Mammalian Genome 7, 380–382 (1996).

Mammalian Genome

Chromosomal mapping of the rat *Slc4a* family of anion exchanger genes, *Ae1*, *Ae2*, and *Ae3*

J.S. Simon,[1] G. Deshmukh,[2] F.J. Couch,[2] S.D. Merajver,[2] B.L. Weber,[2] P. Van Vooren,[3] F. Tissil,[3] J. Szpirer,[3] C. Szpirer,[3] S.L. Alper,[4] H.J. Jacob,[1] F.C. Brosius III[2]

[1]Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, 02129, USA
[2]Department of Internal Medicine, University of Michigan, 1560 MSRB II, 1150 West Medical Center Drive, and Ann Arbor Veterans Administration Hospital, Ann Arbor, Michigan, 48109, USA
[3]Department of Molecular Biology, Université Libre de Bruxelles, Rhode-St-Genese, Belgium
[4]Molecular Medicine and Renal Units, Beth Israel Hospital and Depts. of Cell Biology and Medicine, Harvard Medical School, Boston, Massachusetts, 02215, USA

Received: 23 October 1995 / Accepted: 14 December 1995

The *Slcl4a* (solute carrier family 4, anion exchanger) family, also known as the band 3-related anion exchanger (*Ae*) gene family, comprises at least three distinct members (Alper 1994; Kopito 1990). *Ae* genes are expressed in virtually every mammalian cell, and each of the encoded polypeptides mediates sodium-independent, electroneutral exchange of chloride for bicarbonate across the plasma membrane (Alper 1994). The human *Ae1*, *Ae2*, and *Ae3* genes (more recently identified as solute carrier family 4, anion exchanger, member 1–3; *SLC4a1–3*) have been localized to Chromosomes (Chrs) 17, 7, and 2 respectively (Palumbo et al. 1986; Showe et al. 1987; Yannoukakos 1994). The *Ae1*, *Ae2*, and *Ae3* genes of the mouse have been localized to Chrs 11 (Love et al. 1990), 5, and 1 (White et al. 1994) respectively. The rat *Ae1* gene has been mapped to Chr 10 (Jacob et al. 1995; Serikawa et al. 1992) by genetic mapping, and the rat *Ae3* gene has been localized to Chr 9 with a rat/mouse somatic cell hybrid panel (Deng et al. 1994). Here we report the development of microsatellite genetic markers for the rat anion exchanger genes and the refined chromosomal localizations of the three *Ae* genes by the dual techniques of genetic linkage and fluorescent in situ hybridization (FISH). We also report that the *Ae1* and *Ae2* genes lie within quantitative trait loci for systolic blood pressure in models of rat genetic hypertension.

Rat cosmid genomic DNA clones encoding the three *Ae* genes were gifts of Dr. Gary Shull (University of Cincinnati; Kudrycki and Shull 1993; Linn et al. 1992; and unpublished observations, G. Shull et al.). To determine if microsatellite repeat sequences were present within the *Ae* genes, Southern blots of *Eco*RI-digested cosmid DNA were hybridized with a ^{32}P-labeled $(CA)_{22}$ oligonucleotide probe with standard protocols (Couch et al. 1994; Maniatis et al. 1989). This initial screen indicated the presence of microsatellite CA repeat sequences within each of the *Ae* cosmid clones. Cosmid DNA was subsequently digested with *Sau*3A and ligated into pBluescript KS (+) (Stratagene, La Jolla, Calif.). Subclones were transformed into *Escherichia coli* DH5α, plated, lifted onto nylon filters (DuPont/NEN), and screened with the $(CA)_{22}$ oligonucleotide probe according to manufacturer's directions. Positive colonies were picked, and the plasmid DNA was sequenced. PCR primers flanking the $(CA)_n$ repeats were designed:

Ae1 forward 5′-AGAGGAGGAGCCATGAGAGT-3′,
Ae1 reverse 5′-GGACAGGATGACAATGACAC-3′;
Ae2 forward 5′-TTGGAGGAGTTTGAGGACCC-3′,
Ae2 reverse 5′-AGTCACTGCCTGCAAGCTCT-3′;
Ae3 forward 5′-CCAATCCATTCCATACTTCC-3′,
Ae3 reverse 5′-GATCTTCTACGATGAGGTG-3′.

