

Gene–Environment Interactions in Asthma and Allergies: A New Paradigm to Understand Disease Causation

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Twin studies have suggested that asthma and allergies are the result of a combination of the effects of environmental factors and genetic influences [1]. Until recently, the role of these two potential sources of susceptibility in these conditions was studied separately, or at most, the potential interaction between genes and environment was evaluated by stratifying the effect of exposures by family history [2]. The Human Genome Project has made it possible to assess the potential role of gene–environment interactions as determinants of asthma and allergies in a systematic way. Moreover, and contrary to what was the case during most of the twentieth century, the real limiting step today is the difficulty to comprehensibly assess the degree and timing of environmental exposures.

This article summarizes the theoretical basis on which to insert any studies of gene–environment interactions. It also outlines a specific example of such interactions, as represented by the role of genetic variations in the CD14 gene in relation to exposure to one of the ligands of CD14—namely endotoxin.

The concept of interaction

Interactions between risk factors or causes are not always conceptually easy to grasp. Because in everyday parlance an interaction is often understood as two elements that contribute to the determination of a certain outcome, even when

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acting independently from each other, many authors who comment about gene–environment interactions tend to use the term with this same additive meaning. This is not, however, the meaning with which the word *interaction* is used in this article.

Perhaps an easy way to understand the concept is to express it graphically. In 1909, Wolterek proposed the term *norm of reaction* to represent graphically the different expressions that a certain phenotype could acquire in different environments and in individuals who have different genotypes [3]. In one possible scenario, only additive effects of genetic and environmental influences are present with no real interaction between them. In this case, phenotypic variation (V_p) is simply the addition of the influence of the genetic variation and that of the environmental variation (V_g and V_e , respectively): $V_p = V_g + V_e$.

In this situation (Fig. 1), the influence of the environmental factor on the phenotype is the same for all genotypes and, similarly, the influence of the genotypes on the phenotype is always the same, regardless of the level of environmental exposure. No interactive effect is necessary to explain this particular phenotype. One important consequence of the situation represented in Fig. 1 is that measured heritability will be approximately the same in all environments. Therefore, if the environmental influences represented in Fig. 1 are considered generically as the final result of several different exposures, the genetics of this phenotype can be studied in different environmental contexts without the need for specifically assessing the exposures in those contexts. Well-designed and well-performed genetics studies should provide replicable results, provided that the frequencies of the different genotypes under study are similar in the different populations, and that, if they are not, gene–gene interactions are not crucial determinants of the phenotype.

It is also possible, however, that the extent of the influence of the genotypes on the phenotypes may be different in different environments. In the same context, it is possible that the influence of environmental exposures may be different for

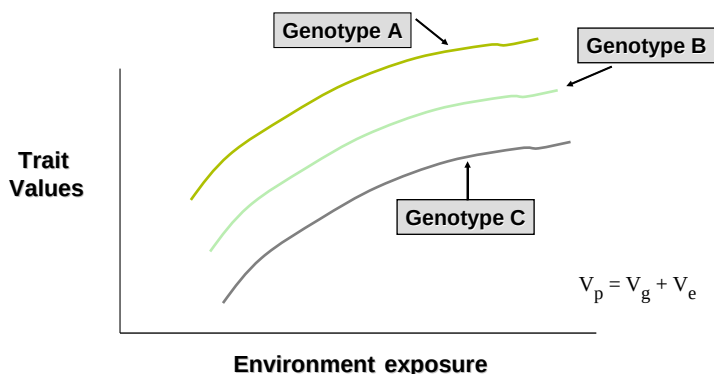


Fig. 1. A “norm of reaction,” in which the association between a phenotype (y axis) and an environmental exposure (x axis) is plotted for different genotypes. In this example, there is no gene–environment interaction, and therefore the curves for the three genotypes are parallel to each other.

