Gene for a serum protein on chromosome 9 in the mouse

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SUMMARY

An electrophoretic variation in a mouse serum protein moving closely to the vitamin D binding protein is described. The variation is determined by two codominant alleles at one locus with the allele in DBA causing fast mobility and that in C57BL causing slow mobility. This locus is located in the proximal part of chromosome 9, 14.3 cM from the d locus and 31.9 cM from the Bgs locus. The protein has not yet been identified but possible candidates among the serum proteins are discussed.

1. INTRODUCTION

Genetic variation and chromosome assignment have been described for some of the proteins in serum and plasma of the house mouse. The structural genes for albumin, Alb-1, and transferrin, Trf, have been mapped on chromosomes 5 (Nichols, Ruddle & Petras, 1975) and 9 (Shreffler, 1963), respectively. Two haemolytic complement component genes (C3 and C4) as well as a plasma protein gene, P1p, have been localized to chromosome 17, close to the H-2 region (DaSilva et al. 1978; Meo, Krasteff & Shreffler, 1975; Whitney, Taylor & Cherry, 1978) and another haemolytic complement factor, Hc, to chromosome 2 (Searle, 1975).

Two new variations associated with serum proteins were briefly described in Mouse News Letter by Eicher & Reynolds (1978) and were given the operational names serum protein-1 and -2, Sep-1 and Sep-2, respectively. Both these genes were found to be located on chromosome 9. Here we report on a serum protein variation that may be identical to Sep-1 as we find associations between this variation and two chromosome 9 markers.

2. MATERIALS AND METHODS

(a) Mice and sample preparation

Mice of strains C57BL/6K1 and DBA/2K1 as well as crosses between these were used as sources for serum, kidney, liver and spleen. The animals were bled by the eye and killed by cervical dislocation.

The blood was allowed to clot at room temperature for 2 h and was kept at +5 °C for an additional 16 h before serum was taken off. Tissue homogenates were prepared as described previously with two parts of distilled water for kidney and
liver and with five parts for spleen (Lundin & Seyedyazdani, 1973). Sera and supernatants were stored at \(-25^\circ\text{C}\) and were typed within 2 weeks.

(b) Electrophoresis

Serum samples were analysed by horizontal polyacrylamide gel electrophoresis. The method of gel casting, sample application and other details of electrophoresis were the same as described by Gahne, Juneja & Grolmus (1977). A step-wise gradient gel of acrylamide concentration 8, 4 and 10\% with a length of 1, 3 and 16 cm, respectively, was used. A discontinuous buffer system at pH 9-0 was used as described by Allen (1974). The gel buffer consisted of Tris-citrate (0-18 M) while the electrode buffer was Tris-borate (0-065 M). Molarity is given with respect to Tris. Samples were placed on the 4\% gel and electrophoresis was conducted until the albumin zone had migrated about 12 cm in the 10\% gel. Electrophoresis was carried out with a constant current of 60 mA and with an initial voltage of 15 V/cm. The final voltage was about 45 V/cm and the total time of electrophoresis was about 4 h. The gels were stained for 1 h in Coomassie blue G 250 in perchloric acid solution, followed by 30 min destaining in 5\% acetic acid.

(c) Autoradiography

The vitamin D binding protein fraction Gc (homologous to the Gc fraction of human serum) was identified after electrophoresis followed by autoradiography as described by Gahne & Juneja (1978).

(d) Detection of antitrypsin, caeruloplasmin and haemopexin

Trypsin inhibitory capacity of the protein fractions after electrophoresis was tested by the method of Uriel & Berges (1968). Caeruloplasmin was stained by using 3-amino-9-ethylcarbazole as substrate in a 1\% agar overlay and with an incubation time of 24 h (Robinson & Lee, 1967). The gels were stained for haemopexin by adding haematatin to the serum samples before electrophoresis, followed by staining with benzidine and $\text{H}_2\text{O}_2$ as used by Stewart & Lovrien (1971).

(e) Beta-galactosidase assay

Beta-galactosidase activities were determined with \(p\)-nitrophenyl-beta-D-galactopyranoside (Sigma) as earlier described (Lundin & Seyedyazdani, 1973). Individual kidney and spleen values were plotted on a two-dimensional diagram from which the different animals could be classified according to \(B_{gs}\) genotype (Lundin & Seyedyazdani, 1973). A few doubtful cases were resolved by using the liver activity values.

3. RESULTS

The variation described here can be seen in the post-albumin zone (Fig. 1). This protein moved just anodic to the Gc protein detected by autoradiography. The protein from DBA animals has a faster mobility than that from C57BL animals. \(F_1\) animals have the two electrophoretic forms of the protein in roughly equal
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amounts. This serum protein was quite resistant to repeated freezing and thawing. There was no difference in phenotype patterns between serum and plasma. The analysis of backcross data (Table 1) showed that this polymorphism was controlled by two codominant, autosomal alleles.

Fig. 1. Polyacrylamide gel electropherogram of mouse serum proteins stained with Coomassie blue G250. Gel buffer: 0.18 M Tris/citric acid, pH 9.0. Electrode buffer: 0.065 M Tris/boric acid, pH 9.0. Acrylamide concentration: 10%. Constant current: 60 mA, 4 h. Electrophoretic types of the variant protein: (A) fast, (B) slow, (C) heterozygote, (D) heterozygote, (E) fast.

