Physiol. Res. 43: 207–208, 1994

LETTERS TO THE EDITOR

Recombinant Inbred Strains in Hypertension Research

The review article of Kuneš and Zicha published in the *Physiological Research* **42:** 225–233, 1993 contains inaccurate genetic terminology and unjustified conclusions. Since we are coauthors of all articles on recombinant inbred (RI) strains cited in this review (moreover, M.P. is the first and/or corresponding author of most of these references), we feel a certain responsibility for the correct presentation and interpretation of the results. We think that the genetics of RI strains needs to be clarified and we therefore decided to write you the following comments.

In paragraph 3, the mathematical models for the analysis of quantitative traits and the derivation of RI strains by Bailey are discussed. *"Various mathematical methods of analyzing quantitative traits controlled by multigenic systems in segregating populations were developed (Falconer 1963, Roderick and Schlager 1966). The main disadvantage of this approach is that a quantitative phenotype cannot be established reliably in a single individual and thus the relationship between phenotype and genotype is very difficult. This difficulty was recognized by Bailey (1965, 1971, 1981) who produced a series of recombinant inbred strains to analyze various quantitative traits".* The authors mentioned mathematical methods for the analysis of quantitative traits and they cited the work of Falconer and Roderick & Schlager. It should be recognized that these references concern so called biometrical genetics that uses the means and variances for the analysis of quantitative traits and not individual values. The main disadvantage of these methods cannot therefore be the unreliability to measure phenotypes in an individual. Also, Bailey did not originally produce his RI strains for the analysis of quantitative traits but, on the contrary, for the analysis of genes determining minor histocompatibility loci with Mendelian inheritance. The usefulness of RI strains for the analysis of multifactorially determined traits was recognized later and has recently received increased attention (e.g. McClearn G.E. et al.: The gene chase in behavioral science. *Psychol. Sci.* **2:** 222–229, 1991). In 1982, we started with the production of RI strains for the analysis of multifactorially determined physiological and morphological traits.

In paragraph 4 (but also in other paragraphs), we can find incorrect genetic terminology, such as *"... polymorphic allele ..."* or *"... the particular homozygous allele ...".* An allele cannot be polymorphic; different variants of genes are called alleles; the presence of different alleles of genes in a frequency >1 % is called polymorphism. An allele also cannot be homozygous; homozygosity is the presence of two identical alleles in a given genetic locus.

In paragraphs 7 and 8, the authors without any context describe the use of recombinant congenic strains and immediately after this they present the results of congenic strains. *"Another way how to test the role of any gene(s) in the determination of a quantitative trait is to develop recombinant congenic strains ..."* (paragraph 7), *"To check how strong is the influence of the genes within RT1 complex on blood pressure we studied SHR.1N congenic strain ..."* (paragraph 8). This makes an impression that the authors do not distinguish two totally different genetic systems, recombinant congenic and congenic strains.

In paragraph 10, the authors discuss their "method of blood pressure-matched strains". *"Recently, we have demonstrated that the major structural alterations in the renin gene ... are accompanied by a reduction in renal renin activity in RI strains inheriting the SHR allele of the renin gene. This could not be a secondary influence of high blood pressure because the same was seen even in blood pressure-matched RI strains."* Using small subpopulations of RI strains, for instance the "blood pressure-matched RI strains", greatly decreases the statistical power of RI strains. When RI strains are typed in multiple genetic markers (at the time of the review submission, several hundred markers were typed), adequately stringent statistical criteria must be used to avoid false positive results. With only 15 RI strains, as were used for comparison of "blood pressure-matched RI strains" (Fig. 7), one can detect statistically significant linkage only when a quantitative trait locus is responsible for at least 64 % of variance among strains. The value of $p=0.01$ is equivalent to approximately 40 % probability of linkage (Neumann P.E.: Inference in linkage analysis of multifactorial traits using recombinant inbred strains of mice. *Behav. Genet.* **22:** 665–676, 1992); usually 95 % probability of linkage is considered as criterion of statistical significance.

In paragraph 12, the authors claim: *"We have solved the question whether cardiac and renal enlargements are primary or secondary events in hypertension by using our set of RI strains".* This conclusion is unjustified. It must be recognized that cardiac and renal weights are multifactorially determined and it is quite possible that in other genetic backgrounds or in different environments, different results might be obtained. It should be kept in mind that the analysis of variance is not sensitive enough to detect interactions (not addition) between genotypes and the environment when sample size is small (Wahlstein D.: Insensitivity of the analysis of variance to heredity-environment interaction. *Behav. Brain. Sci.* **13**: 109–161, 1990).

In conclusion, the authors, a little bit out of context, suggest to use a pedigree analysis in genetic studies of human essential hypertension: *"It could be emphasized that in essential hypertension research each positive correlation ... should be reaffirmed ... e.g. by pedigree analysis".* The pedigree analysis is not the method of choice in genetic studies of essential hypertension because: (1) essential hypertension is genetically heterogeneous, (2) it is practically impossible to obtain reliable blood pressure data from individuals of different generations and (3) some individuals in the pedigree may carry "predisposing" alleles without being hypertensive which could lead to misinterpretation of the analysis. Different strategies than pedigree analysis are therefore used, e.g. the study of affected relatives (Kurtz T.W., Spence M.A.: Genetics of essential hypertension. *Am. J. Med.* **94**: 77–84, 1993).

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Received May 13, 1994