Activating Mutation of the Renal Epithelial Chloride Channel ClC-Kb Predisposing to Hypertension

To the Editor:

We write with respect to the article “Activating Mutation of the Renal Epithelial Chloride Channel ClC-Kb Predisposing to Hypertension” by Jeck et al.1 The authors state that this naturally occurring ClC-KbT481S variation is at position 1441 in the gene accession number NM 000085.1 (altering base A to T). We wish to report agreement with the gene frequency for this polymorphism in a South Australian population. However, we note a discrepancy in the gene position assigned to the allelic variant declared in their paper. The correct position is at 1475 in the same gene accession number.2

This position was determined when our initial efforts to reproduce the findings of the article failed to find this polymorphism at position 1441 in a cohort of our population. The ClC-Kb genotype was determined with an allele-specific polymerase chain reaction (PCR) used previously.3 This PCR method only extends a sequence if it matches at the 3-base pair (i.e., position 1441). This gave PCR product only for the wild type (A) primer, and nothing for the putative 1441(T) mutant. We re-examined the article by Jeck et al and, using the sequence that they published for their primers, found a matching DNA sequence corresponding to a polymorphic site at position 1475 in NM 000085.1.2 A change in our primers to an 18-base sequence, ending at position 1475, succeeded in producing appropriate results for both A and T primers.

We then analyzed the ClC-KbT481S polymorphism in whole genomic DNA samples from a total of 297 control individuals, representing a random selection of people from the South Australian population in the greater Adelaide Metropolitan area who were free of cardiovascular disease. We determined the overall mutant (T) allele frequency to be 14.1% in this population with a prevalence of 25.6% heterozygous and 1.3% homozygous TT individuals. This agrees closely with the data obtained by Jeck et al in 3 Caucasian German population groups (which, when combined, had an average T allele frequency of 12.4%, with 20.4% heterozygous and 2.2% homozygous TT).

We also note a separate but less important error in Table 1 of the article, where the totals (and frequencies) of the A and T alleles are reported by Jeck et al for their Group 3 Southern Bavarian volunteers. These were given as 483 (86.0%) and 79 (14.1%), but should be 547 (87.4%) and 79 (12.6%) respectively, calculated from the data they reported for 313 volunteers.

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Response: ClC-Kb Mutation Revisited

We appreciate the contribution of Milton et al confirming the allele frequency of the ClC-KbT481S polymorphism in a South Australian population.

The discrepant definition of the affected nucleotide position affected by the thymine for adenine replacement is caused by different ways of counting. Milton et al start counting the nucleotides with the first base of exon 1, whereas we started counting with the first base of the annotated ATG Start Codon, which is 34 bases downstream from the first base of exon 1. Counting from the first nucleotide of translated sequence appears more useful for the functional approach on protein level used in our study. The position of the affected nucleotide is also clearly defined by the subsequent amino acid substitution (T481S) and by the respective TaqMan/Light Cycler probes used for genotyping.1 In Table 1 of our publication1 the calculation of allele frequency did indeed not include all wild-type individuals of group 3. Nevertheless, the result was virtually identical to that of the complete subgroup, was similar to that of the other white populations, and significantly different from the African population.

More importantly, 2 other articles failed to demonstrate an association between the ClC-KbT481S polymorphism and blood pressure.2,3 In our population, however, mean arterial blood pressure was significantly (P<0.001) higher in ClC-KbT481S carriers than in wild-type individuals. As the genetic analysis in our study has been strictly blinded and performed in separate laboratories as blood pressure measurements, the results could not have been biased by the study design. On the other hand, the studied population was small (47 carriers and 173 wild-type), thus leaving some uncertainty. We presently attempt to explore the prerequisites for an impact of ClC-Kb on blood pressure.

In a previous study, we had identified a polymorphism of the serum and glucocorticoid inducible kinase SGK1, which in our hands was associated with increased blood pressure.4 Besides ENaC, SGK1 stimulates ClC-Ka5 and ClC-Kb (G. Seebohm, F. Lang, unpublished observations, 2005). A subsequent study on a similarly small group failed to observe an association of the same gene variant with blood pressure.6 A most recent study in a very large group of individuals, however, clearly confirmed the association of the SGK1 gene variant with blood pressure and at the same time disclosed that the association was particularly prominent in individuals with hyperinsulinism.7 This latter observation is in perfect agreement with recent observations in SGK1 knockout mice.8 We do hope that future research will similarly define the mechanisms affecting the influence of ClC-KbT481S on blood pressure.

In view of the analysis of Milton et al it would further be interesting to learn whether the authors have found any difference of the allele frequency between the autochthonic Australian population and successors of European immigration. Compelling evidence points to interethnic differences in genotypes between...
Australian Aborigines and, for example, Caucasians. For instance, we have recently shown such differences for the clinically most relevant cytochrome P450 drug metabolizing enzymes 2D6 and 2C19.9

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Hypertension. 2006;47:e12-e13; originally published online January 30, 2006;
doi: 10.1161/01.HYP.0000203773.85380.1b
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/47/3/e12

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