These PCR primers were used to evaluate the microsatellite sequences for length polymorphisms among various inbred rat strains and to refine the chromosomal locations of *Ae1*, *Ae2*, and *Ae3*. Genotyping was performed as described previously (Jacob et al. 1995). Analysis of DNA from 21 inbred rat strains identified four alleles at the *Ae1* locus ranging from 197 to 203 bp, four alleles at the *Ae2* locus ranging from 114 to 150 bp, and five alleles at the *Ae3* locus ranging from 150 to 214bp (Table 1; for a review of inbred rat strains, see Hedrich 1990).

Table 1. Rat genomic DNA from 21 inbred strains was amplified by PCR primers directed to the microsatellite repeat near the *Ae1*, *Ae2*, or *Ae3* genes. PCR products were analyzed by gel electrophoresis as described in the text. Abbreviations are: Spontaneously Hypertensive/NIH (SHR/Nih), Stroke Prone Spontaneously Hypertensive/Heidelberg (SHRSP/H), Wistar Kyoto/Heidelberg (WKY/H), Salt-Sensitive/John Rapp (SS/Jr), Salt-Resistant/John Rapp (SR/Jr), Lewis/Harlan Sprague-Dawley (LEW/Hsd), Brown-Norway/Harlan Sprague-Dawley (BN/Hsd), Bio-Breeding/Diabetes prone (BB/Dp), Bio-Breeding/Diabetes resistant (BB/Dr), Fischer 344/Kozo Matsumoto (F344/Tj), AxC 9935 Irish/Harlan Sprague-Dawley (ACI/Hsd), Buffalo/Harlan Sprague-Dawley (BUF/Hsd), Spontaneously Hypertensive/Harrap (SHR/Har), Donryu/Harrap (DNY/Har), Wistar to King to Aptekman to Hoq Rat/Kozo Matsumoto. (WKAH/Tj), Castle's Black Rat x Wild Rat/Kozo Matsumoto (LEC/Tj), Otsuka Long-Evans Takamua Fatty/Kozo Matsumoto (OLETF/Tj), Genetically Hypertensive/Stephen Harrap (GH/Nz), Fawn Hooded hypertensive rat/Erasmus University (FHH/Eur), Copenhagen (COP/Uwm), Wistar Furth/University of Wisconsin, Madison (WF/Uwm).

	Allele size (bp)		
Strain	*Ae1*	*Ae2*	*Ae3*
SHR/Nih	199	150	150
SHRSP/H	199	150	172
WKY/H	199	150	172
SS/Jr	199	114	172
SR/Jr	199	114	172
LEW/Hsd	199	114	160
BN/Hsd	201	114	160
BB/Dp	201	114	160
BB/Dr	201	114	172
F344/Tj	199	114	150
ACI/Hsd	201	114	182
BUF/Hsd	203	114	150
SHR/Har	199	150	214
DNY/Har	199	150	172
WKAH/Tj	201	126	172
LEC/Tj	197	134	172
OLETF/Tj	199	134	172
GH/Nz	199	134	172
FHH/Eur	199	126	172
COP/Uwm	—	134	214
WF/Uwm	199	—	—

Correspondence to: F.C. Brosius III, University of Michigan, 1560 MSRBII, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0676. (313) 936-4812, fax: (313) 763-0982. e-mail: fbrosius@umich.edu.

After determination of allele sizes, genotyping of these markers was carried out on 46 F_2 progeny of a Spontaneously Hypertensive rat (SHR) × Brown-Norway rat (BN) intercross (Jacob et al. 1995). Genotype data were analyzed with the MAPMAKER computer program (Lander et al. 1987) and the Kosambi map function. The $(CA)_n$ microsatellite from the *Ae1* clone mapped to Chr 10 (Fig. 1A). No recombination was observed between *Ae1* and the *Band3a* $(TTCA)_8$ microsatellite identified by Serikawa and colleagues (Serikawa et al. 1992), or with *Ppy* and *D10Mit7*. *Ae2* mapped to Chr 4 (Fig. 1B), displayed no recombination with IL6 or with *D4Mgh22*, and was situated 5.7 cM from *D4Mgh1*. *Ae3* mapped to Chr 9 (Fig. 1C), displayed no recombination with *D9Mit5*, and was situated with *D9Mgh2* 6.8 cM to one side and *D9Mit2* (*Crygc*) and *D9Mgh4* (*Crygf*) 6.8 cM to the other side.

