HEMOGLOBIN PHENOTYPES IN MURGESE HORSE

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ABSTRACT

In this note we describe two new equine hemoglobin phenotypes found during a survey of the Murgese horse, a rare Apulian native breed, among whose ancestors the Arabian surely plays an important role. To date we have analysed about 300 individual hemolysates by different chromatographic analyses (PAGIF, IPG, CMC). The results pointed out two unusual patterns where the ratio of the ε24Phe60Gln band to the ε24Phe60Lys band was 93:7 and 70:30 rather than 60:40 which would have been expected of BII homozygote. Given that the three horses exhibiting the unusual patterns shared a common ancestor and that none of the possible combinations of the known haplotypes can account for 7-8% ε24Phe60Lys, reasonably a triplicated arrangement has to be postulated.

Key words: Hemoglobin, Alpha globin gene, Alpha globin gene arrangements, Gene expression, Horse.

RIASSUNTO

FENOTIPI EMOGLOBINICI NEL CAVALLO MURGESE

In questa nota si riportano i risultati di uno studio sul polimorfismo emoglobinico negli equini di razza Murgese, razza autoctona pugliese tra i cui antenati, il cavallo Arabo ha giocato un ruolo di rilievo. All'uopo gli emolisati di 300 soggetti sono stati sottoposti a differenti analisi cromatografiche (PAGIF, IPG e CMC) per la valutazione quali-quantitativa delle emoglobine e delle relative globine. I risultati hanno rivelato la presenza di un polimorfismo ascrivibile per lo più alle varie combinazioni degli aplotipi BI e BII, le cui frequenze calibrate - 0.36 e 0.58 rispettivamente - sono abbastanza simili a quelle riportate per il cavallo Arabo; inoltre accanto alla presenza più o meno sporadica di altri aplotipi quali A, AII e V, tre campioni individuali presentavano rapporti inconsueti tra le bande emoglobine ε24Phe60Gln / ε24Phe60Lys: in particolare rispetto al normale rapporto 60:40, tipico dell’omozigote BII, uno esibiva un pattern nel quale il rapporto tra le bande suddette era di 93:7 e gli altri due di 72:28. Nessuna delle combinazioni possibili sulla base degli aplotipi noti dà ragione dei rapporti rilevati per cui, considerando da un lato la presenza di un antenato comune nei dati anagrafici dei tre cavalli in questione e dall’altro il gradiente di espressione dei geni del cluster alfa globinici, gli autori ipotizzano l’esistenza di un arrangiamento triplicato nel quale il gene in terza posizione codifica per una quota di prodotto pari al 20% del prodotto del gene in seconda posizione che equivale all’8%.

Parole chiave: Emoglobina, Geni alfa globinici, Aplotipi alfa globinici, Espressione genica, Cavallo.
Introduction

It is commonly acknowledged that α globin genes in mammals: i) are present in duplicate copies; ii) the duplicate copies are closely linked on the chromosome; iii) the duplicate loci produce subunits in unequal amounts. Thus, the loci showing characteristic differences in the average level of globin production, the relative densities of the electrophoretic bands can deduce many genotypes.

In horses two common haplotypes, BI and BII, can be found each encoding for two different α-globins, so that there can be up to four different kinds of hemoglobins in a single animal (Kilmartin and Clegg, 1967). In particular the 5' α2 gene encodes a α globin with glutamine (Gln) at position 60 while this position is occupied by lysine (Lys) in the α globin produced by the 3' α1 gene. Moreover, at position 24, in the chains encoded by each of the linked α genes, either tyrosine (Tyr) or phenylalanine (Phe) may be found, which characterise the above haplotypes BI and BII, the former encoding for α24Tyr/60Gln (αTG), and α24Tyr/60Lys (αTL) chains and the latter encoding for α24Phe/60Gln (αPG), and α24Phe/60Lys (αPL) chains. Given that the couple of linked genes displays a gradient of decreasing expression from the 5' to the 3', the quantity of each hemoglobin depends on the number and on the percentage efficiency of the gene producing the relative α globin. In both BI and BII haplotypes the two linked genes are expressed in a 60/40 ratio, the α globin with the Gln in position 60 being produced by the higher-output gene α2 (Bowling et al., 1988). BI and BII are present in all examined horse populations which may however be distinguished on the basis of recombinant haplotypes, possibly different in frequency or in gene arrangements. Various aberrant haplotypes have been generated from BI and BII, probably as a consequence of intrachromosomal conversion - such as AIIa, C or N haplotypes - or of unequal crossover - such as the single α gene haplotype AIIb or the triplicated α gene arrangement V found in Arabians (Bowling et al., 1988).

In this note we give the results of a survey of the hemoglobin polymorphism in the Murgese horse and suggest the presence of an additional equine triplicated α gene arrangement.

Material and methods

The Murgese horse, an Apulian native breed, experienced a bottleneck, which reached its minimum in the 80s. In the last decade, the numerical trend of the breed changed but it is a fact that a very small number of males contributed to the gene pool of the present day population of about 1200 individuals.

A total of 300 Murgese horses from different Apulian farms were analysed as far as hemoglobin phenotype is concerned. In order to define Hb phenotypes, hemolysates, obtained following standard procedures, were routinely analysed by conventional IEF (PAGIF) in a pH gradient 6.7-7.7 (Di Luccia et al., 1991b). Isoelectric focusing on immobilised ultra narrow pH (7.0-7.4) gradient (IPG) (Di Luccia et al., 1991a) was also performed to better define the phenotype of some selected samples. The evaluation of individual Hb band patterns was obtained by scanning the PAGIF gels with a computerised ULTROSCAN XL Enhanced Laser Densitometric equipped with Gelscan 2.0 software from Pharmacia-LKB. Alpha globins of selected samples were isolated from hemoglobins by chromatography on CM-cellulose according to Clegg et al. (1966); three chromatograms for each of the selected samples were carried out. The identification of single hemoglobin bands and relative globins was obtained by comparison with known BI/BI, BI/BII and BII/BII samples.

