Linkage and interdependence of A2MD1 and A2ME2 alpha$_2$-macroglobulin genes in cattle

Jan WĘGRZYN, Elżbieta SKIBA, Piotr KRZYŚCIN

Department of Immuno- and Cytogenetics, National Research Institute of Animal Production, Balice, Poland

Abstract. The transmission of previously described genes A2MD1 and A2ME2 that determine antigenic markers of alpha$_2$-macroglobulins A$_2$mD1 and A$_2$mE2 in cattle was studied. The starting point for the analyses was the lack of individuals negative for both markers in the population of 3551 Black-and-White, Red-and-White, Polish Red and Simmental cattle and interbreed crosses. Controlling of these specificities by allelic genes or genes from closely linked loci was considered. To support or reject this hypothesis, the independence test 2 × 2 and analysis of segregation of A$_2$mD1 and A$_2$mE2 in the offsprings of all phenotypic matings found and of selected matings in which genotypes of sires were determined, were used. It was found that the observed segregation of antigenic markers in the offsprings rules out the possibility that they are determined by allelic genes. The results obtained show that markers A$_2$mD1 and A$_2$mE2 are controlled by the genes A2MD1 and A2ME2 from closely linked loci. Moreover it seems that only those haplotypes are transmitted in which both genes – A2MD1 and A2ME2, or one of them – A2MD1 or A2ME2, are present. No haplotype would then be transmitted (would occur?) in which both genes are in the recessive form.

Key words: alpha$_2$-macroglobulins, antigenic determinants, cattle, genes, markers.

Introduction

Five antigen markers of alpha$_2$-macroglobulin proteins have so far been discovered, which were designated McA1, McA2, McB1, A$_2$mD1 (previously BA7) and A$_2$mE2. The markers McA1 and McA2 (IANNELLI, MASINA 1978) are controlled by allelic codominant genes, while McB1 (IANNELLI, CAPPA-

RELLI 1979) by the dominant gene, which is independent of genes that determine McA1 and McA2. The remaining protein markers of this fraction – A_2mD1 (WILLMANN-WĘGRZYN, WĘGRZYN 1975) and A_2mE2 (WĘGRZYN et al. 1996) are determined by autosomal, dominant genes from open systems. One determinant-one gene relationship is maintained between the antigenic specificities and the genes which control them. Of another macroglobulin marker, described by ABE et al. in 1972, it is only known that in chromatography on sephadex G-200 it was washed out in the first fraction (void volume) and its migration rate equalled that of alpha-globulins. The authors did not specify if this factor was connected with $alpha_2$-macroglobulins or class IgM immunoglobulins (after FABER, STONE 1976).

In the present study, the presence and segregation of markers of $alpha_2$-macroglobulins A_2mD1 and A_2mE2 in cattle were analysed, and genetic relations between genes A2MD1 and A2ME2, which determine the specificities under discussion, were considered.

Material and methods

The genetic relationships between the previously detected antigenic markers of $alpha_2$-macroglobulins A_2mD1 (provisionally designated BA7) and A_2mE2 (WILLMANN-WĘGRZYN, WĘGRZYN 1975, WĘGRZYN et al. 1996) were evaluated on a population of 1753 offsprings of 73 sires and 1725 dams of Black-and-White, Red-and-White, Polish Red, Simmental cattle, and crossbreds of Polish Red with Simmental and Charolais breeds. From 10 to 101 offsprings of one sire were examined.

In the antigenic specificity transmission analysis it was assumed that A_2mD1 (mD1 in short) and A_2mE2 (mE2 in short) are determined by allelic or closely linked genes: A2MD1 (D1 in short) and A2ME2 (E2 in short), respectively. Allelic forms of genes A2MD1 and A2ME2, whose products are not detected yet (recessive by convention) were designated A2MD0 (D0 in short) and A2ME0 (E0 in short), respectively. Gene frequencies in particular breeds were 0.52, 0.36, 0.11 and 0.10 for A2MD1, and 0.57, 0.68, 0.85 and 0.93 for A2ME2, respectively.