Regional chromosome localization of the *Ae* genes was performed by FISH, as described previously (Pinkel et al. 1988; Szpirer et al. 1994). The *Ae1* probe generated bright double chromatid signals on Chr 10 only. The signals were subtelomeric and unambiguously mapped to the last band, that is, 10q32 (Fig. 2A). In the linkage map, *Ae1* is close to one end, thereby allowing us to orient the linkage map with respect to the chromosome: centromere–*D10Mgh13*–. . .–*Ae1*–*D10Mgh1*–telomere (these data imply that the map shown in Jacob et al. (1995) is upside down; the map is correctly oriented in Fig. 1). Our conclusion is in agreement with that of Kuramoto and associates (1993), who assigned *Syb2* to 10q24, i.e. in a more proximal position than *Ae1* (see Fig. 1), and *Gh*, which is closely linked to *Ace* and *Ae1*, to 10q32. We also agree with the results of Yeung and colleagues (1993), who assigned *Tsc2* to 10q12 and *Kid1*, itself closely linked to *D10Mit9*/*RR94* (Hino et al. 1994), to 10q21. These results can be combined as follows: centromere–*D10Mgh13*–*Tsc2*(*q12*)–*D10Mit9*–*Kid1*(*q21*)–*Syb2*(*q24*)–*Ae1/Ace/Gh/D10Mgh1*(*q32*)–telomere.

The *Ae2* probe generated double chromatid signals on Chr 4 close to the centromere, that is, 4q11. Several metaphases showed signals on the two copies of Chr 4 (Fig. 2B). The *Ae2* gene mapped at one end of the linkage group, and thus it was easy to orient the Chr 4 linkage map: centromere–*Ae2, Il6, D4Mgh22*–. . .–*D4Mgh13*–telomere.

The *Ae3* cosmid generated double chromatid signals on the two Chr 9 homologs of several metaphases, and these signals were mapped at 70–75% of the chromosome length, that is, 9q34 (Fig. 2C). The Chr 9 linkage group is relatively short (about 40 cM), and the *Ae3* gene is located in the middle of this map. Thus, it was not

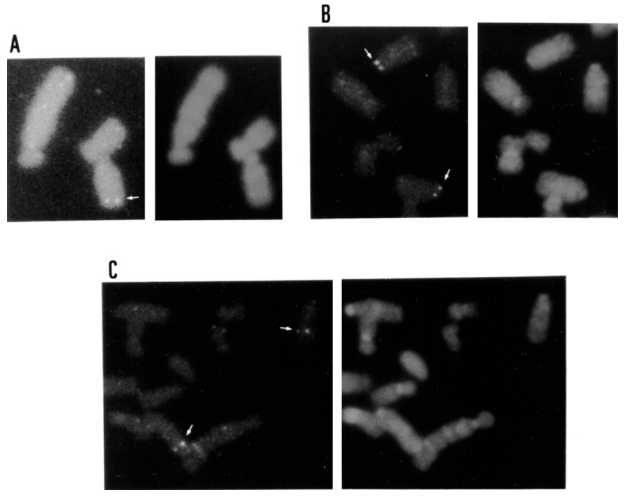

Fig. 2. Regional localization of the rat *Ae/Slc4a* genes by FISH: portions of metaphases showing the signals generated by the probes (left) and the DAPI staining (right). (A) Detection of the *Ae1/Slc4a1* gene at 10q32 (the probe used was the pL2Aδ plasmid, containing the mouse *Ae1/Slc4a1* cDNA sequence from position +362 to +4385). (B) Detection of the *Ae2/Slc4a2* gene at 4q11. (C) Detection of the *Ae3/Slc4a3* gene at 9q34 (the probes used in the two latter cases were pWE15-derived genomic DNA cosmids).