Gene frequencies were estimated by the EH program (Terwillinger and Ott, 1994).

Results and discussion

The AII, BI, BII, and V haplotypes account for most of the phenotypes found in the Murgese population (Tab.1). Accordingly with the above cited origins, frequency data are similar as in Arabians except for the presence of a couple of heterozygous individuals carrying the A haplotype, which is absent in Arabians. However, not all the phenotypes clearly fit into this pattern, an unexpected quantitative polymorphism being observed, some of which will require further examinations.

Among the abnormal quantitative patterns, three samples attracted our attention because of
the unusual band proportions (Fig. 1). In horse 236 (lane 3), band Hb3 ($\alpha_2^{PL} \beta_2$) was extremely low (7%), as opposed to band Hb1 ($\alpha_2^{PG} \beta_2$) which was exceedingly high (93%); in horse 7 (lane 2) and horse 15 (lane 6) the ratios between Hb1 and Hb3 bands, averaged 72:28. None of the possible combinations of the haplotypes described to date can account either for 7% or 28% amount of band Hb3. From examination of the pedigree data, the above three horses resulted to be: the son (236) and two (7 and 15) grandsons of the same stallion. Taking into account that they were adults exhibiting normal hematological values and thus, no developmental or pathological variations might be inferred, a genetic determination was the most reasonable hypothesis for the quantitative phenotypes. This hypothesis is in line with previous investigation on multiple globin genes in sheep (Vestri et al. 1991), suggesting that the 7% protein expression could be justified by the presence of a novel triplicate arrangement in which only the downstream $\alpha$ gene encoded for $\alpha^{GR}$ globin. The two samples characterised by the same band pattern (Hb1 and Hb3) and exhibiting the unusual proportion 72:28 seemed to be in accordance with the above inference. Hence, we focused our analytical work on the 1-3 Hb bands in order to compare the results from the BII homozygotes ($\alpha_2^{PG} \beta_2 : \alpha_2^{PL} \beta_2 = 60 : 40$) with those from individuals showing unusual patterns.

Figure 1. IPG of Hbs from animals with the following phenotypes: (1 and 7) BII/BII reference sample; (2) BII/VI horse 7; (3) AII/VI horse 236; (4 and 5) BI/BII reference sample; (6) BII/VI horse 15; (8) BI/BI reference sample.
In Fig.2 the different ratios between Hb1 and Hb3 bands are shown as the results of PAGIF band densitometry: namely 93:7 (panel a), 72:28 (panel b) and 60:40 (panel c). The results of CMC, indicated that globin structural variants were absent, with the chromatograms demonstrating the existence of one peak corresponding to the β chain and only two peaks corresponding respectively to αm globins and αc (fig.3). As to the relative proportions between the two α chains in the three samples concerned, CMC analyses confirmed the previous estimates.

Multiple α gene arrangements are relatively common in Mammals. Studying the human α globin genes, Liebhaber et al. (1986) demonstrated that the α2 locus plays a dominant role in mRNA and protein synthesis and encodes 2-3 times protein than the α1. Similarly, in sheep one of our co-workers determined both at protein (Vestri et al., 1991) and mRNA level (Vestri et al., 1994) progressive decreases from the 5' to the 3' end. In particular, the level of expression of the 5' α gene does not seem essentially affected, whereas the intermediate genes (one or two) apparently produce the amount of α chain missing to provide a normal output; for example, in the ααα (30:14:6) the efficiency of the 3' is reduced from 18 to 6, approximately.

The 7% protein production found in horse 236 is coherent with the above findings on the expression of the 3' gene in sheep ααα arrangements as well as with that noticed by Bowling et al. (1988) in the case of V haplotype where the third gene expresses 8-9% of α 24Tyr60Lys. As we already mentioned, this ααα haplotype, found in Arabians, was supposed to have been generated by unequal crossover events involving BI chromosomes.

Likewise, a triplicated α haplotype VI, where the upstream genes produce α 24Phe60Gln (α 24Phe60Gln=42-43%) and the third gene expresses 7-8% of α 24Phe60Lys, may have arisen by an unequal crossover between BII chromosomes (α 24Phe60Gln=30% and α 24Phe60Lys=20). Thus, considering that AIIa haplotype encodes only for α 24Phe60Gln, we may infer that the phenotype observed in horse 236 is consistent with a putative AIIa/VI (α24Phe60Gln=50+43% and α 24Phe60Lys=7%) combination, while the one
Figure 3. Elution patterns of globin chains by means of CM-cellulose chromatography. The hemoglobin samples were the same as in figure 2. The different proportion of α globin chain accounts for different proportion found in the hemoglobin samples. Panel a), b) and c) show 8%, 28% and 40% of α^a globin, respectively.

![Elution Patterns](image.png)

observed in horse 7 and 15, seems to correspond to a BII/VI (α24Phe60Gln=30+42% and α24Phe60Lys=20+8%).

On the basis of these results, suggesting the existence of a tentatively named VI ααα arrangement, we are encouraged to perform family studies and experimental work aiming to obtain both genetic and molecular information which may provide conclusive data on the above triplicated haplotype.

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