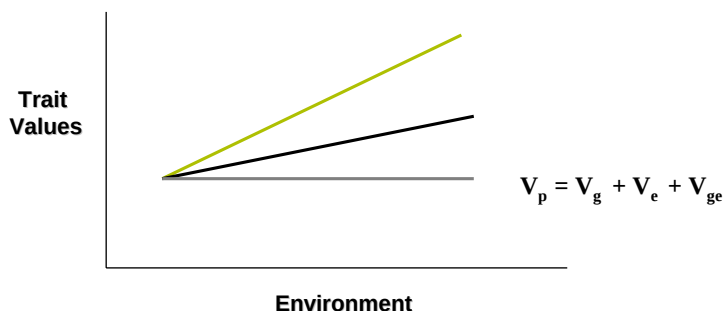


Fig. 2. Norm of reaction for a case in which gene-environment interaction is present. Genotype A (*top*), genotype B (*middle*), and genotype C (*bottom*). V_{ge} , variation explained by gene-environment interaction.

different genotypes. Fig. 2 presents one potential situation of this type, where it can be clearly seen that the influence of the genotype on the phenotype is much higher at high levels of exposure (right side of the graph) than at lower levels of exposure (left side of the graph). In this context, paradoxically, the degree of heritability of this phenotype is strongly determined by the environmental exposure (Fig. 3). Researchers who are studying the influence of this genotype in areas of high exposure are much more likely to find an association between genotype and phenotype than researchers who are studying the same phenotype in relation to the same genotype but at lower levels of exposure. Replication of genetic studies in this context is not a given. In this example, power to find an association at high levels of exposure is much higher than at low levels; therefore, it would be possible that a genotype that has a significant influence on the phenotype may be found, or not found, to significantly determine the phenotype in different studies because the power will change with the degree of the

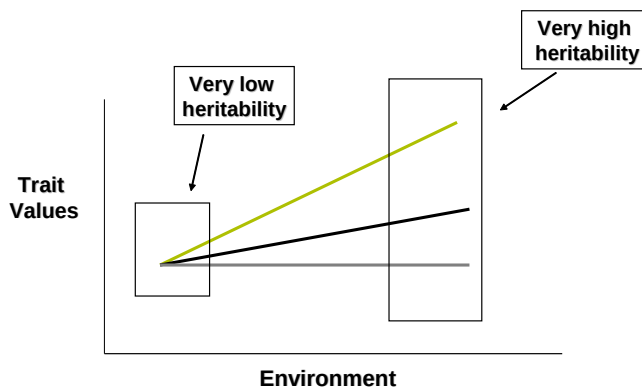


Fig. 3. Measured heritability is paradoxically dependent on environmental exposure when a gene-environment interaction exists. For this reason, power to detect association between a phenotype and a particular genetic polymorphism may depend on the degree of exposure to an environmental factor that interacts with that polymorphism in the population under study.

exposure. The case presented in Fig. 2 is only an example. It could also be possible that, for other gene–environment interactions, the power to detect a genetic association could be higher at lower doses of exposure.

Fig. 2 also illustrates that the influence of an environmental exposure may depend on the genetic characteristics of the population. If a large proportion of the subjects under study, for example, are carriers of genotype C, the environmental exposure will not be found to be strongly associated with the phenotype. Conversely, if the population under study has a large number subjects with genotype A, a very strong effect of the environmental exposure will be found. Therefore, the strength of environmental influences on phenotypes may depend on the genotypes of the individual under study. This can be expressed as: $V_p = V_g + V_e + V_{ge}$, where V_{ge} is the interactive term.

The general principle that a genetic association needs to be consistently replicated by one or more well-designed studies in order to be considered valid [4] presupposes that the environmental contexts in which the phenotypes are expressed have little influence on the genetic determination of those phenotypes. In other words, the penetrance of the genotype should be similar in all environments. For monogenic diseases, in which environmental influences are usually low, this is often the case, but there is no a priori reason why it should be the case for complex diseases such as asthma and allergies, in which exposures play a critical role.