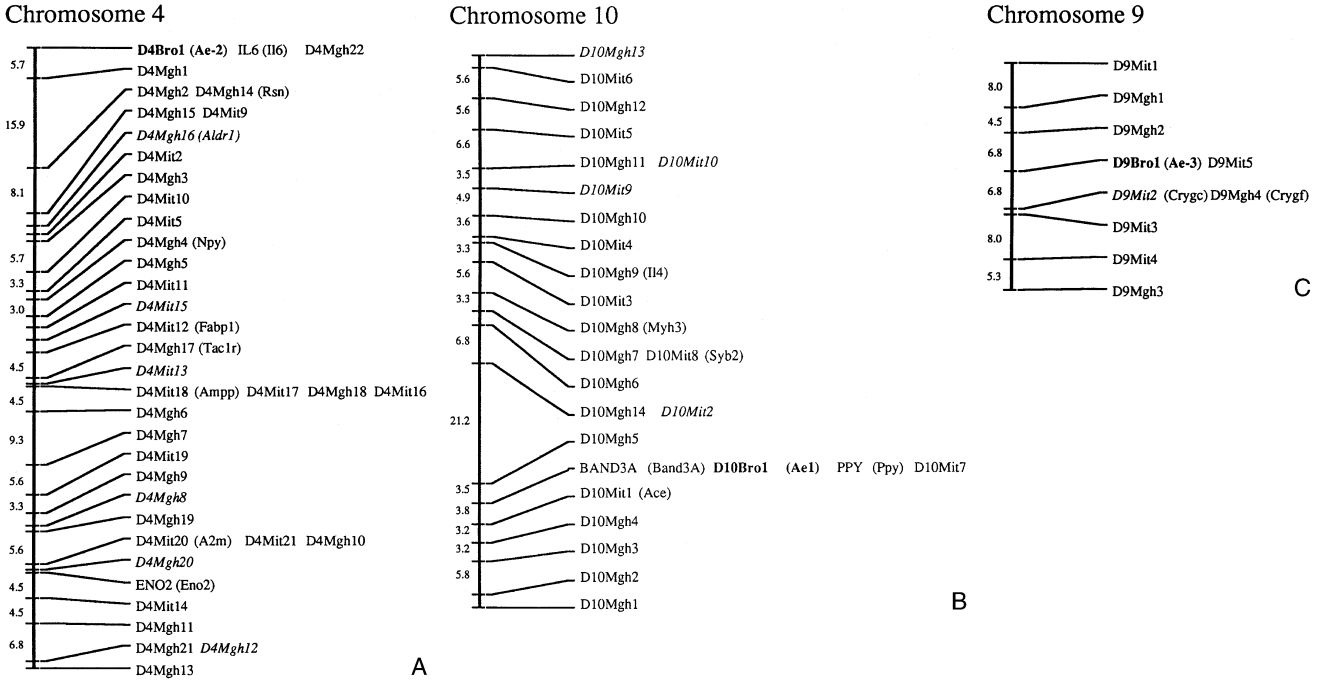

Fig. 1. Genetic linkage maps of rat chromosomes 4, 9, and 10. Formal locus names include the prefix RNO (omitted here), denoting *Rattus Norvegicus* to avoid confusion with loci in other organisms. Gene names (shown in parentheses) refer to loci taken from the literature or GenBank. Markers located on the same horizontal line did not recombine in the 92 meioses studied. Markers for the *Ae*genes generated in this study are shown in bold. Markers whose order is supported by a lod score of at least 2.0 are shown in plain type, the remainder in italics. CentiMorgan distances between markers, calculated using the Kosambi map function, are indicated for distances over 3.3 cM. The precise mapping function for the rat is not known, but the Kosambi map function should be adequate except perhaps in large intervals.

possible to orient the linkage map, although this mapping established the first link between the genetic and the chromosome maps.

