The problem of proper "controls" has been a topic of central interest to researchers in clinical and experimental hypertension for many years. In this issue of *Hypertension*, two groups of investigators examine this question using DNA typing techniques\(^1\),\(^2\) with regard to the comparability of commonly used genetically hypertensive rat strains and their normotensive controls. Confirming earlier data\(^3\) in a more quantitative fashion, the authors point out widespread sequence dissimilarities between sets of hypertensive and control strains. Because of their potential impact on almost any aspect of biomedical research, these reports deserve critical examination of their true implications and relevance.

The question of what constitutes adequate and appropriate experimental controls is obviously one of very general importance and applies to many if not all branches of science. Particularly in biological and medical research, where the aim is to study a specific phenomenon superimposed on a very complex background, it has always been viewed as crucial to design experiments such that the effects of this background are minimized and only the property of interest is compared between cases and controls. The characteristic under investigation, likewise, needs to be well defined to ensure that the same phenomenon is studied in all individuals. Conventional strategies to achieve this goal call for experimental protocols in which, ideally, all variables except for the one under investigation, control strains. Because of their potential impact on almost any aspect of biomedical research, these reports deserve critical examination of their true implications and relevance.

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The quest for representative controls in comparative studies

Two classes of variables determine the phenotype of every individual: genetic factors and environmental influences. For a particular property, either may exert the sole or predominant effect; both classes of factors may act independently; or there may be an interaction between certain genes and certain environmental factors, the nature of which can range from relatively simple to exceedingly complex. When environmental influences are kept constant, which is to some extent feasible under laboratory conditions, the remaining differences between individuals are attributable to the genetic variability, which is innate to all but deliberately inbred populations. This variability obviously extends to all the individual's features (i.e., to both background characteristics and the property of interest). To obtain meaningful results, the individuals selected for the experimental sample must be representative of a well-defined study population. Depending on the experimental question to be answered, one may be able to avoid selecting a separate control group. If the response to an experimental (i.e., environmental) intervention is to be measured, such as in studies aimed at elucidating basic physiological mechanisms or in drug studies, each case can serve as its own control, using a before–after study design. Statistical power in such studies will depend on the appropriateness of the sample selection and on the robustness of the intervention effect vis-à-vis any inhomogeneity of the sample group. Frequently, however, an individual cannot serve as both subject and control. In particular, this is not feasible when one wishes to elucidate information about an existing, inborn characteristic that distinguishes a particular population, such as families with primary hypertension. Then, a second group of individuals must be selected to serve as the reference or control. Under those circumstances careful selection of both cases and controls becomes crucial. The use of stringent selection criteria for the phenomenon under investigation, combined with random sampling techniques to reduce background noise, are generally used to achieve the closest approximation of ideally comparable groups available to the clinical researcher.

Inbred Strains: Interindividual, Not Interstrain, Homogeneity Is Key

It is for this reason that laboratory investigators began using inbred, genetically homogeneous strains of animals. By avoiding the complexities of mixed genetic makeup characteristic of human populations and outbred animal strains, these models allow the development of data bases that will eventually be applicable to guide human studies. The key to the usefulness of inbred animal strains, provided environmental factors are tightly controlled, is the lack of interindividual variation regarding both the property under investiga-
tion and the genetic background. This feature provides the basis for the high degree of statistical power that distinguishes experimentation in inbred strains from studies in genetically heterogeneous populations. Historically, the possibility to compare two inbred strains that differ only with respect to a particular feature of interest, a hereditary trait associated with a particular phenotype, has been viewed as an especially attractive and important aspect of work with inbred strains. Contrary to this still widely held notion, however, experimentation with inbred strains does not depend on the availability of such control strains. The true strength of studies in inbred strains, in fact, relates to the potential of generating genetically well-defined hybrid cohorts, which provide the basis for cosegregation analyses, which then serve as a powerful alternative to matched control strains.

Ideally matched control strains, although theoretically useful, are extremely difficult to obtain for very obvious reasons: An inbred strain displaying a particular phenotype is usually created by selecting individuals with this phenotype from an inhomogeneous (outbred) population (which provides the necessary phenotype variation, especially in the case of polygenic disorders) and mating them, followed by subsequent similar cycles of selective breeding. Once the trait of interest has become sufficiently well established, brother-sister mating is performed to achieve genetic homogeneity by fixing all genes in the homozygous state. As a consequence of this strategy, a host of other genetic characteristics (and, of course, their phenotype correlates) that happened to be present in the animals originally selected become fixed in the disease-carrying inbred strain. Thus, inbred disease strains will differ from their control strain (created analogously by selecting for the absence of the disease phenotype) in more than just the disease-specific genotype and phenotype. Exceptions are transgenic animals, in which a known but as yet often imprecisely defined (e.g., with respect to incorporation site) genetic alteration has been superimposed on an identical genetic background; the theoretical case in which a spontaneous mutation arising in an inbred strain creates a disease model; or carefully bred congenic strains (which, once again, are derived from two inbred strains differing by more than the disease characteristic). In the case of genetically hypertensive animal strains and their normotensive control strains, which were generated by just such selective breeding approaches, a multitude of genetic differences, many more than the few which confer the blood pressure phenotype, must thus be expected to be present. This well-acknowledged fact, which applies to all experimentation in similarly derived inbred disease-model strains, has indeed previously been demonstrated by minisatellite typing (fingerprinting) of genomic DNA where multiple polymorphic bands, many more than would be expected to account for the hypertensive trait, were observed between Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). The papers by St. Lezin and colleagues, and by Johnson and coworkers, present additional, quantitative data for the degree of genetic difference between hypertensive rats and their commonly used reference strains. Although the two papers are not directly comparable (one uses hypervariable regions, the other restriction endonuclease recognition sequences as a measure of genetic divergence) and although different degrees of genetic distance appear to be present between different sets of hypertensive and control strains, both studies in essence render further confirmatory evidence that the genetic differences between inbred hypertensive and normotensive strains are indeed substantial. These studies should convince the last renegade investigators that simple phenomenological experiments comparing disease and control strains are of little if any value.

