The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings?

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Summary

The study of the genetics of complex human disease has met with limited success. Many findings with candidate genes fail to replicate despite seemingly overwhelming physiological data implicating the genes. In contrast, animal model studies of the same genes and disease models usually have more consistent results. We propose that one important reason for this is the ability to control genetic background in animal studies. The fact that controlling genetic background can produce more consistent results suggests that the failure to replicate human findings in the same diseases is due to variation in interacting genes. Hence, the contrasting nature of the findings from the different study designs indicates the importance of non-additive genetic effects on human disease. We discuss these issues and some methodological approaches that can detect multilocus effects, using hypertension as a model disease. This article contains supplementary material, which may be viewed at the BioEssays website at http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html. *BioEssays* 26: 170–179, 2004. © 2004 Wiley Periodicals, Inc.

Introduction

The genetic analysis of complex human disease has been significantly less successful than the analysis and dissection of Mendelian diseases. Even when one or a few studies show promising results with either linkage or association, the findings do not consistently replicate across studies.[1,2] For example, of 101 linkage studies reviewed for 31 complex diseases considerably fewer than half found more than suggestive evidence for linkage and, in many diseases, even the significant findings did not replicate across populations.[1] Similarly, in an analysis of over 600 association studies that had positive findings, only 6 of the 166 studies where replication was attempted produced positive associations more than three times.[2] These inconsistencies in genetic studies of complex human disease are quite disturbing and suggest that either there are no genes that can be found or, more likely, that the approaches generally used to assess the genetic predisposition to disease are inadequate.

In contrast to the human genetic studies, animal studies have found genes that consistently produce disease-like phenotypes, and the underlying genetic basis for the phenotypes in these models have often been elucidated. Animal studies frequently reveal significant single-locus effects that can be reproduced across species and/or strains.[3] Such "disease genes" in animal models can be found relatively easily using linkage mapping techniques in crossed inbred lines.[4] Similarly, transgenic animals or genetically manipulated animals can reveal significant effects of candidate alleles in well-defined genetic backgrounds.[5,6] The difference in the approaches and the success rates between studies using animal models and those in humans is problematic, but may help us to identify factors that prevent genetic dissection of complex human disease and ultimately determine important genetic risk factors in common and complex human disease. One possible explanation for this dichotomy that we will focus on in this paper is the role of the underlying genetic architecture of complex traits and how they differ in the two types of studies. For example, the design of animal studies automatically controls many variables that can confound human studies. Specifically, the use of isogenic animal models of complex disease identifies genes with physiological role(s) in phenotypes related to the diseases. In the animal studies, the associations can be functionally elucidated because the animals can be experimentally manipulated and many other variables can be controlled. In contrast, in human studies, it is impossible to

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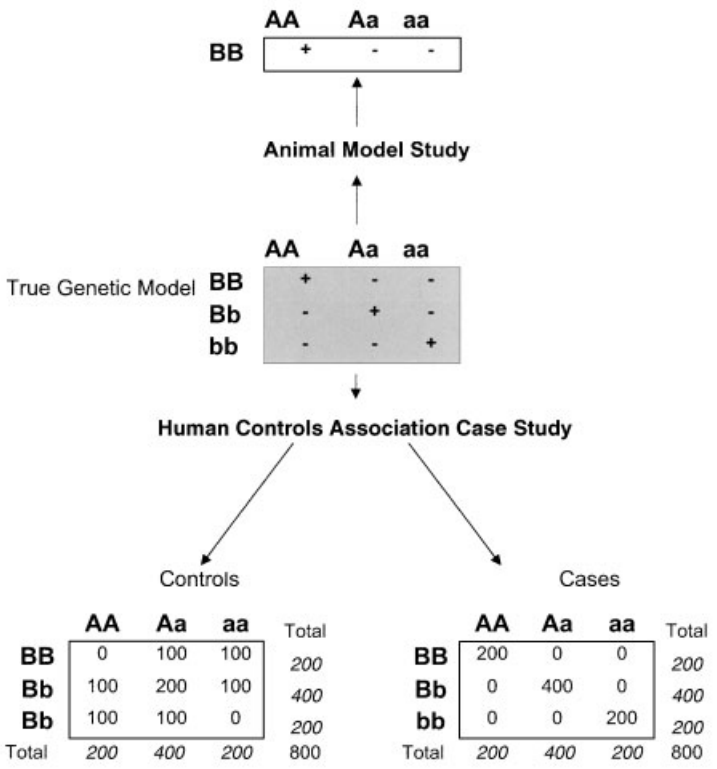

Figure 1. The effects of epistasis on detection of association. In the middle section of the figure, the true 2 locus genetic model is represented in the matrix as either high risk (+) or low risk (−). The matrix indicates that no single locus genotype confers high risk on all genetic backgrounds. The major point regarding the model is that it is a completely interactive model; there are no marginal effects. One way to view the model is that locus A and locus B are both players in a biochemical or signal transduction pathway and the product of these two loci need to be balanced in a very particular way to maintain "normalcy" (non-disease state). Any deviations from this balance increase risk of disease. Therefore, **AABB**, **AaBb** and **aabb** can all be high risk because of the balance of the two products encoded by the respective genotypes. For example, **A** and **B** can be high-throughput alleles, and **a** and **b** low-throughput alleles in a pathway. If they are in the appropriate combinations, then the phenotype is balanced. The effects of this model in both animal and human studies can be seen in the results of two hypothetical studies. The first an animal study (upper section) where the second locus is fixed for variant **B**. In this case a single locus effect will be detected for locus A. In a hypothetical case-control association study (bottom section) where only singe locus effects are examined no effect will be detected for either of the loci, studied independently. The numbers represent the number of subjects of a given genotype who are either controls (left side) or cases (right side) who fall into each genotype class. Note that increased risk is defined as in the true genetic model. There are no single locus genotypes that associate with high risk (Italics in bottom section of the figure for A locus and for B locus), but again 2-locus genotypes do. If this hypothetical scenario represents a true case, then simply increasing sample size would never permit the detection of single locus effects. However, if locus A were fixed for the **a** allele in the study population, a single locus effect at the B locus should be detectable with **bb** associating with increased risk. This may be true even in very small sample sizes.