It is important to stress, however, that in the example illustrated in Fig. 2, the genetic association would eventually be detected if a sufficient number of subjects who had some degree of exposure to the environmental factor were present in the population. In other words, one would expect that a meta-analysis of all studies of the relation between this particular phenotype and the genotypes under study would eventually show that the association was present, and would be able to detect the marginal effect represented by V_g in the above equation. A different case is illustrated in Fig. 4, where the genotypes have completely different and even opposite effects at different levels of exposure. At low levels,

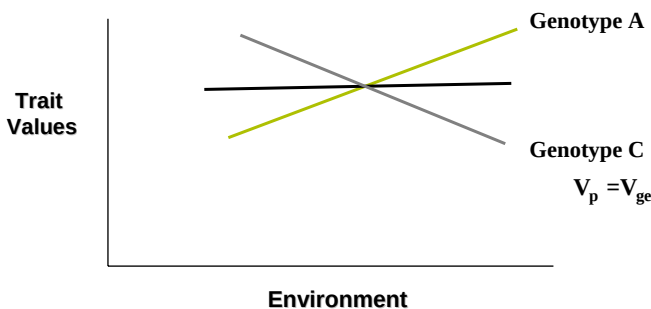


Fig. 4. In situations such as represented here, the allele associated with increased expression of the phenotype will depend on the degree of exposure. At low levels of exposure, expression of the phenotype is higher for genotype C; at lower levels of exposure, expression of the phenotype is higher for genotype A.

carriers of genotype C are more likely to have the phenotype under study, whereas carriers of genotype A are protected. At high levels, genotype A is the risk factor and genotype C the protective factor. In this case, $V_p = V_{ge}$. In other words, there are no marginal effects of either the genotype or the environmental exposure, and all the effects are interactive. In this situation, the effects of the environment and genotype are strictly dependent on the context in which they occur. For example, the exposure is protective for genotype C, but increases the risk for developing the phenotype for genotype A. It is indifferent for genotype B.

Interactive versus marginal effects in human genetics

Until recently, most examples of gene-environment interaction had been studied in plants and invertebrates [3]. The concept of phenotypic plasticity—the development of different phenotypes for the same genotype in different environments—has been extensively studied in plants because it has important consequences for the yields of genetically manipulated crops in different climates and external conditions. In contrast, the study of gene-environment interactions is conspicuously absent from mammal and especially human genetics. For example, a recent search for papers in the *American Journal of Human Genetics* using the term *gene-environment* yielded only two hits. This finding is understandable partly because exposures are very difficult (and in humans, often impossible) to standardize. But beyond these practical issues, the most important reason for this absence is that environmentalists and geneticists have addressed the impact of these two influences on most phenotypes in separate ways, with each individual considering the other's focus of interest as background noise with respect to the effects being studied.

It is the author's contention that the separate study of genetic and environmental influences has considerably hindered understanding of the role of these influences in determining most complex diseases. A good example is the current status of genetic studies of allergies. Hoffjan and colleagues [5] recently published a comprehensive study of the hundreds of polymorphisms in dozens of genes reportedly associated with asthma or allergies. The results are sobering; most of these reports have either not been replicated or replication has been inconsistent. Only for eight genes have the findings of one group in one population been corroborated in three or more different populations, and several of these genes are involved in the terminal, effector arm of the process leading to the expression of allergies (eg, IL-13, IL-4 receptor alpha, IL-4). Moreover, no single gene has been consistently found to be associated with the same phenotype in all populations studied. Similar conclusions have been reached by reviews of linkage studies of asthma and allergies [6].