A total of 72 back-cross animals were typed, 56 F1 × DBA and 16 F1 × C57BL. A rather strong association between this variation and coat colour was immediately apparent and was demonstrated to be due to linkage with the d locus on chromosome 9. A three-point test was made by determination of the Bgs types of the animals (Table 1). A calculation of the map distances gives 12.5 ± 4.4 cM between
and $Bgs$, 14.3 ± 4.7 cm between $d$ and the serum protein gene and 31.9 ± 6.2 cm between $Bgs$ and the serum protein gene.

The most likely order of the three loci is thus

serum protein ←—14.3—→$d$←—12.5—→$Bgs$.

This serum protein is not yet identified. It was possible to show that it is not the antitrypsin nor the vitamin D binding (Gc) protein (Fig. 1) or the serum esterase (Es-1).

Table 1. Mice from two types of back-crosses between C57BL/6K1 and DBA/2K1 and their distribution among different phenotypes: $F_1 \times C57BL$: 6 ♀, 10 ♂; $F_1 \times DBA$: 29 ♀, 27 ♂

| Back-cross $F_1 \times DBA$ | Coat colour |
|----------------------------|-------------|
|                            | Black and chocolate (Dd) | Blue and dilute chocolate (dd) |
| Galactosidase activity     | $Bgs^{dd}$ | 4* | 24 |
|                           | $Bgs^{da}$ | 25 | 3* |
| Serum protein              | Fast       | 6* | 25 |
|                           | Heterozygote | 23 | 2* |
| Galactosidase activity     | $Bgs^{da}$ | 9* | 22 |
|                           | $Bgs^{dd}$ | 19 | 6* |
| Serum protein              | Fast       | 9* | 22 |
|                           | Heterozygote | 19 | 6* |
| Back-cross $F_1 \times C57BL$ | Galactosidase activity |
|                            | $Bgs^{da}$ | 4* | 3 |
|                           | $Bgs^{dd}$ | 5  | 4* |

4. DISCUSSION

This serum protein variation is inherited in a classical codominant mode as can be seen in Fig. 1. This pattern is well-known for structural loci of many monomeric proteins like transferrin and albumin (Manwell & Baker, 1970). The heterozygote carries the parental components in roughly equal amounts and each electromorph in the heterozygote has a concentration of about half of that found in the homozygotes. Thus it seems likely that this variation is determined by the structural gene locus of the protein.

Associations are found between this electrophoretic serum protein variation and two chromosome 9 loci, $d$ and $Bgs$ (Table 1). The $d$, dilute, allele in homozygous condition causes dilution of the coat colour to make animals blue or dilute chocolate that would otherwise be black or chocolate. The $Bgs$ locus affects the
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beta-galactosidase activities in many tissues (Lundin & Seyedyazdani, 1973; Felton, Meisler & Paigen, 1974).

The likely location of the serum protein locus based on the recombination frequencies is the proximal part of chromosome 9, about 25 cM from the centromere. The distance measured between Bgs and d in this investigation is only 12.5 ± 4.4 which is much smaller than previous estimates which were 25.0 cM (Seyedyazdani & Lundin, 1973) and 21.2 (Felton et al. 1974). In a large unpublished material (324 back-cross animals) we find the recombination distance between Bgs and d to be 20.7 ± 2.3 cM. It is not possible to offer a good explanation for the deviating value in the present material except the limited size of the sample. Our earlier data are based on crosses between the very same mouse strains.

We have, so far, not been able to identify the protein. It was, however, possible to demonstrate that it is not the vitamin D binding protein called Gc in man. The variant protein moves very closely to the Gc protein (Daiger, Scanfield & Cavalli-Sforza, 1975) which does not in itself vary between the two mouse strains (Fig. 1).

We could also show that our variant protein does not have anti-trypsin activity as all components with strong anti-trypsin activity were found in the albumin zone in our electrophoretic system. It was further found to lack the enzyme activity of both haemopexin and caeruloplasmin. It must be remembered that the methods used here to demonstrate binding and activity are at best semiquantitative. This makes it possible for weaker binding and activity to be hidden after separation in polyacrylamide gels.

There are a limited number of candidates for identity with this protein that moves in the alpha2-region. As it occurs in moderate but more than trace amounts in serum and plasma it is not likely to be either Zn-alpha2-glycoprotein or thyroxine-binding globulin. Due to its fairly rapid movement in rather dense polyacrylamide gels it ought to have a molecular weight that is well below 100000. This excludes several other proteins from the list (Putman, 1975). The most likely candidates to our mind are antithrombin III which is a single polypeptide chain and alpha2-HS-glycoprotein. Both of these move in the alpha2-region and have molecular weights around 65000 and 49000, respectively.

On the basis of the likely location of the serum protein gene in the proximal part of chromosome 9 it seems possible that it could be identical to Sep-I, briefly described by Eicher & Reynolds (1978). The electrophoretic mobilities of the protein in C57BL and DBA mice are, however, reversed in comparison with the information given by these authors. A decision on this point of possible identity must await further information about the Sep-I variation.

Another variant serum protein (Sep-2) was described by Eicher & Reynolds (1978) but its preliminary localization to a more distal region of chromosome 9 makes it a less likely alternative.

A final point which we like to raise concerns nomenclature. It seems unsatisfactory to use names such as serum protein-1 and -2 other than as provisional names. As soon as the real identities of these proteins are known they should be
renamed preferably to conform as much as possible with designations used in other mammals, especially in man.

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