control for many intrinsic (i.e., genetic) and extrinsic (i.e., environmental) factors. Most important from the perspective of identifying genetic risk factors, in human studies there is likely to be substantial variability in genetic backgrounds within and across study populations, and many of the alleles in these variable backgrounds may have substantial effects on the phenotypes under study. If this reasoning is correct, then it is necessary to incorporate genetic background effects and population genetic variation into any study analyzing genetic predisposition to complex disease because variation at interacting and/or compensating loci may mask the effects of single-locus variation. Simply put, although a given allele may be of physiological significance as demonstrated in the animal studies, variation in allele frequencies of interacting loci can mask an effect in a given study.[7] Therefore, it is possible that studied mutations do not replicate from animal models in human studies because of effects of other, presumably interacting, alleles that have not been considered in a disease study (Fig. 1). Similarly, animal model studies may detect effects that are not the true genetic model because other loci that are important in human populations have been fixed for one of the variants (Fig. 1).

Such a position is certainly substantiated by numerous classical studies identifying the enhancement or suppression of a mutation's effect by variation in a second or third locus for characters such as eye color[8] or bristle shape in *Drosophila*.[9] More recently, the isolation of modifiers has been used extensively in *Drosophila* to dissect developmental and signal

transduction pathways. An excellent example is the genetic dissection of the RAS signal transduction pathway.[10,11]

We reason that the contrast between animal and human studies points to the necessity of multilocus analyses in humans in order to understand the genetic basis of complex human disease. If this is correct, it would mean that animal models are not truly models of human disease where more than one gene predisposes to disease, but rather models of physiological pathways that are factors in human diseases.

Another alternative is that there may be many genes with small effects that cannot be identified in human studies as they are presently designed because these effects are too small to be found, as is discussed in a recent review.[12] In the present review, we will focus our discussion on the role of genetic interaction in the genetic basis of complex disease to elucidate the issues and some potential solutions to the underlying problems in the genetic analyses of complex disease. We will focus on one disease, essential hypertension, but the general principles that we present will be applicable to many others.

The genetic models of rodent hypertension: uncommon consistency but increasing complexity

A substantial number of genes and/or chromosomal regions have been identified in rodent studies as being important in the development of hypertension (see Schork et al. for a comprehensive review[3]). Several investigators, using different strains of animals, have validated many of the positive results, yielding a remarkably consistent pattern. A few such results, regarding the renin–angiotensin system (RAS) are presented in the Table 1. These results may be taken at face value as demonstrating that this ''complex'' disease has a simple genetic basis, since most of the findings have been replicated in several laboratories, using a variety of experimental designs. However, it is unlikely that this is the case for at least three reasons: first, if there were simple genetic susceptibilities to complex disease, it is likely that they would have already been discovered in humans given the substantial effort made in a number of diseases; second, the phenotypes studied in animals are not truly identical to human disease but

Table 1. Results from rodent system for the renin–angiotensin system gene variation and the effects on blood pressure

Gene	Animal	Effects	Design and references
ACE	Rat	+	Linkage[47,48]
	Rat	+	Antisense protection[49]
	Rat	+	Cross-strain association[50]
	Mouse	–[a]	Gene titration[24]
AGT	Mouse	+	Gene titration[6]
	Mouse	+/–[b]	Gene titration[13]
	Mouse	+/–[c]	Human transgene[5,51,52]
	Rat	+/–[d]	Human transgene[19]
	Mice	+/–[c]	Rat transgene[23]
REN	Mouse	+	Genetic ''Clamping''[18]
	Mouse	+/–[c]	Rat transgene[23]
	Mouse	+/–[c]	Human transgene[5]
	Rat	+	Mouse transgene[53–55]
	Rat	+/–[d]	Human transgene[19]
	Rat	+	Linkage[56,57]
	Rat	+	Mouse transgene and modifer mapping[16]
	Rat	+	Mouse transgene[54]
Angiotensin II Receptor, Type 1a			
	Mouse	+	Gene knockout[58–60]
	Rat	+	Drug response[61]
	Rat	+	Antisense[62]
	Rat	+	Gene knockout[62]
	Mouse	+	Gene knockout[63]

[a]It was shown that changes in ACE copy number were compensated for by increased expression of the renin gene, indicating that effects on blood pressure will require environmental or genetic changes in addition to variation in ACE.
[b]Genetic variation was associated with variable expression in several other genes indicating the presence compensation mechanisms, but blood pressure was only associated with AGT genotype in females.
[c]Renin and AGT transgenes or one transgene and an infusion of the second protein. Blood pressure only increased when the action of both gene products was included.
[d]Renin and AGT transgenes were placed into rats and HT only developed in mice with both transgenes.

are limited representations of them. However, the fact that animals may not truly reproduce human disease does not minimize their importance in ultimately elucidating the mechanisms of human disease, but only suggests that there are limitations that we need to consider. Third, there is increasing evidence that when interactions are examined in animal studies they do play an important role. This last finding is likely to be important in understanding genetic risk factors in human disease.

Despite the substantial, although not complete, consistency across studies, there are increasing data that suggest more complex interactions are operating in animal systems when they are looked for.[5,13–17] Even in homogeneous genetic backgrounds varying a single factor, such as the copy number of a gene, can have enormous effect on a variety of parameters, in an interactive way.[13] Kim et al.[13] have demonstrated that as angiotensin gene (AGT) copy number increases not only does the plasma level of this protein increase, but mRNA levels of several other genes change (5 of 10 mRNAs assayed were significantly different among AGT copy number animals). These changes are also affected by gender, in some but not all of the cases. These changes may be viewed as the organism's compensatory response to perturbation. Such evidence may suggest that single genetic changes are insufficient to cause a strong or lasting phenotype.

One way to circumvent the physiological response to a genetic change is to fix or clamp the expression of a transgene of interest, thereby providing an excellent system to study its physiological effects. This has been done with the renin gene in a mouse model and, as expected, a hypertensive phenotype can be produced.[18] Therefore, single locus effects can be found in a system that would normally respond physiologically to maintain homeostasis in a proximal sense.

