities in the peptide-binding groove, ignoring serologic differences. Class II molecules have 4 primary pockets (P1, P4, P6, and P9) and 3 minor pockets (P3, P7, and P10) within their binding groove. Recently, there is an appreciation of significant homologies within the peptide-binding groove that are shared among serologically disparate HLA alleles. These homologies within the peptide-binding groove have been called "shared epitopes,"¹⁶ "restrictive supertype patterns" or "functional groups."¹⁷ Blanco and colleagues¹⁸ recently used this approach to investigate the HLA class II linkage for the latex-fruit syndrome in a Spanish population. Within the *HLA-DQ* alleles, they found that the presence of allele *DQB1*0201* was associated with susceptibility to fruit reactivity. The initial evaluation of the *DRB1* alleles did not reveal any statistical association, but when these data were reanalyzed by grouping the known alleles into 7 functional groups based on peptide-binding groove motifs in pocket 4 as described by Ou et al,¹⁷ they found that the presence of *HLA-DRB1* alleles belonging to functional group E confirmed susceptibility to fruit allergy. Blanco et al had a distinct advantage over studies of peanut allergy, because they were examining a subgroup of latex and fruit allergic

patients who were sensitized to a single or limited number of proteins. Nonetheless, a similar method of exploring HLA associations in patients with severe peanut allergy may be revealing.

An alternative hypothesis is that MHC class II genes are at most minor players in the inheritance of peanut allergy. If this is true, what other genetic elements may be important? The answer to this question rests within the larger question of why a subgroup of the population (approximately 20%) develops IgE-mediated diseases and only a small percentage of these develop peanut allergy. This is not just bad luck, because there is clearly increased risk among family members and tremendously increased risk between identical twins. Sampson⁴ has characterized food allergy as a failure of the mucosal immune system, which is a reasonable hypothesis. A handful of known genetic elements may contribute to a failure of mucosal immunity, including genes of the innate immune system that encode for the toll-like receptors, modulating the interactions between the immune system and bacteria in the gut. Polymorphisms in the toll-like receptors possibly combined with unknown determinants of gut permeability and yet to be discovered factors governing the mucosal immune system may be at play.^{4,7}

In summary, the genetic basis of peanut allergy is still a wide-open question. The importance of this article by Shreffler and colleagues is to highlight the complexity of the immune response to a common food. The aberrant immunologic response to food allergens may well involve the HLA system, but this is difficult to demonstrate. The importance of other genetic elements, environmental influences, and their interactions is yet to be delineated.

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