The most frequently invoked explanations for these discrepancies have been technical [4]: studies are either marred by type I error (many comparisons, overt or hidden, are made in the first report of an association, thus annulling the value

of the conventional $P = .05$ value as a threshold for significance) or by type II error (replicative studies are too small to provide enough power). A more subtle explanation is the divergent definitions used to study asthma- and allergy-related phenotypes; assuming that different forms of these diseases will have common genetic backgrounds (or common environmental backgrounds, for that matter) underestimates the heterogeneity of the phenotypes under study. Although these technical objections may be valid in certain cases, they are unlikely to entirely explain the consistent inconsistency of association studies of complex diseases such as asthma and allergies.

One problem could be the fact that gene–gene interactions could be much more important determinants of risk for complex diseases than previously thought. Analyzing these interactions goes beyond the scope of this article, and good examples regarding linkage analysis have been recently reported in yeast [7]. More to the point, Howard and colleagues [8] reported a cogent example of such interactions regarding asthma for IL-13 and IL-4 receptor alpha. In both cases, only the study of the interactive effect between genes yielded significant results, with each gene that was assessed separately being unrelated to the phenotypes under study. In the case of gene–gene interactions, as illustrated by Howard and colleagues, the interactive effects can be observed at any level of the causation pathway under study, even at the distal extreme of the causation process.

Similar to gene–gene interactions, considering gene–environment interactions may be necessary to detect the effects of certain exposures and germ-line variations, specifically for those in which the relative value of the interactive term is high with respect to the marginal V_g and V_e effects. Unfortunately, it is not possible to determine a priori or from limited empirical data which genetic effect will be environmentally modified and to what extent. As explained earlier and graphed in Fig. 3, separate studies performed in environmental conditions that represent the extreme left part and the extreme right part of Fig. 2 will yield weak and strong heritability estimates, respectively, and only through combining the results of both studies (and perhaps others at intermediate levels of exposure) will the true, complex nature of the gene–environment interaction become apparent. It is also difficult to standardize exposures and their measurement in humans, for ethical and practical reasons. For an extremely mobile species like humans, and one that cannot be purposely subject to coerced, fixed, and potentially harmful exposures at pre-established doses and in a randomized way, the true exposure (ie, one that includes measurement of dose and timing) to any environmental stimulus is often impossible to estimate with any accuracy.

Perhaps the only exception to this rule is the study of the genetic determinants of responses to therapeutic interventions. The controlled and random nature of therapeutic trials offers a unique opportunity to study gene–environment interactions in humans. These trials provide ample evidence that there is a wide variability in responses to commonly used antiasthma medicines [9], and it has been suggested that part of this variability is because of genetic variation in the population. The main focus of pharmacogenetics today is the genes that encode for protein directly involved in the response system and metabolism of the drugs

under study. In the case of asthma and allergies, special attention has been paid to the genes for the β -2-adrenergic receptor [10] and the leukotriene receptor system [11].

Gene-environment interactions in asthma: the case for endotoxin and CD14

Because modern pharmacogenetic studies assume that the genes most likely to show variations that would modify the response to exposures (in this case, drugs) are those directly involved in the response system for that drug, the author's group explored the role of endotoxin-responsive genes in the pathogenesis of asthma and allergies. During the 1990s, the discovery that certain populations presumably more heavily exposed to microbial products were less likely to have allergies [12] suggested the idea (dubbed the *hygiene hypothesis*) that biologic products present in the surface of bacteria and other microbes could influence the type of immune responses mounted against allergens in subjects exposed to those products [13]. Subsequently, studies in rural communities in central Europe demonstrated that the likelihood of becoming sensitized against aeroallergens and of having atopic asthma was inversely proportional to the concentration of endotoxin present in home dust [14]. At the same time, studies in animal models showed that exposure to endotoxin before (but not after) sensitization procedures that usually triggered a Th2-type response abolished those responses [15]. These results suggested that activation of the endotoxin-receptor system, if it occurred at the right time during the sensitization process, could prevent the development of allergic responses.