In studies that have looked explicitly for epistasis in animal models, it is often,[5,14–16,19–21] but not always found.[22] For example, in one of these of hypertension studies, rats transgenic for either the human renin or human angiotensinogen were not hypertensive, but rats carrying both transgenes were severely hypertensive.[19] Similar results have been found in studies of doubly transgenic mice for the human genes[5] as well as mice doubly transgenic for rat renin and angiotensinogen transgenes.[23] These data are supportive of a role of context dependence in the etiology of hypertension, and that, even in animal models, it may be important to have multiple genetic changes in order to change a phenotype such as blood pressure. Such context dependency may also be seen in one of the negative studies. A gene titration study of the angiotensin I-converting enzyme (ACE) gene in mice showed a significant change in ACE plasma levels, but no concordant change in blood pressure. However, the changes in ACE levels were accompanied by significant changes in renin levels, another enzyme in the same physiological pathway.[24] The authors concluded from these findings that there are substantial homeostatic mechanisms that can compensate for variation in one gene and that additional changes either genetic or environmental are required to produce hypertension.

Another factor that may play a role in the reproducibility of animal studies is that the genetic manipulations in animal models are often extreme—gene titration or knockouts may be more severe than one would see in most human populations. In a well-integrated natural genetic system, such as the human genome, one does not expect to find, for example, null alleles, in a gene that plays a critical physiological role. Therefore, despite evidence from model systems that such manipulations affect phenotype, it is unexpected to find this kind of extreme variation in most human studies, although some rare Mendelian forms of hypertension do exist.[25] Hence the effects of any naturally occurring variation are likely to be less clear than in the animal experimental evidence. This is emphasized by the fact that, in most cases, animal studies do not assess the role of naturally occurring variation and its effects on phenotypes. One exception to this is the production of a transgenic mouse with naturally occurring variants in the human G-coupled protein receptor kinase-4 (GRK-4) gene.[26] The putative disease-causing variant produces a hypertensive animal but the wild-type allele does not. These findings suggest a functional role of the specific SNP.

Human studies and the failure to consistently replicate the animal studies

Human studies often do not have the clear and simple findings found in animal studies. Using the renin–angiotensin–system (RAS) as an example, we will document the failure to replicate the positive findings in animal studies, and the failure to replicate positive findings in human studies across populations. At least four human genes have now been studied in multiple populations that have evidence of a role in animal hypertension: angiotensinogen (AGT), ACE, renin (REN), and the angiotensin II type 1 receptor (AT2R1) (Tables 1 and 2; a more extensive version of Table 2 may be viewed at the BioEssays website at http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html). The evidence in human studies is at best inconsistent and at worst meaningless. There is a tendency to fail to replicate across ethnic groups, suggesting different genetic risk factors across populations. However, replication failed even in some cases within an ethnic group (e.g. the Japanese populations for association with AGT variation or Caucasians for REN). Of course, the studies in the same ethnic groups may uncover differences within groups that were previously unknown.

How might these findings be interpreted? One alterative is to simply reject the positive findings as non-representative of true genetic risk factors. This would imply that the positive results are artifactual or type 1 errors and that the data from the animal models is of little or no use in human disease. We find this conclusion unsatisfactory based on the multiple sets of

Table 2. Results of linkage and/or association studies in humans for essential hypertension (a fuller version of table can be viewed at the BioEssays website at http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html)

Gene	Result	Method/Marker[a]	Population(s)
ACE	+	I/D association	Afro-Caribbean[64], African-American[65], Chinese[66], Caucasian[67,68]
	+	SNP linkage/association[c]	African[69]
	−	I/D association	Caucasian[64,70,71], Japanese[72], Afro-Caribbean[73], Chinese[74]
	−	I/D association[d]	Caucasian, African-American[75]
	−	I/D association[e]	African[33], Caucasian[76]
	+/−[f]	Variance components linkage	Caucasian[29]
	+/−[f]	I/D association	Caucasian[30,31]
REN	+	MboI and/or *Bgl*I RFLP association	Caucasian[77,78]
	+	G1051 SNP association	Japanese[79]
	−	*Taq*I/*Hin*fI/*Hin*dIII RFLP haplotype sibpair linkage	Caucasian[80]
	−	*Taq*I/*Hin*fI/*Hin*dIII haplotype association	Caucasian[81]
AGT	+	Association/linkage M235T SNP/STRP	Caucasian[27,82]
	+/−[f]	M235T SNP association	Caucasian[67,83]
	+	Linkage positive; M235T SNP association negative	Afro-Caribbean[84]
	+	M235T/Promoter SNP haplotype association	Japanese[85]
	+	M235T SNP association	Chinese[86]
	+	9-marker haplotype association	Japanese[28]
	+[g]	SNP haplotype association	Chinese[87]
	−/+[h]	M235T SNP association	Caucasian[75]
	−	M235T/T174M SNP transmission disequilibrium test	Chinese[34]
	−	Sibpair linkage of M235T, T174M, 2 dinucleotide repeats	Chinese[88]
	−	M235T; G-6A SNP association	Caucasian[89]
	+/−[i]	M235T/G-6A SNP association and meta-analysis	Japanese[90]
		Sibpair linkage/linkage	Caucasian[91]
	−	Sibpair linkage & SNP association	Caucasian[92]
	−	M235T SNP association	Japanese[93], African-American[75,94,95], Caucasian[67,70,95], Japanese[72], African[33],[e], Caucasian[76],[i]
AT2R1	+[j]	−535T SNP association	Japanese[32]
	+	Linkage and A1166C SNP association	Caucasian[91,96]
	+	A1166C SNP association	Caucasian[67]
	+/−[f]	A1166C SNP association	Caucasian[83]
	+[b]	Regression on A1166C SNP	Caucasian[97]
	−	A1166C SNP association	Caucasian[98], Japanese[99], African[33],[e]