**Matched Control Strains Are Not Essential**

Every single one of the genetic differences present between disease and control strains represents a polymorphic marker that distinguishes with equal statistical power between strains, yet only a tiny fraction, presenting disease-related genes, are of interest to the investigator. Taking hypertension in SHR as an example, only an estimated three to six loci will purportedly be polymorphic sites will represent blood pressure–relevant markers. This implies that differences observed on any level (physiological, biochemical, or molecular genetic) between the hypertensive and the normotensive strains are, in fact, much more likely unrelated than related to hypertension. The long-appreciated problem of widespread genetic heterogeneity between normotensive and hypertensive strains and the task at hand, identifying those polymorphisms (i.e., genes) that in fact are contributing to the disease process, led geneticists many years ago to propose, as a seminal test for a polymorphic marker’s relevance to the hypertensive phenotype, that the marker must show cosegregation with blood pressure in a freely segregating F2 cohort bred from the two parental strains. This approach effectively filters out all polymorphic markers except the ones that are relevant to the phenotype of interest. By taking this strategy a step further, one can, in fact, make use of the large number of polymorphisms that distinguish two strains and test for cosegregation with randomly selected markers, thereby examining at a multitude of loci dispersed across the genome. Thus, the very property that precludes perfectly matched controls serves as a powerful instrument to effectively screen the genome for new, previously unknown disease-relevant genes. Therefore, if proper cosegregation studies are performed, the question of “appropriate control” strains is indeed all but relegated to a nonissue: One can take any two genetically homogeneous strains that differ in a phenotype of interest (e.g., blood pressure) and breed an F2 cohort. As long as precise quantification of intermediate phenotypes is assured and provided a large enough number of animals and markers is screened, one will, by default, find loci contributing to the phenotype difference between the particular strains examined. Each of these loci identifies a chromosomal region (which may be quite large) within which the causative gene is located and where it may ultimately be characterized by using complementing molecular genetic and mapping approaches. Several such cosegregation studies in hypertension, examining both polymorphic markers representing certain candidate genes and random (tandem repeat) polymorphisms, have been published over the last few years documenting the validity and feasibility of this approach. Thus, the renin gene locus (i.e., the renin gene or another closely linked...
gene) has been found to be linked to hypertension in the Dahl strains of sodium-sensitive and sodium-resistant rats, and loci on chromosomes 10, 18, and X have been found to contribute to blood pressure variance between WKY and stroke-prone SHR. Efforts are currently underway in a number of laboratories to conduct similar experiments in additional strains of hypertensive and normotensive animals and in the same strains previously tested, but examining different candidate genes or chromosomal regions.

A Call for International Coordination

This raises an issue that may prove to be considerably more important than the nonspecific genetic heterogeneity between disease and control strains: To compare information provided by different investigators and to synthesize individual contributions into a larger picture, a certain standardization of investigational approaches, which will also assure reproducibility of experimental results, will become crucial. This relates to both environmental and genetic variables. As Kurtz et al previously have shown, there exists genetic heterogeneity between WKY rats from different sources, in one case even from the same supplier. Since we do not know if this heterogeneity affects blood pressure--relevant markers, it is from an experimental standpoint of far greater concern than the heterogeneity between SHR and WKY rats, which we know how to deal with. Similarly, inconsistencies in environmental factors between individual experiments may lead to controversial results, particularly when examining a trait such as hypertension that shows extremely complex multifactorial modulation patterns. Add to this the problem of quantifying a trait that is represented by a continuous rather than a discrete variable, and in particular the controversies regarding the appropriate method of measuring blood pressure in rats, and it is easy to anticipate the emergence of a Babylon of irreconcilable data. As research laboratories around the world are gearing up to pursue cosegregation and mapping studies, some measure of coordination will become essential to achieve long-term goals. Thus, by taking great care to characterize precisely and as far as possible to standardize the genetic and environmental variables introduced into a given experiment, we may greatly enhance the impact of each individual study by being able to put it into context with others. Only this kind of approach will eventually yield truly comprehensive information about polygenic, multifactorial diseases of such complexity as hypertension. The time is ripe for an internationally coordinated program to study the rat genome, which could provide the framework for this kind of concerted effort in rat models of genetic hypertension. The prospect of using experimental data from rat models to study directly analogous paradigms in humans emphasizes the importance of scientific coordination in this area. Such an approach, as applied to this and other disease states, holds promise for bridging the gap between experimental and clinical medicine on many fronts and circumvents the problem of controls on yet a different level.

Modern molecular genetics has finally provided us with the tools needed to tackle the pathogenetic background of primary hypertension. In this new era of molecular medicine, it is our responsibility to use these powerful methods wisely and efficiently to expand our knowledge rather than to create more confusion.

References

Genetic variation in hypertensive and 'control' strains. What are we controlling for anyway?

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