

## Close linkage between albumin and vitamin D binding protein (Gc) loci in chicken: a 300 million year old linkage group

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### SUMMARY

Evidence for close genetic linkage between the structural loci for serum albumin (*Alb*) and serum vitamin D binding protein (*Gc*) in chicken is presented. The results are based on a study of a single sire family comprising 36 informative offspring. No recombinants have been observed. It is concluded that this linkage in the chicken is homologous to the close linkage of the albumin and *Gc* loci reported in man and the horse. Thus, this linkage group has most probably been conserved for at least 300 million years.

### 1. INTRODUCTION

Close genetic linkage between the structural loci coding for serum albumin and serum vitamin D binding protein (*Gc*) has been reported in man (Weitkamp, Rucknagel & Gershowitz, 1966) and in horses (Sandberg & Juneja, 1978; Weitkamp & Allen, 1979). The recombination frequency between the two loci was estimated at about 2% in man (Weitkamp *et al.* 1970) and about 1% in the horse (Sandberg & Juneja, 1978).

In the chicken, serum albumin (*Alb*) polymorphism was demonstrated by McIndoe (1962). Four alleles (*Alb<sup>F</sup>*, *Alb<sup>S</sup>*, *Alb<sup>C</sup>* and *Alb<sup>C1</sup>*) have so far been reported (reviewed by Baker *et al.* 1970). Recently we detected genetic polymorphism of chicken serum vitamin D binding protein (*Gc*). Two autosomal alleles designated *Gc<sup>F</sup>* and *Gc<sup>S</sup>* were observed (Juneja *et al.* 1982). This finding prompted us to test for linkage between *Alb* and *Gc* in chicken. The present report documents close linkage of the two loci also in this species.

### 2. MATERIALS AND METHODS

#### (i) *Chicken*

One cock (Rhode Island Red breed) heterozygous at the albumin and *Gc* loci (*Alb<sup>F</sup>/Alb<sup>S</sup>*, *Gc<sup>F</sup>/Gc<sup>S</sup>*) was mated to hens of White Leghorn breed by artificial insemination. All the hens were homozygous at the two loci (*Alb<sup>F</sup>/Alb<sup>F</sup>*, *Gc<sup>S</sup>/Gc<sup>S</sup>*). The linkage phase of the cock was unknown but its gametic contribution could be determined from the phenotypes of the offspring. Altogether 36 resulting chicks were phenotyped for albumin and *Gc*.

(ii) *Electrophoresis*

Serum albumin phenotyping was done by acid starch gel electrophoresis according to Gahne (1966). Serum Gc phenotypes were determined by two-dimensional agarose gel-polyacrylamide gel electrophoresis as described by Juneja *et al.* (1982).

Table 1. *Segregation data for Alb and Gc in one sire family*

Sire's genotype	Gamete transmitted from sire to offspring				$\Sigma$
	$Alb^F Gc^F$	$Alb^F Gc^S$	$Alb^S Gc^F$	$Alb^S Gc^S$	
$Alb^F Alb^S, Gc^F Gc^S$	22	0	0	14	36

## 3. RESULTS

Fourteen of the chicks studied had the phenotypes Alb FS, Gc S while the remaining 22 chicks were of the phenotypes Alb F, Gc FS. The segregation data (Table 1) provide evidence for close genetic linkage between *Alb* and *Gc* in chicken. The single locus segregation for each of the loci did not deviate significantly from the expected 1:1 ratio. The *Alb/Gc* haplotypes of the cock studied evidently were  $Alb^F Gc^F / Alb^S Gc^S$ . No recombinant was observed among the 36 informative offspring.

In the absence of recombinants, an upper limit of the recombination frequency ( $r$ ) between two loci can be estimated by using the formula  $P = (1-r)^n$ , where  $P$  is the probability of seeing no recombination by chance alone and  $n$  is the number of potentially recombinant chromosomes studied (Dizik & Elliott, 1977). In the present data  $n = 36$ . If we set  $P = 0.05$  (5%), then  $r = 0.08$ . Hence there is a 95% probability that the recombination frequency between *Alb* and *Gc* does not exceed 8%. Thus, the recombination frequency in chicken seems to be similar to the 1–2% observed in man and the horse.

## 4. DISCUSSION

The detection of linkage between the loci for serum albumin and serum vitamin D binding protein (Gc) in the present study, establishes a new linkage relationship in chicken. Neither of these two loci has been assigned to any of the ten linkage groups previously detected in chicken (reviewed by Somes, 1979). Furthermore, the albumin locus was reported to segregate independently of 13 other loci (see Etches & Hawes, 1979).

The close linkage of the albumin and Gc loci in man, horse and chicken strongly suggests that these species share a homologous chromosome segment conserved since the time of divergence from a common ancestor (approximately 300 million years ago – cf. Jarvik, 1980). Interspecies homologies of the genes for each of these two proteins are based on previous biochemical studies. It has been shown that avian albumin and Gc have similar physicochemical characteristics, molecular weights and amino acid compositions to that of mammalian albumin and Gc, respectively (Peters, 1975; Bouillion *et al.* 1980). However, each of these proteins has, in the course of evolution, changed to the extent that it shows only very little immunological cross-reaction between the vertebrate classes (Peters, 1975; Bouillion *et al.* 1980).

Gene families arisen by regional gene duplication such as the  $\beta$ -globin cluster have often remained intact during the course of evolution (see e.g. Ingram, Scott & Tilghman, 1981). Gene mapping has revealed several linkage homologies among mammalian species even for loci not in close proximity on the chromosome (cf. Ohno, 1973; Minna, Lalley & Francke, 1976; Lundin, 1979; Searle, 1981). There

are some indications that these linkage homologies extend beyond the class of mammals. For instance the loci coding for 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase/hexose-6-phosphate dehydrogenase are linked in *Drosophila*, the fish genus *Xiphophorus*, and mouse, and are syntenic in a frog species (see Morizot, Wright & Siciliano, 1977). The result of the present study clearly proves the existence of linkage homologies between species of different vertebrate classes and further corroborates the concept of stable linkage relationships in the vertebrate genome.

The karyotypes of avian and mammalian species are strikingly different. Birds have in general a few macrochromosomes and a large number of microchromosomes whereas mammals have macrochromosomes exclusively. This fact suggests that extensive chromosomal rearrangements have taken place during the course of evolution. The chromosome segment containing the genes for serum albumin and Gc appears, however, to have remained intact. Close linkage between these two loci is thus expected in most mammalian and avian species. An obvious topic for future research is to test for linkage of the two loci in other vertebrate classes; serum albumin and a serum vitamin D binding protein have been detected in amphibians and teleosts (see Peters, 1975; Bouillion, Van Baelen & de Moor, 1980).

The basis for the observed stability of linkage relationships in the vertebrate genome is not yet clarified. In the case of the remarkable conservation of *Alb/Gc* linkage, it is interesting to note that the genes for  $\alpha$ -fetoprotein and albumin which most probably arose by an ancient gene duplication are still in tandem in the mouse genome (Ingram, Scott & Tilghman, 1981). These genes are sequentially expressed during development and code for the major serum protein in the fetus and the adult, respectively. The *Alb/Gc* linkage group in chicken may contain the locus for an avian homologue to mammalian  $\alpha$ -fetoprotein. A fetal-specific  $\alpha$ -globulin with properties and tissue distribution similar to those of mammalian  $\alpha$ -fetoprotein has been reported in chicken (Lindgren, Vaheri & Ruoslahti, 1974). These findings could be significant for the explanation of possible constraints on chromosome rearrangements in the *Alb-Gc* region.

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