The potential role of exposure to endotoxin in the development of allergies provided a paradigm analogous to that pursued in pharmacogenetic studies. The receptor system for endotoxin has been amply studied because of its crucial role in the development of innate immune responses. A central component is the toll-like receptor 4 (TLR4), which when engaged, activates the intracellular signaling mechanism involved in endotoxin responses. For TLR4 to respond to femtomolar doses of ligand, however, endotoxin needs to interact with CD14, a molecule that is found in membrane-bound (mCD14) and soluble (sCD14) circulating forms [16]. The author's group reasoned that if genetic variations could alter the structure of the CD14 molecule or the amount of CD14 available for interaction with endotoxin, the potential for the latter to influence the development of allergies could be substantially modified. The group thus screened the CD14 gene for polymorphisms [17] and found none of any significance in the coding region, but several in the 5' genomic region, and one single nucleotide polymorphism (SNP) in particular: a C-to-T conversion at position -159 (this same SNP is also called CD14/-260, depending on the position from which the nucleotides are counted). The group reported that this SNP was associated with the level of circulating sCD14 in a large population sample in Tucson, Ariz, with homozygotes for the T allele having higher levels than carriers of the C allele [17]. Homozygotes for the T allele were found to be less likely to be

atopic, as determined by the number of positive skin tests against aeroallergens. This finding was replicated by researchers studying another urban population in the Netherlands [18]. Moreover, studies in different populations confirmed the author's group's report of increased levels of circulating sCD14 in carriers of the T allele as compared with carriers of the C allele for CD14/–159 [19], whereas others reported increased expression of mCD14 in the surface of antigen-presenting cells of TT homozygotes as compared with carriers of other CD14/–159 genotypes [20].

Complex studies of the functional consequence of the SNP on CD14 transcription rates and on transcription factor interaction with the CD14/–159 genomic region were undertaken to determine the molecular basis for the association between the CD14/–159 SNP and expression of mCD14 and sCD14 [21]. These studies showed that, in a relevant cell type (MonoMac cells), a luciferase reporter assay showed that the T allele was associated with higher transcription rates than the C allele. The effects were reproducible but modest in size, as could be expected given the small effects in protein expression. Electromobility shift assays (EMSA) showed that these modifications in transcription rates could potentially be attributed to alterations caused by CD14/–159 in the complex interactions between this genomic region and the SP1 and SP3 transcription factors [21].

The results of these studies thus seemed to confirm the author's original hypothesis that, among individuals presumably exposed to low doses of endotoxin in urban environments, variations in the CD14 gene that could potentially increase sensitivity to endotoxin by increasing receptor availability could protect against the development of allergies. However, much like for most other genetic variations reportedly associated with asthma and allergies, not all well-performed studies in which the association between CD14 and allergies was tested could replicate the group's findings [19]. Often no explanation can be found for these discrepancies, but in the case of CD14, one potential explanation could be that, unbeknownst to the investigators, the populations they were studying could be exposed to different levels of endotoxin. For example, if the type of gene–environment interaction present in the case of CD14 was the one illustrated in Fig. 2, studies in populations at very low levels of exposure could find no association of CD14/–159 with allergies, whereas at levels above the minimum, the T allele could become protective.

The subsequent report by Gern and colleagues [22] of an interaction between CD14/–159 and exposure to dogs in the home in determining early-life atopic dermatitis provided further support for this assumption. In these studies, only homozygotes for the T allele showed a protective effect against atopic dermatitis among infant dwellers of homes with dogs. If the same result is expressed from the point of view of the exposure, only among children living in homes with dogs was there a protective effect of the CD14/–159 T allele. Exposure to dogs has been shown to be associated with increased levels of endotoxin in home dust [23], and therefore the results may reveal a gene–environment interaction of the type illustrated in Fig. 2.