[a]If several polymorphisms were analyzed and one showed a positive association, only that one is given and the results are shown as positive.
[b]There were marginal effects on blood pressure in normal adults and evidence for an interaction between ACE and ATR1 on diastolic blood pressure.
[c]There was no evidence of linkage but association was found for both systolic and diastolic BP using a two-locus additive model for two ACE SNPs.
[d]Although there was no evidence for a marginal effect in any of the cohorts, there was evidence for interaction of the ACE DD genotype with the AGT 235 TT genotype in one of the study samples (Framingham).
[e]Although there were no associations with single variants, there was evidence of an interactions between renin-angiotensin system genes.
[f]Findings are context dependent—either gender, age, or BMI.
[g]There was also evidence for an interaction with the ACE I/D polymorphism.
[h]One Caucasian population sample replicated a second did not; but result may be dependent on other variables and there was evidence for epistasis with the ACE gene in at least one of the study populations.
[i]The case control study was negative, but meta-analysis suggested an association with the M235T polymorphism.
[j]There was also evidence of an interaction with the ACE I/D polymorphism.

positive data in both animal and human studies, and the well-defined physiological pathways through which these genes can act. An alternative is that the genes are in fact risk factors, but that detecting risk is only possible using single gene analysis if the genetic backgrounds in the study populations are conducive to detecting such single gene effects. Since most investigators only test for marginal or single gene effects, then the lack of reproducibility may arise from the effects of single loci that are dependent on the genotypes at unstudied and/or unanalyzed genes.[7]

Another factor to consider is that often the negative findings are for single polymorphisms but do not consider the entire gene. If the positive findings are due to linkage disequilibrium with a functional variant in one population that is in equilibrium in the second population, the association will disappear. Such may be the case for the AGT gene. Caulfield et al.[27] studied

the M235T variant and microsatellites linked to the AGT gene. They found evidence for linkage but none for association with the M235T variant previously found to associate with hypertension. A later study revealed complete linkage disequilibrium between the M235T variant and a −6 variant,[28] indicating that the M235T variant may not be functional and that the linkage relationship of this variant may differ among populations.

Additionally, it is possible that some of the genetic effects of the candidate loci are context dependent, playing a significant role in only males or females or in young vs. old individuals, or in people of a specific body mass index.[29–31] Since these characteristics are not measured or analyzed in many of the studies, it is possible that the failure to replicate is due to interactions between genes and environmental factors as well as to gene–gene interactions. It is obvious that the environments in animal studies are easier to control and therefore there is less environmental variation than in any human association studies. If there are interactions between environmental risk factors and genotypes, then it can be difficult to both detect and replicate genetic associations in human populations.

Clearly, the determination of the genetic risk factors of complex diseases such as hypertension does not have a simple genetic basis. Of course, this is not surprising. However, many investigators continue to collect and analyze data under the assumption that a carefully designed experiment or a large enough sample will succeed in identifying "the gene of major effect." We suggest that as a research agenda this is fatally flawed, and the preponderance of conflicting data supports this view. For example, increasing sample size in order to increase the ability to detect small marginal effects will only work if the genetic effects are primarily additive or multiplicative (Box). If there is true epistasis, then simply increasing sample size will still fail to detect associations (Fig. 1). This can lead to expensive studies that are doomed to fail. We argue that it may be a better strategy to look at more genes and to test for epistasis before going to the expense of increased subject recruitment in association studies.

If our hypothesis is correct, it will be necessary to design experiments with the intention of identifying gene–gene and gene–environment interactions as risk factors. This will not solve all of the problems but we hypothesize that it should help substantially in identifying results that can be replicated. Of course there are still substantial problems with the design of experiments intended to identify such complex interactions, but it is necessary to start such endeavors.

Some data already support the hypothesis that gene–gene interactions will play a role in human hypertension. Cases for epistasis have been made in both the presence and absence of marginal effects. Takahashi et al.[32] and Williams et al.[33] found evidence of interactions in the presence and absence of main effects, respectively. These studies were in two distinct populations: a Japanese and Ghanaian population. In a Chinese population, there was no evidence for similar interactions.[34] Such results point to one problem with candidate gene studies examining epistasis—the failure to study even one critical gene may cause other genes critical to the phenotype to go undetected. This can also be population dependent because variation in allele frequency can cause effects to

Box 1. There are many kinds of gene–gene interactions with different phenotypic implications. Gene–gene interaction may be one of several types. With epistatic interactions, as classically defined, genes interact to distort Mendelian ratios. The effect of this type of interaction is for one or more genes to mask or suppress the effects of other gene or genes. Such a case is illustrated in Fig. 1, where disease risk effects of one locus can be completely hidden by the genotype at a second locus. Alternatively, one or more loci may alter the phenotypic effects of alleles at other loci via changes in magnitude or kind as described below. A clear description of the ways that genotype effects can be masked is presented by Phillips.[100]

Another type of interaction is possible in which each genotype may have a distinct phenotype, but that the phenotype is not the same for all genetic backgrounds. For example, the classic example of agouti coat color in mammals. Gene A encodes hair patterning with allele **A** encoding an agouti pattern and allele **a** solid pattern. Similarly gene B encodes color of coat pigment, with allele **B** coding for black pigment and **b** for brown pigment. In this case, **A**−**/B**− individuals are agouti, **A**−**/bb** are cinnamon, **aa/B**− are black and **aa/bb** are brown. All genotype combinations can be detected (given dominance at each locus), but the phenotypes are not the same if the genotype at the second locus genotype varies.

Multiplicative and additive effects can also occur such that the genotype at a second locus is affected, but that the effects of the first locus effects are detectable. For example, assume gene A affects height in a codominant fashion. Further, allele **A** increases height by x units, and allele **a** by y units and **Aa** by $(x + y)/2$ units. Locus B may modify this effect either additively (B operates similarly to A but its effects are added to the effects of gene A). For example, allele **B** may increase height by z units and allele b by zero units. If these two alleles are codominant **Bb** genotype increases height by z/2 units. If this is the case, both loci affect the ultimate phenotype but they do not mask the effects of each other. A similar effect can be with a multiplicative model such that locus B amplifies the effects of locus A by increase the size change by 1-, 2-, or 3-fold depending on the number of B alleles.

disappear in the analysis.[7] The role of population variation is a critical one for human studies, but much less so for those in animals.

