Immunogenetics (1995) 42: 153–155 © Springer-Verlag 1995

BRIEF COMMUNICATION

Aileen Butler · Julie M. Rochelle · Michael F. Seldin
Alexander S. Whitehead

The gene encoding the mouse serum amyloid A protein, apo-SAA5, maps to proximal chromosome 7

Received: 24 February 1995

The serum amyloid A (SAA) proteins, which are apolipoproteins of the high-density-lipoprotein (HDL) complex, may be broadly categorized, on the basis of differential expression, as either acute-phase (A-SAAs) or constitutive [(C-SAAs) (Steel and Whitehead 1994)]. Three *A-SAA* genes (*SAA1, SAA2, SAA3*) have been described in the mouse, and are characterized by rapid and dramatic induction following an inflammatory stimulus (Lowell et al. 1986a, b). These contrast with *SAA4*, which is a pseudogene, and the recently identified mouse SAA5 protein, which exhibits only moderate induction during the acute-phase response and is therefore considered to be constitutively expressed, a feature which may reflect functional diversity (de Beer et al. 1991). Apo-SAA_5 may contribute to the normal physiological role(s) of HDL, since it associates predominately (>90%) with non-acute-phase HDL, particles (de Beer et al. 1994). More specifically, SAA may have significant influence on the interaction of HDL with lecithin cholesterol acyltransferase (LCAT), one of the critical enzymes in cholesterol esterification (Fielding et al. 1972). It is therefore of considerable interest to further characterize both the mouse *SAA5* gene and its product.

The mouse *SAA* genes have been previously localized to proximal chromosome 7, between the pink eye dilution locus (*p*) and the glucose phosphate isomerase locus (*Gpi-1*) (Taylor and Rowe 1984; Stubbs et al. 1994). However, the mouse *SAA5* gene, whose product exhibits only 48% amino acid identity to SAA1/2/3 proteins, has not yet been mapped (de Beer et al. 1994). As part of our goal of producing a high-resolution map of the mouse *SAA* genes, we have defined the chromosomal location of *SAA5* by means of a haplotype analysis of a mouse interspecific backcross panel. Furthermore, we have isolated and sized a yeast artificial chromosome (YAC) containing all five mouse *SAA* genes.

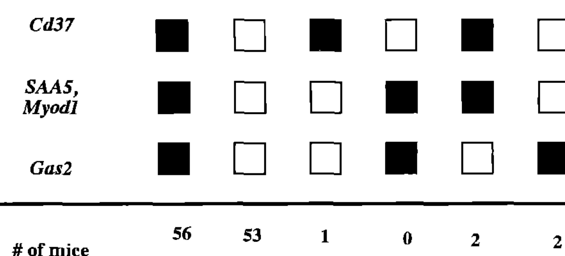

Fig. 1 Segregation of *SAA5* on mouse Chr 7 in [(C3H/HeJ-*gld* × *M. spretus*) F_1 × C3H/HeJ-*gld*] interspecific backcross mice. *Filled boxes* represent the homozygous C3H pattern and *open boxes* the F_1 pattern. The mapping of the reference loci, cluster designation 37 (*Cd37*), myoblast differentiation factor-1 (*Myod1*), and growth arrest specific gene-2 (*Gas2*) in this cross have been previously described (Wright et al. 1992; Columbo et al. 1992). For *SAA5*, informative *Bgl* I RFLVs (C3H 3.4 kb; *M. spretus*, 3.6 kb) were determined in the current study by hybridization of Southern blots using an [α-^{32}P]-labeled 207 bp probe (generated by PCR, under conditions described in the text, using the primers MSAA5L: 5′-GTCTGCCACTCAGACAGC-3′ and MSAA5R: 5′-TTATTTTCTGTGATCCAT-3′) which specifically hybridizes to mouse *SAA5*

To establish a chromosomal location for the *SAA5* gene, a haplotype analysis was performed using a panel of DNA samples from an interspecific cross that has been characterized for over 800 genetic markers throughout the genome (for examples see Saunders and Seldin 1990, and Watson et al. 1992). Initially, DNA from the two parental mice [C3H/HeJ-*gld* and (C3H/HeJ-*gld* × *Mus spretus*) (*M. spretus*) F_1] were digested with various restriction endonucleases and hybridized with an *SAA5* probe (see Figure 1 legend) to determine restriction fragment length variants (RFLVs). An informative *Bgl* I RFLV was detected [(C3H/HeJ-*gld*, 3.4 kilobase (kb); *M. spretus*, 3.6 kb and was used to characterize the backcross mice.

Comparison of the haplotype distribution of the *SAA5* RFLV indicated that this gene cosegregated in all 114 meiotic events with the myoblast differentiation-1 (*Myod1*) locus on mouse chromosome (Chr) 7 (Fig. 1).