Nonlinear interactive effects

All of the earlier results still point to an association between CD14/–159 and atopic phenotypes in which protective effects attributable to the T allele of CD14/–159 could be detected if sufficient numbers of subjects who were exposed to more than minimal levels of endotoxin were included in the sample. However, reports regarding subjects heavily exposed to endotoxin later emerged that challenged this assumption. Ober and colleagues [24] studied allergic sensitization among the Hutterites, a rural population living in a communal setting in the northern United States. They found that, contrary to findings in urban populations, the C allele of CD14/–159 was protective against sensitization in these subjects. Similarly, Amelung and colleagues [25] reported that, among workers in the animal facilities of the Jackson laboratories, presumably heavily exposed to endotoxin, the C allele of CD14/–159 was also protective. These findings were clearly unexpected, and could have been interpreted as the result of simple random variation around the null. An alternative explanation, however, was that the effects being observed in these different populations could follow the type of gene–environment interaction model schematically described in Fig. 4. In that case, one would expect that, at different levels of exposure, different CD14/–159 alleles would show protective effects, and there could also be exposure ranges in which no association could be found between CD14/–159 and allergy-related phenotypes.

A study by Zambelli-Weiner and colleagues [26] provided important new information in support of this second scenario. These authors studied the association between prevalence of asthma and CD14/–159 in a population of African descent in the island of Barbados. Contrary to other studies, however, concentration of endotoxin in house dust was also measured, and the effects of CD14/–159 were assessed at different levels of exposure. They found that the T allele was protective at low levels of exposure to endotoxin, whereas the C allele was protective at high levels of exposure (Fig. 5). They could not distinguish between the effects on asthma and those on atopic sensitization because most subjects who had asthma were atopic. A similar pattern was observed when the children enrolled in the study of the association between endotoxin exposure and prevalence of asthma and allergies in rural communities in central Europe were genotyped for CD14/–159 [27]. Much like in the study in Barbados, the T allele of CD14/–159 was protective against atopy at low levels, unrelated to it at intermediate levels, and was a risk factor for allergic sensitization at high levels of exposure. Much like in the study by Gern and colleagues [22], having a dog in the home was protective among carriers of the T allele, but this effect seemed to be independent of the concentration of endotoxin measured in house dust. It is thus likely that having a pet in the home may have effects on allergic sensitization that are only partially explained by the concomitant increased exposure to endotoxin.

The results of the studies by Zambelli-Weiner and colleagues [26] and by Eder and colleagues [27] clearly suggest a nonlinear pattern (of the type illustrated in Fig. 4) for the interaction between CD14/–159 and endotoxin exposure in

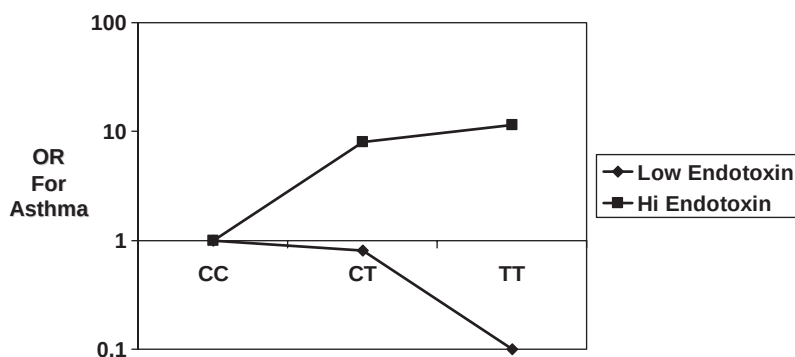


Fig. 5. Association between CD14/–159 and atopic asthma in individuals who have high exposure and low exposure to endotoxin in the island of Barbados. At higher levels of exposure, the T-allele is a risk factor for asthma, whereas at lower levels of exposure, the C-allele is a risk factor. CC, homozygotes for C allele; CT, heterozygotes; OR, odds ratio; TT, homozygotes for T allele. (Data from Zambelli-Weiner A, Ehrlich E, Stockton ML, et al. Evaluation of the CD14/–260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005;115(6):1203–9.)