Another potential explanation of the abundance of positive findings in the animal studies but inconsistent results in the human studies may result from publication bias. One may suppose that negative results in animal systems are less likely to be published because it is generally imperative that basic biological findings be positive, whereas it is not yet uncommon to publish negative findings in studies of human populations. The role of this factor is difficult to judge, but cannot be completely disregarded.

Approaches to determining the nature of genetic risk factors in complex disease

If our hypothesis is correct regarding the importance of context in assessing genetic risks of complex disease, then we will need to develop methods that are capable of detecting such interactions.[35] The identification and characterization of genes that increase susceptibility to human disease partially or solely through interactions with other genes is both a statistical and a computational challenge. The statistical challenge is due to the sparseness of the data when multiple genetic risk factors are considered simultaneously. This curse of dimensionality[36] can lead to an increase in type I errors with methods such as logistic regression due to parameter estimates that have very large standard errors.[37,38] Further, stepwise model-fitting procedures can lead to more type II errors and a decrease in power because interaction effects are only tested for those variables that have an independent main effect. Those polymorphisms that have an interaction effect but not a main effect will be missed.

The computational challenge is due to the size and complexity of the search space. As the number of candidate gene polymorphisms to be evaluated increases, the number of possible interaction terms involving those polymorphisms increases exponentially. For example, with just 100 polymorphisms there are $1.27 * 10^{30}$ possible combinations to evaluate. Carrying out an exhaustive search of all these combinations is not computationally feasible. The alternative is to carry out a machine learning search using a strategy such as genetic algorithms.[39] However, these strategies do not guarantee that an optimal solution will be found and may be no better than a random search when the polymorphisms have no independent main effects that are above the background noise associated with nonfunctional polymorphisms.

New analytical strategies to address these challenges need to be and are being developed for studies of human disease susceptibility in epidemiological study designs.[40] For example, the multifactor dimensionality reduction (MDR) method was recently developed specifically to address the limitations of logistic regression that result from the curse of dimensionality.[41] The MDR method is nonparametric and does not assume any genetic model. Simulation studies suggest that it has good power to detect gene–gene interactions in reasonable sample sizes even in the presence of genotyping error and/or missing genotypes.[42] Application of MDR to case-control data ($n = 400$) for sporadic breast cancer identified a statistically significant four-locus interaction among estrogen metabolism genes that would not have been possible using logistic regression.[42] This method and others[43–46] show promise for improving our ability to detect gene–gene interactions over that provided by traditional parametric statistical approaches. These methods will have to be further developed and expanded in order to approach the higher levels of reproducibility and phenotype prediction that is necessary in genetic studies of complex disease.

Conclusions

Results of complex disease studies in animal models are often substantially different from that in human studies. We have proposed that this is due to underlying structures of the study designs and that, when examined in appropriate ways, the complexity of the systems are comparable (Fig. 1). This latter point implies that there is a great need to directly address the role of multilocus genetics in the study of complex disease. In this review, we list a few methods that can do this and have had success in identifying genetic effects when simpler approaches have not.

Additionally, it is important to fully utilize the natural experiments that human population based studies describe to gain a better understanding of the underlying biological interactions. In fact, it would be impossible a priori to design the best experiments that incorporate multiple genetic factors. There are simply too many multilocus combinations possible to determine which is the best one(s). However, if we use results from human multilocus studies, we can narrow the potential field of functional interactions significantly.

Of course, it is possible that results from animal systems cannot be applied to humans because of differences in biology, but this would stand in contrast to numerous findings that have successfully used gene conservation to define functional regions of the genome and to identify candidates for diseases with simpler genetic risks, i.e., single locus or Mendelian diseases. We suggest that the role of animal models to define good candidates is still well suited to the study of complex disease. Perhaps even more important is the fact that we can use human studies to define complex interactions, using the methods that we have mentioned and that these new hypotheses can be tested for functional interactions in animal systems.

References

1. Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M. Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet 2001;69:936–950.
2. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002;4:45–61.