To support/reject this hypothesis, the presence of both specificities in the whole population studied was analysed as well as the distribution of these markers in the offsprings of all phenotypic matings and of selected matings, in which sire genotypes could be determined based on the segregation of traits in the offsprings. Independence test 2×2 was also used with simplified formulas for χ^2 and a correlation coefficient for alternative traits r_a (ŻUK 1989).

The abbreviated symbols of the markers under discussion and the genes which control them, used here have only been introduced for the sake of this publication.

Results

In the material under study, which included parents and their offsprings, there were individuals having both antigenic specificities or one of them. There were no individuals negative for both factors. In the 2×2 contingency test (Table 1), used to evaluate the independent presence of mD1 and mE2, highly significant values of χ^2 (328.74) and correlation coefficient r_a (0.30) were found.

Table 1. 2×2 independence test of A_2mD1 and A_2mE2 antigenic markers

Marker		A_2mE2	
		+	–
A_2mD1	+	1591	360
	–	1600	0

$\chi^2 = 328.74$; $r_a = 0.30$

Among the parents only six phenotypic matings were found of the type:

$\sigma\, mD1^+mE2^+ \times \varphi\, mD1^+mE2^+$, $\sigma\varphi\, mD1^+mE2^+ \times \varphi\sigma\, mD1^+mE2^-$,

$\sigma\varphi\, mD1^+mE2^+ \times \varphi\sigma\, mD1^-mE2^+$, $\sigma\, mD1^+mE2^- \times \varphi\, mD1^+mE2^-$,

$\sigma\varphi\, mD1^+mE2^- \times \varphi\sigma\, mD1^-mE2^+$ and $\sigma\, mD1^-mE2^+ \times \varphi\, mD1^-mE2^+$

(Table 2).

In four of them (I, II, III and V), both the segregation and the lack of some phenotypes in the offsprings were inconsistent with the values which should occur if these specificities were determined by allelic genes.

From among 73 sires studied, individual genotypes of 32 $mD1^+mE2^+$ bulls were determined, in the first stage independently for mD1 or mE2, through analysis of markers distribution in the offsprings of each of them and in the dams which had no mD1 and mE2, respectively. It was found that 22 bulls were heterozygous for both D1 and E2. The next two groups of 5 sires each had genotypes D1/D0 and E2/E2, and D1/D1 and E2/E0, respectively.

Analysing the number and segregation of both specificities in the offsprings of 770 dams of phenotypes $mD1^+mE2^-$, $mD1^+mE2^+$ and $mD1^-mE2^+$ it was deduced that the sires of particular groups in fact had the following genotypes: 22 ♂♂ D1E0/D0E2; 5 ♂♂ D1E2/D0E2 and 5 ♂♂ D1E2/D1E0 (Table 3).

Table 2. The distribution of A_2mD1 and A_2mE2 in the offsprings of all phenotypic matings in Black-and-White, Red-and-White, Polish Red and Simmental cattle

Mating type*	No. of ma-tings	Phenotypes observed and number of offsprings		
		$mD1^+mE2^-$	$mD1^+mE2^+$	$mD1-mE2^+$
I ♂ $mD1^+mE2^+$ × ♀ $mD1^+mE2^+$	416	80	270	66
II ♂ ♀ $mD1^+mE2^+$ × ♀ ♂$mD1^+mE^-$	194	68	125	2
III ♂ ♀ $mD1^+mE2^+$ × ♀ ♂$mD1^-m2^+$	566	3	290	273
IV ♂ $mD1^+mE2^-$ × ♀ $mD1^+mE^-$	37	37	–	–
V ♂ ♀ $mD1^+mE2^-$ × ♀ ♂$mD1^-mE2^+$	91	–	85	6
VI ♂ $mD1^-mE2^+$ × ♀ $mD1^-mE2^+$	403	–	–	403

* $mD1^{+/-}$ – presence/absence of A_2mD1.
$mE2^{+/-}$ – presence/absence of A_2mE2.