Under physiologic conditions, *Ae* polypeptides mediate transport of bicarbonate out of the cell, hence acidifying the cell interior and increasing intracellular chloride concentration (Alper 1994; Ganz et al. 1989). Abnormalities in regulation of intracellular pH and chloride concentrations have been implicated in the development of genetic hypertension in rats and in humans (Aalkjaer 1990). Therefore, we examined the segregation of *Ae1–3* genes with the hypertensive phenotype in several large, inbred crossed rat models of hypertension developed by us and others. Interestingly, the *Ae1* gene segregated within a quantitative trait locus, *Bp1,* shown to have a major effect on systolic and diastolic blood pressure after sodium load in F_2 progeny of a stroke-prone SHR/H (SHRSP) × Wistar-Kyoto/H intercross (Hilbert et al. 1991; Jacob et al. 1991). In addition, the *Ae2* gene has been mapped to an area on Chr 4 that has been demonstrated to segregate with blood pressure in recombinant inbred rats derived from crossing SHR/NIH and Brown-Norway (BN) rats (Pravenec et al. 1994). However, neither *Ae1, Ae2,* nor *Ae3* genes cosegregated with any blood pressure phenotype in F_2 progeny from intercrosses of Salt-Sensitive/John Rapp × BN/Hsd (Harlan Sprague-Dawley) and Genetically Hypertensive/Nz × BN/Nz (Jacob et al. unpublished observation).

The combination of genetic linkage and fluorescent in situ hybridization data confirm and refine the localization of the *Ae* genes in the rat. Moreover, linkage analysis suggests that *Ae1* and *Ae2* are candidate genes that may contribute to the development and/or maintenance of hypertension in both the SHRSP and SHR models of genetic hypertension. The development of polymorphic PCR markers for the rat *Ae* genes should facilitate further examination of these genes and their potential roles in rat models of high blood pressure.

Acknowledgments. The authors wish to thank Dr. G. Shull for the generous gift of cosmid clones. We also thank Eric Winer for expert technical assistance. This work was supported by Bristol-Myers Squibb and NIH/NIDDK (H.J. Jacob, S.A. Alper), the FRSM (Belgium) and the ''Communaté Française de Belgique'' (ARC), NIH/NHLBI grant HL-18575 (F.C. Brosius III) and a Veterans Affairs Merit Review grant (F.C. Brosius III). (J.S. Simon is supported by an Individual National Research Service Award. C. Szpirer is a Research Director of the FNRS (Belgium). S.L. Alper is a recipient of an Established Investigator Award from the American Heart Association.

References

Aalkjaer, C. (1990). Regulation of intracellular pH and its role in vascular smooth muscle regulation. J. Hypertens. 8, 197–206.

Alper, S.L. (1994). The band 3-related AE anion exchanger gene family. Cell. Physiol. Biochem. 4, 265–281.

Couch, F.J., Kiousis, S., Castilla, L.H., Xu, J., Chandrasekharappa, S.C., Chamberlain, J.S., Collins, F.S., Weber, B.L. (1994). Characterization of ten new polymorphic dinucleotide repeats and generation of a high-density microsatellite-based physical map of the BRCA1 region of chromosome 17q21. Genomics 24, 419–424.

Deng, A.Y., Gu, L., Rapp, J.P., Szpirer, C., Szpirer, J. (1994). Chromosomal assignment of 11 loci in the rat by mouse-rat somatic hybrids and linkage. Mamm. Genome 5, 712–716.

Ganz, M.B., Boyarsky, G., Sterzl, R.B., Boron, W.F. (1989). Arginine vasopressin enhances pH_i regulation in the presence of HCO_3^- by stimulating the three acid-base transport systems. Nature 337, 648–651.

Hedrich, H. (1990). *Genetic Monitoring of Inbred Strains of Rats,* (New York: Gustav Fischer Verlag).

Hilbert, P., Lindpaintner, K., Beckmann, J.S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., De Gouyon, B., Julier, C., Takahasi, S., Vincent, M., Ganten, D., Georges, M., Lathrop, G.M. (1991). Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. Nature 353, 521–525.

Hino, O., Kobayashi, T., Tsuchiya, H., Kikuchi, Y., Kobayashi, E., Mitani, H., Hiramaya, Y. (1994). The predisposing gene of the Eker rat inherited cancer syndrome is tightly linked to the tuberosous sclerosis (Tsc2) gene. Biochem. Biophys. Res. Commun. 203, 1302–1308.

Jacob, H.J., Lindpaintner, K., Lincoln, S.E., Kusumi, K., Bunker, R.K., Mao, Y.-P., Ganten, D., Dzau, V.J., Lander, E.S. (1991). Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67, 213–224.