3. Schork NJ, Nath SP, Lindpaintner K, Jacob HJ. Extensions to quantitative trait locus mapping in experimental organisms. Hypertension 1996;28:1104–1111.
4. Kreutz R, Hubner N. Congenic rat strains are important tools for the genetic dissection of essential hypertension. Semin Nephrol 2002; 22:135–147.
5. Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD. Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. J Clin Invest 1996;97:1047–1055.
6. Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, Jennette JC, Coffman TM, Maeda N, Smithies O. Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci USA 1995;92:2735–2739.
7. Templeton AR. Epistasis and complex traits. In: Wolf JB, Brodie ED III, Wade MJ, editors. Epistasis and the Evolutionary Process. New York, NY: Oxford University Press; 2000. 41–57.
8. Bridges CB. Specific modifiers of eosin eye color in Drosophila melanogaster. J Exp Zool 1919;28:337–384.
9. Lancefield DE. An autosomal brislte modifier, affecting a sex-linked character. Am Nat 1918;52:462–464.
10. Rubin GM, Chang HC, Karim F, Laverty T, Michaud NR, Morrison DK, Rebay I, Tang A, Therrien M, Wassarman DA. Signal transduction downstream from RAS in Drosophila. Cold Spring Harbor. Symp Quant Biol 1997;62:347–352.
11. Therrien M, Morrison DK, Wong AM, Rubin GM. A genetic screen for modifiers of a kinase suppressor of ras-dependent rough eye phenotype in Drosophila. Genetics 2000;156:1231–1242.
12. Smithies O, Kim HS, Takahashi N, Edgell MH. Importance of quantitative genetic variations in the etiology of hypertension. Kidney Int 2000;58: 2265–2280.
13. Kim HS, Lee G, John SW, Maeda N, Smithies O. Molecular phenotyping for analyzing subtle genetic effects in mice: application to an angiotensinogen gene titration. Proc Natl Acad Sci USA 2002;99:4602–4607.
14. Rapp JP, Garrett MR, Deng AY. Construction of a double congenic strain to prove an epistatic interaction on blood pressure between rat chromosomes 2 and 10. J Clin Invest 1998;101:1591–1595.
15. Ohno Y, Tanase H, Nabika T, Otsuka K, Sasaki T, Suzawa T, Morii T, Yamori Y, Saruta T. Selective genotyping with epistasis can be utilized for a major quantitative trait locus mapping in hypertension in rats. Genetics 2000;155:785–792.
16. Kantachuvesiri S, Haley CS, Fleming S, Kurian K, Whitworth CE, Wenham P, Kotelevtsev Y, Mullins JJ. Genetic mapping of modifier loci affecting malignant hypertension in TGRmRen2 rats. Kidney Int 1999;56:414–420.
17. Tremblay J, et al. TA repeat variation, Npr1 expression, and blood pressure: impact of the Ace locus. Hypertension 2003;41:16–24.
18. Caron KM, James LR, Kim HS, Morham SG, Sequeira Lopez ML, Gomez RA, Reudelhuber TL, Smithies O. A genetically clamped renin transgene for the induction of hypertension. Proc Natl Acad Sci USA 2002;99: 8248–8252.
19. Bohlender J, Fukamizu A, Lippoldt A, Nomura T, Dietz R, Menard J, Murakami K, Luft FC, Ganten D. High human renin hypertension in transgenic rats. Hypertension 1997;29:428–434.
20. Garrett MR, Rapp JP. Two closely linked interactive blood pressure QTL on rat chromosome 5 defined using congenic Dahl rats. Physiol Genomics 2002;8:81–86.
21. Schlager G, Chao CS. The role of dominance and epistasis in the genetic control of blood pressure in rodent models of hypertension. Clin Exp Hypertens 1991;13:947–953.
22. Cordell HJ, Todd JA, Hill NJ, Lord CJ, Lyons PA, Peterson LB, Wicker LS, Clayton DG. Statistical modeling of interlocus interactions in a complex disease. Rejection of the multiplicative model of epistasis in type 1 diabetes. Genetics 2001;158:357–367.
23. Ohkubo H, et al. Generation of transgenic mice with elevated blood pressure by introduction of the rat renin and angiotensinogen genes. Proc Natl Acad Sci USA 1990;87:5153–5157.
24. Krege JH, Kim HS, Moyer JS, Jennette JC, Peng L, Hiller SK, Smithies O. Angiotensin-converting enzyme gene mutations, blood pressures, and cardiovascular homeostasis. Hypertension 1997;29:150–157.
25. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell 2001;104:545–556.
26. Felder RA, et al. G protein-coupled receptor kinase 4 gene variants in human essential hypertension. Proc Natl Acad Sci USA 2002;99:3872–3877.
27. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P, Clark AJ. Linkage of the angiotensinogen gene to essential hypertension. N Engl J Med 1994;330:1629–1633.
28. Sato N, et al. Nine polymorphisms of angiotensinogen gene in the susceptibility to essential hypertension. Life Sci 2000;68:259–272.
29. Fornage M, Amos CI, Kardia S, Sing CF, Turner ST, Boerwinkle E. Variation in the region of the angiotensin-converting enzyme gene influences interindividual differences in blood pressure levels in young white males. Circulation 1998;97:1773–1779.
30. Turner ST, Boerwinkle E, Sing CF. Context-dependent associations of the ACE I/D polymorphism with blood pressure. Hypertension 1999;34: 773–778.
31. O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. Circulation 1998;97:1766–1772.
32. Takahashi N, Murakami H, Kodama K, Kasagi F, Yamada M, Nishishita T, Inagami T. Association of a polymorphism at the 5′-region of the angiotensin II type 1 receptor with hypertension. Ann Hum Genet 2000; 64:197–205.
33. Williams SM, et al. Combinations of variations in multiple genes are associated with hypertension. Hypertension 2000;36:2–6.
34. Niu T, et al. Angiotensinogen gene polymorphisms M235T/T174M: no excess transmission to hypertensive Chinese. Hypertension 1999;33: 698–702.
35. Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Human Heredity 2003;56:73–82.
36. Bellman RE. Adaptive control processes: a guided tour. Princeton, NJ: Princeton University Press; 1961.
37. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996;49:1373–1379.
38. Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley; 2000.
39. Goldberg DE. Genetic algorithms in search, optimization, and machine learning. Reading, Mass: Addison-Wesley Pub Co; 1989.
40. Moore JH, Williams SM. New strategies for identifying gene-gene interactions in hypertension. Ann Med 2002;34:88–95.
41. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, Moore JH. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet 2001;69:138–147.
42. Ritchie MD, Hahn LW, Moore JH. Power of multifactor dimensionality reduction for detecting gene-gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. Genet Epidemiol 2003;24:150–157.
43. Zee RY, et al. Multi-locus interactions predict risk for post-PTCA restenosis: an approach to the genetic analysis of common complex disease. Pharmacogenomics J 2002;2:197–201.
44. Moore JH, Hahn LW. A cellular automata approach to detecting interactions among single-nucleotide polymorphisms in complex multifactorial diseases. Pac Symp Biocomput 2002; 53–64.
45. Moore JH, Hahn LW. Cellular automata and genetic algorithms for parallel problem solving in human genetics. In: Merelo JJ, Panagiotis A, Beyer H-G, editors. Lecture Notes in Computer Science. Berlin: Springer-Verlag; 2002; 821–830.
46. Nelson MR, Kardia SLR, Ferrell RE, Sing CF. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. Genome Research 2001;11:458–470.
47. Hilbert P, et al. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. Nature 1991;353:521–529.
48. Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES. Genetic mapping of a gene causing