From the matings of 22 sires to 51 dams $mD1^+mE2^-$ and with 227 dams $mD1^-mE2^+$, there were offsprings with phenotypes of their parents – $mD1^+mE2^+$ and $mD1^+mE2^-$ and $mD1^+mE2^+$ and $mD1^-mE2^+$, respectively, the proportions in both cases being close to 1:1. There were no individuals with phenotypes $mD1^-mE2^+$ and $mD1^+mE2^-$. From the matings of these sires with 276 dams $mD1^+mE2^+$, the number of offsprings with determined three phenotypes were approximate to 1:3:1.

From the matings of 5 sires to 13 dams $mD1^+mE2^-$ the whole offsprings had a phenotype of their sires – $mD1^+mE2^+$. From the matings of these sires with the remaining 51 and 42 dams there were offsprings $mD1^+mE2^+$ and $mD1^-mE2^+$ in proportions approximate to 2:1 and 1:1, respectively.

Finally, from the matings of the last group of 5 sires there were offsprings $mD1^+mE2^-$ and $mD1^+mE2^+$ from 14 and 72 dams in proportions approximate to 4:1 and 1:2, respectively, and only individuals $mD1^+mE2^+$ from 24 dams $mD1^-mE2^+$.

Table 3. Segregation of A_2mD1 and A_2mE2 in the offsprings of fathers with deduced genotypes mated to dams with phenotypes mD1$^+$mE2$^-$, mD1$^+$mE2$^+$, mD1$^-$mE2$^+$ *

Deduced genotypes of sires**	No. of sires	Phenotypes of dams	No. of dams	Phenotypes observed and number of offsprings		
				mD1$^+$mE2$^-$	mD1$^+$mE2$^+$	mD1$^-$mE2$^+$
A2MD1 A2ME0 / A2MD0 A2ME2	22	mD1$^+$mE2$^-$	51	30	21	–
		mD1$^+$mE2$^+$	276	53	177	46
		mD1$^-$mE2$^+$	227	–	109	118
A2MD1 A2ME2 / A2MD0 A2ME2	5	mD1$^+$mE2$^-$	13	–	13	–
		mD1$^+$mE2$^+$	51	–	35	16
		mD1$^-$mE2$^+$	42	–	18	24
A2MD1 A2ME2 / A2MD1 A2ME0	5	mD1$^+$mE2$^-$	14	11	3	–
		mD1$^+$mE2$^+$	72	23	49	–
		mD1$^-$mE2$^+$	24	–	24	–

* see Table 2.

** genotypes were determined on the basis of A_2mD1 and A_2mE2 segregation in the offsprings for each specificity separately.

There were no offsprings mD1$^+$mE2$^-$ after any of 106 (13+51+42) dams mated to the first group of five sires, just as there were no offsprings mD1$^-$mE2$^+$ after any of 110 (14+72+24) dams mated to the second group of five sires.

Discussion

In the population under study there were no individuals devoided of both mD1 and mE2, which suggests that the markers are not transmitted independently. This conclusion is confirmed by the highly significant values of χ^2 and of the correlation coefficient for alternative traits in the 2×2 independence test. The dependent transmission is also indicated by the distribution of mD1 and mE2 among the offsprings of four phenotypic matings found among the parents. The results of these matings also preclude the possibility that mD1 and mE2 are controlled by allelic genes.