A. Butler · A. S. Whitehead (✉)
Department of Genetics and Biotechnology Institute, Trinity College, University of Dublin, Dublin 2, Ireland

J. M. Rochelle · M. F. Seldin
Department of Medicine, Duke University Medical Centre, Durham, NC 27710, USA

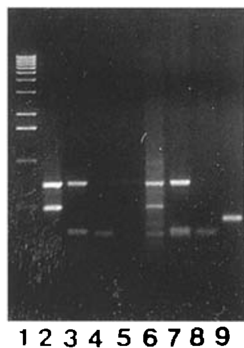

Fig. 2 Locus-specific PCR amplification of fragments from the mouse *SAA1, SAA2, SAA3, SAA4*, and *SAA5* genes, and the gene encoding a Shaw-type voltage-gated potassium channel, *Kcnc1* (Wymore et al. 1994), which serves as a negative control. The *Kcnc1*-specific primers were (MKCNC1L: 5′-GGCCTGTCCTCAAAAGCC-3′, and MKCNC1R: 5′-GTCAGCACACCAGCCAGA-3′), yielding a PCR product of 392 bp. *SAA*-specific primers were as described in the text. PCR products were analyzed on a 1.2% agarose gel. Lane **1**, 1-kb ladder (Lifetechnologies, Inc., Gaithersburg, MD; Lanes **2–5**, products of PCR with YAC κB8 as template, using primers for *SAA1* and *SAA3* (multiplex PCR), *SAA2* and *SAA4* (multiplex PCR), *SAA5*, and *Kcnc1*, respectively; Lanes **6–9**, products of PCR with mouse genomic DNA as template, using primers for *SAA1* and *SAA3* (multiplex PCR), *SAA2* and *SAA4* (multiplex PCR), *SAA5*, and *Kcnc1*, respectively. PCR control reactions with no added template DNA yielded no specific products

The best gene order (Bishop 1985) ± the standard deviation (Green 1981) indicated the gene order: (centromere) *Cd37* – 0.9 cM ± 0.9 cm – *SAA5/Myod1* – 3.5 cM ± 1.7 cM – *Gas2*. This mapping placed the *SAA5* locus in the same region of mouse Chr 7 previously shown to contain the *SAA1, SAA2, SAA3*, and *SAA4* loci (Taylor and Rowe 1984; Stubbs et al. 1994).

To further define the relationship among the *SAA* genes, mouse YAC libraries were screened and analyzed. The combined I. C. R. F. and St. Mary's YAC libraries, partial *Eco* RI libraries constructed with the pYAC4 vector and representing a total of six genomic equivalents (Brown 1992), were screened by polymerase chain reaction (PCR) using SAA locus-specific primer pairs for *SAA1* (MSAA1,2L: 5′-CTCCTAAGTTTCTTTCTGCA-3′ and MSAA1R: 5′-TTGAAGTATTTGTCTGAGTT-3′; PCR product of 724 base pairs (bp), *SAA2* (MSAA1,2L and MSAA2R: 5′-TGGAAGTATTTGTCTCCATC-3′; PCR product of 724 bp), *SAA3* (MSAA3,4L: 5′-AGACAAATACTTCCATGCTC-3′ and MSAA3R: 5′-GTCCACTCCGGCCCCACTCA-3′; PCR product of approximately 500 bp), *SAA4* (MSAA3,4L and MSAA4R: 5′-AGATAGGCAGGACTGAGAAT-3′; PCR product of 317 bp), and *SAA5* (MSAA5L2: 5′-GGATTGGAAACCCTGCAG-3′ and MSAA5R: 5′-TTATTTTCTGTGATCCAT-3′; PCR product of 309 bp). The PCR assay (10 mM Tris-HCl, pH 8.3, 2 mM $MgCl_2$, 50 mM KCl, 200 mM each dNTP, 0.5 pmol/µl each forward and reverse primers, and 4 µg/ml genomic DNA) was carried out at 94 °C for 7 min to denature, followed by 40 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.2 min. A YAC clone (stack I, κB8) was isolated which was demonstrated by PCR to contain the mouse *SAA1, SAA2, SAA3, SAA4*, and *SAA5* genes (Fig. 2).

Pulse field gel electrophoresis (PFGE) of agarose blocks containing YAC κB8 DNA, followed by Southern blotting onto Hybond-N (Amersham International PLC; Amersham, Buckinghamshire, UK), hybridization with an [α-^{32}P]-labelled 2.7 kb probe specific for the left (trp) end of the YAC vector (Sellar et al. 1994), and visualization by standard autoradiography established the YAC to be approximately 600 kb. This result confirms that mouse *SAA5* maps to proximal Chr 7, by virtue of its close linkage to the *SAA1, SAA2, SAA3* and *SAA4* genes that have previously been mapped to this region (Taylor and Rowe 1984; Stubbs et al. 1994). It furthermore indicates that all of the known mouse SAA genes are very closely linked (i. e., within 600 kb).