hypertension in the stroke-prone spontaneously hypertensive rat. Cell 1991;67:213–224.
49. Wang H, Reaves PY, Gardon ML, Keene K, Goldberg DS, Gelband CH, Katovich MJ, Raizada MK. Angiotensin I-converting enzyme antisense gene therapy causes permanent antihypertensive effects in the SHR. Hypertension 2000;35:202–208.
50. Ocaranza MP, Piddo AM, Faundez P, Lavandero S, Jalil JE. Angiotensin I-converting enzyme gene polymorphism influences chronic hypertensive response in the rat Goldblatt model. J Hypertens 2002;20:413–420.
51. Cvetkovic B, Keen HL, Zhang X, Davis D, Yang B, Sigmund CD. Physiological significance of two common haplotypes of human angiotensinogen using gene targeting in the mouse. Physiol Genomics 2002;11:253–262.
52. Morimoto S, Cassell MD, Beltz TG, Johnson AK, Davisson RL, Sigmund CD. Elevated blood pressure in transgenic mice with brain-specific expression of human angiotensinogen driven by the glial fibrillary acidic protein promoter. Circ Res 2001;89:365–372.
53. Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. Nature 1990;344:541–544.
54. Lee MA, Bohm M, Paul M, Bader M, Ganten U, Ganten D. Physiological characterization of the hypertensive transgenic rat TGR(mREN2)27. Am J Physiol 1996;270:E919–E929.
55. Langheinrich M, Lee MA, Bohm M, Pinto YM, Ganten D, Paul M. The hypertensive Ren-2 transgenic rat TGR (mREN2)27 in hypertension research. Characteristics and functional aspects. Am J Hypertens 1996;9:506–512.
56. Rapp JP, Wang SM, Dene H. A genetic polymorphism in the renin gene of Dahl rats cosegregates with blood pressure. Science 1989;243:542–544.
57. Kurtz TW, Simonet L, Kabra PM, Wolfe S, Chan L, Hjelle BL. Cosegregation of the renin allele of the spontaneously hypertensive rat with an increase in blood pressure. J Clin Invest 1990;85:1328–1332.
58. Oliverio MI, Best CF, Smithies O, Coffman TM. Regulation of sodium balance and blood pressure by the AT(1A) receptor for angiotensin II. Hypertension 2000;35:550–554.
59. Cervenka L, Horacek V, Vaneckova I, Hubacek JA, Oliverio MI, Coffman TM, Navar LG. Essential role of AT1A receptor in the development of 2K1C hypertension. Hypertension 2002;40:735–741.
60. Ito M, Oliverio MI, Mannon PJ, Best CF, Maeda N, Smithies O, Coffman TM. Regulation of blood pressure by the type 1A angiotensin II receptor gene. Proc Natl Acad Sci USA 1995;92:3521–3525.
61. Ito S, Komatsu K, Tsukamoto K, Kanmatsuse K, Sved AF. Ventrolateral medulla AT1 receptors support blood pressure in hypertensive rats. Hypertension 2002;40:552–559.
62. Galli SM, Phillips MI. Angiotensin II AT(1A) receptor antisense lowers blood pressure in acute 2-kidney, 1-clip hypertension. Hypertension 2001;38:674–678.
63. Oliverio MI, et al. Reduced growth, abnormal kidney structure, and type 2 (AT2) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT1A and AT1B receptors for angiotensin II. Proc Natl Acad Sci USA 1998;95:15496–15501.
64. Barley J, Blackwood A, Miller M, Markandu ND, Carter ND, Jeffery S, Cappuccio FP, MacGregor GA, Sagnella GA. Angiotensin converting enzyme gene I/D polymorphism, blood pressure and the renin-angiotensin system in Caucasian and Afro-Caribbean peoples. J Hum Hypertens 1996;10:31–35.
65. Duru K, Farrow S, Wang JM, Lockette W, Kurtz T. Frequency of a deletion polymorphism in the gene for angiotensin converting enzyme is increased in African-Americans with hypertension. Am J Hypertens 1994;7:759–762.
66. Jeng JR, Harn HJ, Jeng CY, Yueh KC, Shieh SM. Angiotensin I converting enzyme gene polymorphism in Chinese patients with hypertension. Am J Hypertens 1997;10:558–561.
67. Hingorani AD, Jia H, Stevens PA, Hopper R, Dickerson JE, Brown MJ. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. J Hypertens 1995;13:1602–1609.
68. Zee RY, Lou YK, Griffiths LR, Morris BJ. Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. Biochem Biophys Res Commun 1992;184:9–15.
69. Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, Luke A, Chen G, Elston RC, Ward R. Linkage and association analysis of angiotensin i-converting enzyme (ace)-gene polymorphisms with ace concentration and blood pressure. Am J Hum Genet 2001;68:1139–1148.
70. Mondorf UF, Russ A, Wiesemann A, Herrero M, Oremek G, Lenz T. Contribution of angiotensin I converting enzyme gene polymorphism and angiotensinogen gene polymorphism to blood pressure regulation in essential hypertension. Am J Hypertens 1998;11:174–183.
71. Schmidt S, van Hooft IM, Grobbee DE, Ganten D, Ritz E. Polymorphism of the angiotensin I converting enzyme gene is apparently not related to high blood pressure: Dutch Hypertension and Offspring Study. J Hypertens 1993;11:345–348.
72. Kato N, Sugiyama T, Morita H, Kurihara H, Furukawa T, Isshiki T, Sato T, Yamori Y, Yazaki Y. Comprehensive analysis of the renin-angiotensin gene polymorphisms with relation to hypertension in the Japanese. J Hypertens 2000;18:1025–1032.
73. Forrester T, McFarlane-Anderson N, Bennett FI, Wilks R, Cooper R, Rotimi C, Morrison L, Ward R. The angiotensin converting enzyme and blood pressure in Jamaicans. Am J Hypertens 1997;10:519–524.
74. Chiang FT, Lai ZP, Chern TH, Tseng CD, Hsu KL, Lo HM, Tseng YZ. Lack of association of the angiotensin converting enzyme polymorphism with essential hypertension in a Chinese population. Am J Hypertens 1997;10:197–201.
75. Borecki IB, Province MA, Ludwig EH, Ellison RC, Folsom AR, Heiss G, Lalouel JM, Higgins M, Rao DC. Associations of candidate loci angiotensinogen and angiotensin-converting enzyme with severe hypertension: The NHLBI Family Heart Study. Ann Epidemiol 1997;7:13–21.
76. Vasku A, et al. Angiotensin I-converting enzyme and angiotensinogen gene interaction and prediction of essential hypertension. Kidney Int 1998;53:1479–1482.
77. Frossard PM, Lestringant GG, Elshahat YI, John A, Obineche EN. An MboI two-allele polymorphism may implicate the human renin gene in primary hypertension. Hypertens Res 1998;21:221–225.
78. Frossard PM, Malloy MJ, Lestringant GG, Kane JP. Haplotypes of the human renin gene associated with essential hypertension and stroke. J Hum Hypertens 2001;15:49–55.
79. Hasimu B, Nakayama T, Mizutani Y, Izumi Y, Asai S, Soma M, Kokubun S, Ozawa Y. Haplotype analysis of the human renin gene and essential hypertension. Hypertension 2003;41:308–312.
80. Jeunemaitre X, Rigat B, Charru A, Houot AM, Soubrier F, Corvol P. Sib pair linkage analysis of renin gene haplotypes in human essential hypertension. Hum Genet 1992;88:301–306.
81. Soubrier F, Jeunemaitre X, Rigat B, Houot AM, Cambien F, Corvol P. Similar frequencies of renin gene restriction fragment length polymorphisms in hypertensive and normotensive subjects. Hypertension 1990;16:712–717.
82. Jeunemaitre X, et al. Molecular basis of human hypertension: role of angiotensinogen. Cell 1992;71:169–180.
83. Tiret L, et al. Gene polymorphisms of the renin-angiotensin system in relation to hypertension and parental history of myocardial infarction and stroke: the PEGASE study. Projet d'Etude des Genes de l'Hypertension Arterielle Severe a moderee Essentielle. J Hypertens 1998;16:37–44.
84. Caulfield M, Lavender P, Newell-Price J, Farrall M, Kamdar S, Daniel H, Lawson M, De Freitas P, Fogarty P, Clark AJ. Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. J Clin Invest 1995;96:687–692.
85. Sato N, Katsuya T, Rakugi H, Takami S, Nakata Y, Miki T, Higaki J, Ogihara T. Association of variants in critical core promoter element of angiotensinogen gene with increased risk of essential hypertension in Japanese. Hypertension 1997;30:321–325.
86. Chiang FT, Hsu KL, Tseng CD, Hsiao WH, Lo HM, Chern TH, Tseng YZ. Molecular variant M235T of the angiotensinogen gene is associated with essential hypertension in Taiwanese. J Hypertens 1997;15:607–611.
87. Tsai CT, Fallin D, Chiang FT, Hwang JJ, Lai LP, Hsu KL, Tseng CD, Liau CS, Tseng YZ. Angiotensinogen gene haplotype and hypertension: interaction with ACE gene I allele. Hypertension 2003;41:9–15.
88. Niu T, et al. Angiotensinogen gene and hypertension in Chinese. J Clin Invest 1998;101:188–194.