In the material studied the distribution of both antigenic determinants in the offsprings from standard test matings of the type double heterozygote × recessive homozygote could not be analysed, as such matings were not found. Segregation of the factors studied in the offsprings of 32 sires, whose genotypes were determined, were analysed. Segregation of both determinants in the offsprings of 22 bulls, double heterozygotes, indicates that the genes which determine mD1 and mE2 are located on the chromosomes of the same pair, opposite each to another. The distribution of the traits in the offsprings of

the next two groups of sires of 5 individuals each, does not only confirm the opposing arrangement of genes D1 and E2, but also provides evidence that these genes can also be located next to each other on one of the homologous chromosomes. The statement that among these sires $mD1^+mE2^+$ there are homozygotes D1/D1 or E2/E2, that is individuals having both genes (D1E2) on one chromosome, enables one to ultimately reject the possibility that A_2mD1 and A_2mE2 are determined by allelic genes.

The lack of individuals with phenotypes $mD1^-mE2^-$, the exclusion of allelism between D1 and E2, the high (0.30) correlation coefficient between mD1 and mE2, and the genotypes of the sires (second and third group in Table 3) clearly indicate that the characters analysed are determined by closely linked genes.

The determination of markers mD1 and mE2 by the genes D1 and E2 from closely linked loci can explain some of the observed numbers of offsprings with particular phenotypes. For example, 22 sires with genotypes D1E0/D0E2, mated to 276 dams $mD1^+mE2^+$ produced offsprings with three different phenotypes whose number was approximate to the 1:3:1 proportion. Such distribution of traits results from the genotypes of dams, in which under phenotypes $mD1^+mE2^+$ there should be, in addition to at least double heterozygotes, also homozygotes for D1 or E2 or for both genes.

The linkage of genes controlling mD1 and mE2 also explains the lack of offsprings $mD1^+mE2^-$ from all dams mated to sires D1E2/D0E2 (second group of sires) and the lack of offpring $mD1^-mE2^+$ from all dams mated to sires D1E2/D1E0 (third group). In both cases the offsprings' phenotypes are determined by sire genotypes: the offsprings of sires homozygous for a given gene must receive one of the alleles of this gene.

The lack of offsprings of phenotypes $mD1^-mE2^+$ and $mD1^+mE2^-$ among progeny of 22 sires D1E0/D0E2 mated to 51 dams $mD1^+mE2^-$ and 227 dams $mD1^-mE2^+$, respectively, was observed. Moreover, the lack of offsprings $mD1^-mE2^+$ within progeny of 5 sires D1E2/D0E2 mated to 13 dams $mD1^+mE2^-$ as well as the lack of offsprings $mD1^+mE2^-$ within progeny of 5 sires D1E2/D1E0 mated to 24 dams $mD1^-mE2^+$ were noticed. In the above-mentioned matings, mD1 and mE2 segregated in the offsprings as if all dams $mD1^+mE2^-$ were homozygotes D1/D1, while dams $mD1^-mE2^+$ were homozygotes E2/E2 and transmitted either gene D1 or E2, respectively. In this case under phenotypes of 51 and 13 dams $mD1^+mE2^-$ and of 227 and 24 dams $mD1^-mE2^+$ there would be only individuals with genotypes D1E0/D1E0 and D0E2/D0E2, respectively. Therefore, among these dams there would be no genotypes D1E0/D0E0 and D0E2/D0E0, respectively, in which both genes

occur in the recessive form in one haplotype. In consequence only those haplotypes would be transmitted in which both genes (D1 and E2) or one of them (either D1 or E2) occurred.

Such concept of linkage and interrelation between both genes seems to explain the characteristic segregation mD1 and mE2 in the offsprings from the matings of sires with deduced genotypes and dams with all phenotypes found, as well as the lack of individuals mD1⁻mE2⁻ in the whole population studied (3551 animals).

The analysis of the presence and segregation of antigenic markers of $alpha_2$-macroglobulins A_2mD1 and A_2mE2 in cattle ruled out the possibility that these factors are determined by allelic genes. It was found, however, that genes A2MD1 and A2ME2 are in closely linked loci. The results obtained also indicate that only those haplotypes are transmitted in which both genes – A2MD1 and A2ME2, or one of them – A2MD1 or A2ME2, occur. No haplotype would then be transmitted (would occur?) in which both genes are in the recessive form.

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