Jacob, H.J., Brown, D.M., Bunker, R.K., Daly, M.J., Dzau, V.J., Goodman, A., Koike, G., Kren, V., Kurtz, T., Lernmark, Å., Levan, G., Mao Y-P., Pettersson, A., Pravenec, M., Simon, J.S., Szpirer, C., Szpirer, J., Trolliet, M.R., Winer, E.S., Lander, E.S. (1995). A genetic linkage map of the laboratory rat, *Rattus norvegicus.* Nature Genet. 9:63–69.

Kopito, R.R. (1990). Molecular biology of the anion exchanger (AE) gene family. Int. Rev. Cytol. 123, 177–199.

Kudrycki, K.E., Shull G.E. (1993). Rat kidney band 3 Cl-/HCO3-exchanger mRNA is transcribed from an alternative promoter. Am. J. Physiol. 264, F540–F547.

Kuramato, T., Serikawa, T., Hayasaka, N., Mori, M., Yamada, J. (1993). Regional mapping of the Rowett nude gene (RONU) to rat chromosome 10q24-q32 by localizing linked Syb2 and Gh loci. Cytogenet. Cell Genet. 63, 107–110 (1993).

Lander, E.S., Green, P., Abrahmson, J., Barlow, A., Daly, M., Lincoln, S., Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing genetic linkage maps of experimental and natural populations. Genomics 1, 174–181.

Linn, S.C., Kudrycki, K.E., Shull, G.E. (1992). The predicted translation product of a cardiac AE3 mRNA contains an N terminus distinct from that of the brain AE3 Cl-/HCO3-exchanger. J. Biol. Chem. 267, 7927–7936.

Love, J.M., Knight, A.M., McAleer, M.A., Todd, J.A. (1990). Towards construction of a high resolution map of the mouse genome using PCR-analyzed microsatellites. Nucleic Acids Res. 18, 4123–4130.

Maniatis, T., Fritsch, E.F., Sambrook, J. (1989). *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Palumbo, A.P., Isobe, M., Huebner, K., Shane, S., Rovera, G. (1986). Chromosomal localization of a human band 3-like gene to region 7q35–7q36. Am. J. Hum. Genet. 39, 307–316.

Pinkel, D., Landegent, J., Collins, C., Fuscoe, J., Segraves, R., Lucas, J., Gray, J. (1988). Fluorescent in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci. USA 85, 9138–9141.

Pravenec, M., Gauguier, D., Schott, J., Buard, G., Kren, V., Wong, A., Szpirer, C., Szpirer, J., Wang, J-M., St. Lezin, E., Spence, M.A., Flodman, P., Lathrop, M., Vergnaud, G., Kurtz, T. (1994). Chromosome mapping of blood pressure regulatory loci in the SHR by total genome scanning of recombinant inbred strains. Hypertension 24, 373 (abstract).

Serikawa, T., Kuramoto, T., Hilbert, P., Mori, M., Yamada, J., Dubay, C.J., Lindpaintner, K., Ganten, D., Guenet, J.L., Lathrop, G.M., Beckmann, J.S. (1992). Rat gene mapping using PCR-analyzed microsatellites. Genetics 131, 701–721.

Showe, L.C., Ballintine, M., Huebner, K. (1987). Localization of the gene for the erythroid anion exchange protein band 3 to human chromosome 17. Genomics 1, 71–76.

Szpirer, C., Tissir, F., Rivière, M., Levan, G., Szpirer, J. (1994). Assignment of rat *Jun* family genes to chromosome 19 (*Junb*), chromosome 5q31–33 (*Jun*), and chromosome 16 (*Jund*). Mamm. Genome 5, 361–364.

White, R.A., Geissler, E.N., Adkison, L.R., Dowler, L.L., Alper, S.L., Lux, S.E. (1994). Chromosomal location of the murine anion exchanger genes encoding AE2 and AE3. Mamm. Genome 5, 827–829.

Yannoukakos, D., Stuart-Tilley, A., Fernandez, H.A., Fey, P., Duyk, G., Alper, S.L. (1994). Molecular cloning, expression, and chromosomal localization of two isoforms of the AE3 anion exchanger from human heart. Circ. Res. 75, 603–614.

Yeung, R.S., Buetow, K.H., Testa, J.R., Knudson, A.G. (1993). Susceptibility to renal carcinoma in the Eker rat involves a tumor suppressor gene on chromosome 10. Proc. Natl. Acad. Sci. USA 90, 8038–8042.