89. Rodriguez-Perez JC, Rodriguez-Esparragon FJ, Hernandez-Perera O, Fiuza-Perez MD, Anabitarte-Prieto A, Losada-Cabrera A. Effects of the angiotensinogen gene M235T and A(-6)G variants on blood pressure and other vascular risk factors in a Spanish population. J Hum Hypertens 2000;14:789–793.
90. Kato N, Sugiyama T, Morita H, Kurihara H, Yamori Y, Yazaki Y. Angiotensinogen gene and essential hypertension in the Japanese: extensive association study and meta-analysis on six reported studies. J Hypertens 1999;17:757–763.
91. Kainulainen K, Perola M, Terwilliger J, Kaprio J, Koskenvuo M, Syvanen AC, Vartiainen E, Peltonen L, Kontula K. Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension.PG-844–9. Hypertension 1999;33:844–849.
92. Wang WY, Glenn CL, Zhang W, Benjafield AV, Nyholt DR, Morris BJ. Exclusion of angiotensinogen gene in molecular basis of human hypertension: sibpair linkage and association analyses in Australian anglo-caucasians. Am J Med Genet 1999;87:53–60.
93. Kishimoto T, Suyama A, Igarashi A, Osaki Y, Okamoto M, Yamamoto T, Nanba E, Kurosawa Y, Fukumoto S. Angiotensinogen gene variation and hypertension in a cohort study in Japanese. J Epidemiol 2001;11:115–119.
94. Rotimi C, Morrison L, Cooper R, Oyejide C, Effiong E, Ladipo M, Osotemihen B, Ward R. Angiotensinogen gene in human hypertension. Lack of an association of the 235T allele among African Americans. Hypertension 1994;24:591–594.
95. Barley J, Blackwood A, Sagnella G, Markandu N, MacGregor G, Carter N. Angiotensinogen Met235 → Thr polymorphism in a London normotensive and hypertensive black and white population. J Hum Hypertens 1994;8:639–640.
96. Bonnardeaux A, Davies E, Jeunemaitre X, Fery I, Charru A, Clauser E, Tiret L, Cambien F, Corvol P, Soubrier F. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension 1994;24:63–69.
97. Henskens LH, Spiering W, Stoffers HE, Soomers FL, Vlietinck RF, De Leeuw PW, Kroon AA. Effects of ACE I/D and AT1R-A1166C polymorphisms on blood pressure in a healthy normotensive primary care population: first results of the Hippocates study. J Hypertens 2003;21: 81–86.
98. Schmidt S, Beige J, Walla-Friedel M, Michel MC, Sharma AM, Ritz E. A polymorphism in the gene for the angiotensin II type 1 receptor is not associated with hypertension. J Hypertens 1997;15:1385–1388.
99. Takami S, Katsuya T, Rakugi H, Sato N, Nakata Y, Kamitani A, Miki T, Higaki J, Ogihara T. Angiotensin II type 1 receptor gene polymorphism is associated with increase of left ventricular mass but not with hypertension. Am J Hypertens 1998;11:316–321.
100. Phillips PC. The language of gene interaction. Genetics 1998;149:1167–1171.