determining allergy-related phenotypes. The most important consequence of these findings, from the point of view of genetic epidemiology, is that it is not possible to study either of these main effects independent of the other. Because this pattern of gene–environment interaction has rarely been studied in humans, it is currently unknown if it is unique to CD14/–159 or if other genetic variants show similar types of gene–environment interactions. However, a recent report Hoffjan and colleagues [28] strongly suggests that the latter may be true. These investigators studied the association between polymorphisms in immune genes and several early-life immune phenotypes related to atopy, and assessed the way in which these associations were modified by attending day care in early life—an exposure known to protect against the development of atopy [29]. For polymorphisms in three of these genes (those for the IL-4 receptor alpha, nitric oxide synthase-3, and high affinity receptor for IgE), opposite alleles showed association with early-life immune phenotypes depending on whether the child was or was not exposed to day care. These results thus suggest that, much like for CD14/–159, the association between these immune gene polymorphisms and atopy-related phenotypes cannot be studied independently from the day care–related exposures that modify their effects. It remains to be elucidated what role these gene–environment interactions play in the inconsistency between genetic studies of atopy-related phenotypes.

The biology of gene–environment interactions

Although the earlier results appear compelling, the biologic mechanisms that underlie the differential effects of genetic variations in different environments

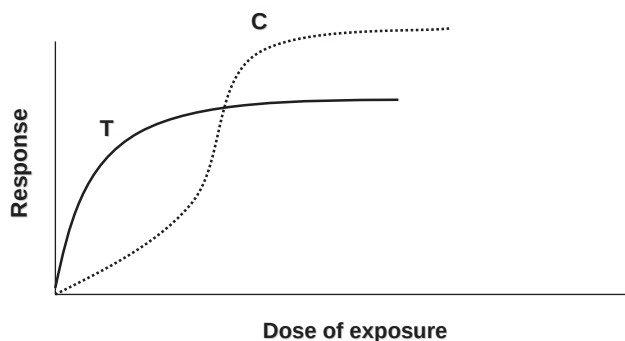


Fig. 6. Hypothetical biologic scenario that could explain the gene-environment interaction observed in Fig. 5. C, C allele; T, T allele.

have not been studied in humans. Recent observations among subjects experimentally exposed to endotoxin may provide a clue (Tricia LeVan, PhD and colleagues, unpublished data, 2005). These studies show that homozygotes for the CD14/−159 T allele had higher basal levels of circulating sCD14 than carriers of the C allele, but showed no significant change in circulating sCD14 levels 24 hours after being exposed to endotoxin by inhalation. Conversely, homozygotes for the CD14/−159 C allele had lower levels of basal sCD14 levels, but showed significant increases in sCD14 after exposure. These results could be explained by a hypothetical different shape in the dose-response curve between carriers of different CD14/−159 alleles. At lower levels of exposure, carriers of the T allele would have higher responses that may reach an early peak (Fig. 6). Beyond a certain dose of endotoxin, carriers of the C allele would have higher responses than carriers of the T allele. In this scenario, responsiveness to endotoxin would still be present in carriers of the C allele at levels of exposure beyond which responses would have already plateaued among homozygotes for the CD14/−159 T allele. Although this hypothetical scenario is plausible, it requires empiric demonstration.

Summary

The example of the complex interactions between environmental exposures and polymorphisms in the CD14 gene in predisposing for allergy-related conditions offers a good indication of the complexity of the mechanisms that determine susceptibility to these conditions. Contrary to what has been the rule for monogenic diseases, the association between genetic variations and polygenic conditions such as asthma and allergies may not always be unidirectional; that is, not always will the same alleles be associated with the conditions under study. Concepts of penetrance of genetic variations that ignore these nonlinear influences (which may affect gene-gene and gene-environment interactions) may

hinder a better understanding of the mechanisms of disease involved, and therefore may delay the development of preventive strategies for these common conditions. Discrepancies between well-designed genetic studies of asthma and allergies, therefore, may be suggesting something fundamental about how these diseases develop and how it will be possible to abolish them in the future.

