Letters to the Editor

Can Knockout Mice Help Dissect Relevant Genes in Hypertension? Evidence and Confounding Factors

To the Editor:

In a recent article, Rhaleb et al.1 showed that bradykinin B2 receptor gene knockout (B2-KO) mice at 2 to 3 months of age have normal basal blood pressure (BP) levels and heart weight compared with control 129/SvEvTac mice. In addition, they did not find any difference between B2-KO mice and controls regarding the ability of ACE inhibition to prevent hypertension and heart hypertrophy in response to aorta coarctation or mineralocorticoids. The authors state that these results cannot confirm those obtained by others,2–4 leaving unexplained the reason(s) for such discrepancies. On the other hand, they agree that B2-KO mice are predisposed to develop salt-sensitive hypertension.1,4,5

In the case of the B2-KO mouse, gene targeting was performed by transfecting embryonic stem cells derived from J129Sv mice with a vector designed to disrupt the entire coding sequence of the B2 receptor gene.6 Chimerical mice were then bred with J129SvEv mice and only the offspring that were heterozygous for the mutation (thus having both sets of chromosomes of J129Sv origin) were used for subsequent mating to homozygosity. Both J129Sv and 129/SvEvTac substrains (used as controls) show lower basal BP levels compared with the B2-KO mice studied by us. This observation indicates that discrepancies cannot be attributed to the use of different controls. Unfortunately, as correctly stated by Rhaleb et al.,1 either J129Sv or 129/SvEvTac mice may differ from null mutants at a limited number of loci other than the site of the mutation. Backcross breeding of the mutation into other inbred strains has been proposed to circumvent the problems that arise from the incomplete homogeneous genetic background of knockout models. However, since the new strain will contain a relative large amount of original DNA even after 10 generations of backcrossing, this approach has a reduced rigor due to the possibility of inheritance of gene sequences close to the mutation. Another limitation of the backcross breeding approach, which may be relevant in the case of B2-KO mice, is that the null mutation is usually transferred into the genetic background of C57Bl/6J mice, which are characterized by lower BP compared with other mouse strains. Therefore, in C57Bl/6J mice, the impact of disrupting the B2 receptor gene might be compensated by redundancy of other vasodilator mechanisms.

It should be considered that from generation of the knockout to phenotyping, null-mutant mice are subjected to “genetic stress” that may eventually lead to divergent cardiovascular phenotypes in colonies that originate from the same strain. Random mutations in the genome of B2-KO colonies could have contributed to this divergence. Moreover, in relatively small colonies (100 to 500 mice), breeding procedures are under the strong influence of random genetic drift, leading to a very rapid fixation of allele frequency. This could be sufficient to cause quantitative differences in the phenotype of two segregated populations harboring the same null mutation. For example, assume that disruption of the vasopressor gene β is responsible for a differential expression of alleles a and A of vasopressor gene α. Such a regulatory influence on gene α might eventually lead to different BP phenotypes depending on which allele (a or A) is fixed as a result of genetic drift. This phenomenon might help explain the greater susceptibility of our B2-KO mice to develop hypertension, either spontaneously or in response to exposure to mild increases in dietary salt, compared with the extremely high salt excess shown to induce hypertension in another colony of the same knockout model as demonstrated by Alfie et al.5,6 Our studies point to the renin-angiotensin-aldosterone system as the vasopressor, salt-retaining mechanism that, in the absence of a functional bradykinin B2 receptor signaling, causes hypertension in B2-KO mice.6 Instead, a clear-cut conclusion cannot be drawn from the study as borderline BP difference (P<0.07) was observed between B2-KO and wild-type mice exposed to an excess of mineralocorticoids for 4 weeks.1

The conclusive statement that the B2 receptor gene does not participate in the maintenance of normal BP cannot be granted by studies limited to young animals, particularly when considering that hypertension and its complications often develop with aging in humans. The importance of phenotyping the mutant animals from early developmental phases to adulthood is outlined by our finding that B2-KO mice develop hypertensive left ventricular remodeling and reparative fibrosis leading to dilated, failing cardiomyopathy at 12 months of age.7 Moreover, heterozygous mice develop hypertension later in life compared with B2-KO mice and show compensatory ventricular hypertrophic growth with normal mass/chamber volume ratio.7 Thus, interactions between the bradykinin B2 receptor, other regulatory genes, and environmental factors are developmentally regulated. Data from mice with 0, 1, or 2 copies of the bradykinin B2 receptor gene and transgenic mice harboring the human B2 receptor transgene together with the wild-type receptor gene strongly indicate that BP is inversely correlated with the number of B2 receptor alleles.4,8

In conclusion, developmental analysis appears to be most appropriate when phenotyping knockout models of aging-related diseases. Rather than a matter of argument, the observation of phenotypic differences between small-size colonies that carry the same mutation should be regarded as an unique occasion to dissect the influence of selection, genetic drift, and epistatic interactions on the pathogenesis of hypertension and related target-organ damage.

Note: After acceptance of this letter for publication, at the occasion of the 53rd Annual Fall Conference of the Council for High Blood Pressure Research, Pierre Meneton et al.9 communicated that disruption of the tissue kallikrein gene triggers cardiac abnormalities typical of a dilated cardiomyopathy in mice even at earlier stages of the life compared with our B2-KO. These data reinforce the view that the kallikrein-kinin system is indeed important for normal cardiovascular development.

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In response to the letter to the editor concerning our recent study on the role of kinins in blood pressure (BP) and cardiac hypertrophy under normal conditions or in experimental hypertension,1 we chose 129/SvEvTac mice as our controls based on a recent study examining simple sequence-linked polymorphisms (SSLP) among various 129 substrains,2 showing fewer differences between 129/SvEvTac and the two 129 substrains comprising the B2-KO compared with those used by Madeddu et al3 (J129/Sv, assumed to be 129/SvJ) and Borkowski et al4 (J129/SvEv, being undetermined). In addition, 129/SvJ mice have been officially renamed 129X1/SvJ to indicate that it is not a pure strain, but rather genetically contaminated,5 and is not the original source of the ES cell line AB 2.1.6

We disagree about the utility of the standard backcrossing of the gene of interest to a standard genetic background (here C57BL/6J). However, we agree that there will be some remaining 129-derived loci linked to the B2-KO locus, but there will be relatively few. In addition, use of C57BL/6J avoids a second renin gene, Ren2, which is unique to some mouse strains (including 129/SvJ and 129/SvEvTac mice at 1, 2, 3, and 6 months, but found no significant difference between direct mean arterial pressure (MAP), heart rate (HR), and heart weight (HW) than wild-type mice. Our findings do not support the hypothesis that B2 kinin receptors are a major component in the maintenance of normal BP. It would be useful if our laboratories could exchange B2-KO mice and see whether their phenotypes are conserved. If phenotypes of each strain are conserved, then we could assume that genetic drift occurred.

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