We have successfully mapped the most recently described member of the mouse *SAA* gene superfamily, *SAA5*, to Chr 7, and demonstrated an *SAA1/2/3/4/5* linkage group on YAC κB8, with a maximum length of 600 kb. The latter will facilitate the generation of a fine-map of the mouse *SAAs*, through partial restriction digest analyses of YAC κB8, and subsequent integration into the published map of the *Ldh3-Myod1* region of mouse Chr 7 (Stubbs et al. 1994). Interspecies comparisons can subsequently be employed to further elucidate the evolution of the *SAA* gene family in mammals, since the organization of the human *SAA* gene cluster on 11p15.1 has recently been described (Sellar et al. 1994). These and other approaches are currently being pursued in our laboratory, as part of our longterm goal to characterize the mouse and human *SAA* genes and their encoded proteins.

Acknowledgments We gratefully acknowledge the support of St. Mary's Hospital Medical School, Norfolk Place, London, UK for the use of the I. C. R. F./St. Mary's YAC screening service. We thank Ms. Clarissa Uhlar for technical assistance. This work was supported by a grant from the Health Research Board of Ireland and Wellcome Trust Programme Grant #034345/Z/91/Z (to A. S. W.) and N. I. H. Grant #HG00734 (to M. F. S.).

References

Bishop, D. T. The information content of phase-known matings for ordering genetic loci. *Genet Epidemiol 2:* 349–361, 1985

Brown, S. D. M. The mouse genome project and human genetics. *Genomics 13:* 490–492, 1992

DeBeer, M. C., Beach, C. M., Shedlofsky, S. I., and deBeer, F. C. Identification of a novel serum amyloid A protein in BALB/c mice. *Biochem J 280:* 45–49, 1991

DeBeer, M. C., Kindy, M. S., Lane, W. S., and deBeer, F. C. Mouse serum amyloid A protein (SAA5) structure and expression. *J Biol Chem 269:* 4661–4667, 1994

Columbo, M. P., Martinotti, A., Howard, T. A., Schneider, C., D'Eustachio, P., and Seldin, M. F. Localisation of growth arrest-specific genes on mouse chromosomes 1, 7, 8, 11, 13, and 16. *Mammalian Genome 2:* 130–134, 1992

Fielding, C. J., Shore, V. G., and Fielding, P. E. A protein cofactor of lecithin cholesterol acyltransferase. *Biochem Biophys Res Commun 46:* 1493–1498, 1972

Green, E. L. Linkage, recombination and mapping. *In* E. Green (ed.): *Genetics and Probability in Animal Breeding Experiments*, pp. 77, Macmillan, New York, 1981

Lowell, C. A., Stearman, R. S., and Morrow, J. F. Transcriptional regulation of serum amyloid A gene expression. *J Biol Chem 261:* 8453–8461, 1986a

Lowell, C. A., Potter, D. A., Stearman, R. S., and Morrow, J. F. Structure of the murine serum amyloid A gene family. *J Biol Chem 261:* 8442–8452, 1986b

Saunders, A. M. and Seldin, M. F. A molecular genetic linkage map of mouse chromosome 7. *Genomics 8:* 524–535, 1990

Sellar, G. C., Oghene, K., Boyle, S., Bickmore, W. A., and Whitehead, A. S. Organization of the region encompassing the human serum amyloid A (SAA) gene family on chromosome 11p15.1. *Genomics 23:* 492–494, 1994

Steel, D. M. and Whitehead, A. S. The major acute phase reactants: C-reactive protein, serum amyloid P component, and serum amyloid A protein. *Immunol Today 15:* 81–88, 1994

Stubbs, L., Rinchik, E. M., Goldberg, E., Rudy, B., Handel, M. A., and Johnson, D. Clustering of six human 11p15 homologs within a 500-kb interval of proximal mouse chromosome 7. *Genomics 24:* 324–332, 1994

Taylor, B. A. and Rowe, L. Genes for serum amyloid A proteins map to chromosome 7 in the mouse. *Mol Gen Genet 195:* 491–499, 1984

Watson, M. L., D'Eustachio, P., Mock, B. A., Steinberg, A. D., Morse, H. C., Oakey, R. J., Howard, T. A., Rochelle, J. M., and Seldin, M. F. A linkage map of mouse chromosome 1 using an interspecific cross segregating for the *gld* autoimmunity mutation. *Mammalian Genome 2:* 158–171, 1992

Wright, M. D., Rochelle, J. M., Tomlinson, M. G., Seldin, M. F., and Williams, A. F. Gene structure, chromosomal localisation, and protein sequence of mouse CD53 (*Cd53*): evidence that the transmembrane 4 superfamily arose by gene duplication. *Inter Immunol 5:* 209–216, 1992

Wymore, R. S., Korenberg, J. R., Kinoshita, K. D., Aiyar, J., Coyne, C., Chen, X., Hustad, C. M., Copeland, N. G., Gutman, G. A., Jenkins, N. A., and Chandy, K. G. Genomic organisation, nucleotide sequence, biophysical properties, and localisation of the voltage-gated K^+ channel gene *KCNA4/Kv1.4* to mouse chromosome 2/human 11p14 and mapping of *KCNC1/Kv3.1* to mouse chromosome 7/human 11p14.3-p15.2 and *KCNA1/Kv1.1* to human 12p13. *Genomics 20:* 191–202, 1994