References

- [1] Duffy DL, Martin NG, Battistutta D, et al. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990;142(6 Pt 1):1351–8.
- [2] Remes ST, Castro-Rodriguez JA, Holberg CJ, et al. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. *J Allergy Clin Immunol* 2001;108(4):509–15.
- [3] Pigliucci M, Schlichting C. Phenotypic evolution: a reaction norm perspective. Sunderland (MA): Sinauer Associates, Inc.; 1998.
- [4] Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361(9360):865–72.
- [5] Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. *Respir Res* 2003;4(1):14.
- [6] Los H, Koppelman GH, Postma DS. The importance of genetic influences in asthma. *Eur Respir J* 1999;14(5):1210–27.
- [7] Brem RB, Storey JD, Whittle J, et al. Genetic interactions between polymorphisms that affect gene expression in yeast. *Nature* 2005;436(7051):701–3.
- [8] Howard TD, Koppelman GH, Xu J, et al. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *Am J Hum Genet* 2002;70(1):230–6.
- [9] Malmstrom K, Rodriguez-Gomez G, Guerra J, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. *Ann Intern Med* 1999;130(6):487–95.
- [10] Israel E, Drazen JM, Liggett SB, et al. The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. *Am J Respir Crit Care Med* 2000;162(1):75–80.
- [11] Drazen JM, Yandava CN, Dube L, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999;22(2):168–70.
- [12] Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299(6710):1259–60.
- [13] Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;354(Suppl 2):S112–5.
- [14] Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347(12):869–77.
- [15] Tulic MK, Wale JL, Holt PG, et al. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;22(5):604–12.
- [16] Ulevitch RJ, Tobias PS. Recognition of endotoxin by cells leading to transmembrane signaling. *Curr Opin Immunol* 1994;6(1):125–30.
- [17] Baldini M, Lohman IC, Halonen M, et al. A polymorphism in the 5'-flanking region of the CD 14 gene is associated with circulating soluble CD14 levels and with total serum IgE. *Am J Respir Cell Mol Biol* 1999;20(5):976–83.
- [18] Koppelman GH, Reijmerink NE, Colin Stine O, et al. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163(4):965–9.
- [19] Kabesch M, Hasemann K, Schickinger V, et al. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. *Allergy* 2004;59(5):520–5.

- [20] Hubacek JA, Rothe G, Pit'ha J, et al. C(−260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999; 99(25):3218–20.
- [21] LeVan TD, Bloom JW, Bailey TJ, et al. A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001;167(10):5838–44.
- [22] Gern JE, Reardon CL, Hoffjan S, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004;113(2):307–14.
- [23] Park JH, Spiegelman DL, Gold DR, et al. Predictors of airborne endotoxin in the home. *Environ Health Perspect* 2001;109(8):859–64.
- [24] Ober C, Tsalenko A, Parry R, et al. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet* 2000;67(5):1154–62.
- [25] Amelung PJ, Weisch DG, Xu J, et al. A polymorphism in CD14 is associated with high IgE levels in a population with laboratory animal allergy. *Am J Respir Crit Care Med* 2000; 161(3):A927.
- [26] Zambelli-Weiner A, Ehrlich E, Stockton ML, et al. Evaluation of the CD14/−260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005;115(6):1203–9.
- [27] Eder W, Klimecki W, Yu L, et al. Opposite effects of CD14/−260 on serum IgE levels in children raised in different environments. *J Allergy Clin Immunol* 2005;116:601–7.
- [28] Hoffjan S, Nicolae D, Ostrovnaya I, et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. *Am J Hum Genet* 2005;76(4):696–704.
- [29] Ball TM, Castro-Rodriguez JA, Griffith KA, et al. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–43.