[Regular Paper]An additional classification for equine Hb- α types

Michinari Yokohama

SUMMARY

Equine hemoglobin- α (Hb- α) types have been classified into ten genotypes using the four haplotypes of A, AII, BI and BII which have been identified internationally. However, Hb- α types with the new haplotype not being able to be applied to the ten known Hb- α types, were detected in Hokkaido native horses. These new variants were also observed in other foreign breeds of horses as well. In addition, since all of these new Hb- α types are clearly controlled by the same new haplotype, this new haplotype was given the name of CII, and then, CI as another haplotype compared with the supposed haplotype CII type. At the same time, an additional classification for the Hb- α types with the new haplotype was demonstrated, and then, some problems for typing them and the difference in the Hb types between donkeys and horses were also discussed.

Key words: equine Hb- α type, new haplotype CII, IEF.

INTRODUCTION

Equine Hb- α is separated into the I region with high mobility and II region with low mobility by isoelectric focussing (IEF) of pH range from 5.5 to 7.7. Then, the I and II regions can be separated into the two bands, respectively, with the equine Hb- α types being composed of a maximum of four bands.

Equine Hb- α types are classified ten genotypes by four haplotypes of A, AII, BI and BII. As haplotypes BI and BII form variants in which their bands can be detected in both the I and II regions, haplotypes A and AII characterize variants which their bands can not be detected in the II region. Accordingly, haplotypes without bands in the I region are not internationally recognized yet.

This report presents the results which shows the existence of the new haplotype, and at the same time, introduces and discusses about an additional classification method for equine Hb- α types with new variants.

MATERIALS AND METHOD

Samples: The Hb- α types in this report were detected with blood samples collected formerly from thoroughbreds, angloarabs, arabs, Japanese native horses (8 breeds) and heavy horses (Percheron and Breton lines),^{1,2)} and then ponies, donkeys and hinnys used newly recently. Each sample was used after adjusting the washed-packed red cell to 2-3% lysates with distilled water.

IEF method: The Hb- α types were characterized by the acrylamide gel IEF with a pH

ウマ Hb- α 型の新しい分類法

横濱道成; Laboratory of Animal Resources, Faculty of Bio-Industry, Tokyo University of Agriculture.
Correspondence address: Michinari Yokohama, Laboratory of Animal Resources, Faculty of Bio-Industry,
Tokyo University of Agriculture, 196, Aza-Yasaka, Abashiri 099-24, Japan.

(Received July 16, 1990, Accepted August 27, 1990)

range of 5.5-7.7 which Yokohama et al. already reported.^{1,2)}

RESULTS AND DISCUSSION

1. Classification by the four known haplotypes

As equine Hb- α types can be classified into ten genotypes using the four haplotypes A, AII, BI and BII (showed at the top in Fig. 1), nine Hb- α phenotypes with the exception of the AI/AI type are shown on photos A and B in Fig. 2. As is also evident from each Hb- α type pattern in these photos, the haplotypes BI and BII characterize variants in which their bands can be detected in both the I and II regions, but all variants with haplotypes A and AII in which their bands can not be observed in the II region with low mobility. Moreover, four bands of Hb- α types were marked respectively by Arabian figures from 1 to 4 with 1 designating the highest mobility.

As AII/AII type (1 band pattern) in which is homozygote of haplotype AII which was proposed

at first by Gahne et al. at the 19 th International Conference on Animal Blood Groups and Biochemical Polymorphism (Wageningen) in 1980 could not be detected in this time, but they mistakenly interpreted that the genotype AII/AII for demonstrating haplotype AII is expressed by the 1, 2 band pattern (this is actually the A/AII type) at that time.

For hemoglobin concentration of each type, bands of the heterozygotic types in which haplotype A and B series overlapped each other showed the strongest Hb color, and the remaining heterozygotes had secondary concentrations. When the bands of the haplotype B series forming heterozygotes with the haplotype A series are located in the II region, their bands showed the weakest color. Also, the Hb components in the I region were generally observed to have a higher color concentration than those in the II region.

Haplotypes A and AII variants were not recognized in light breed horses like thoroughbreds. Also, haplotypes A and AII are equivalent to the

Fig. 1. Diagram of equine Hb- α types by IEF.

Fig. 2. Equine Hb- α phenotypes.

Photo A: 1(BI/BII), 2(BII/BII), 3(BI/BI), 4(A/BII), 5(A/BI) and 6(A/A). Photo B: 1(A/AII), 2(AII/BI) and 3(AII/BII). Arrows show minor bands from Hb components of the I region.

Hb^a gene which had been detected by starch gel electrophoresis (Fig. 3), and their variants were also generally observed in other foreign horses as well as in Japanese native horses, heavy horses and ponys in view of literature.²⁾ However, haplotypes A and AII variants were not recognized in Noma native horses under the small-sized population and Misaki native horses under closed breeding in the limited area of Japanese native horses. As racing horses like thoroughbreds are also fixed genetically haplotypes BI and BII variants, these are very interesting when considering the relationships between each Hb- α type and the functional advantages of the Hb components.

2. Proposal of new haplotype CII

Nos. 1-5 individuals on Photo A (just like Fig. 3) are variants without bands in the II region detected by starch gel electrophoresis. On the one hand, No. 6 is a variant without a band in the I region and recognized first in Hokkaido native horses in 1980.³⁾ Since it can not be compared directly with Photo B, because this X type was not detected by IEF at that time, recently a variant which is thought to be expressed by the same genetic mode as the X variant was shown in No. 2 in Photo B was recognized in same horse breed. To demonstrate these new variants, haplotypes CII (3 band pattern) or CI (4 band pattern) are presumed

Fig. 3. Evidence showing new Hb haplotype named as CII.

Photo A: Hb types by starch gel electrophoresis. 1(A/A), 2 and 3(A/a), 4 and 5(a/a) and 6(new type). Photo B: Hb- α types by IEF. 1(BI/BII) and 2(BI/CII).

as showed below in Fig. 1, and an additional eleven Hb- α types capable of being formed between the two new haplotypes and the four known haplotypes were then prepared.

A new genotype corresponding to the X type was thought as either one of the CII/CII type (3 band pattern), CI/CI type (4 band pattern) or CI/CII type (3, 4 band pattern) of these eleven Hb types. As a result of researching whether these variants with haplotypes CI and CII are or not in horse breeds other than Hokkaido native horses, Braend et

al. (1983) had been reported four individuals with genetic variants without bands in the I region, and they called it the abnormal Hb haplotype "N" in native Norwegian trotter breed.⁴⁾ These variants were one *BI/CII* type (2, 3, 4 band pattern) and three *BII/CII* types (1, 3 band pattern) respectively according to the additional classification of Fig. 1. Then, Patterson and Bell (1985) reported on AIII variant which appears to be identical to the haplotype "N",⁵⁾ this was *BII/CII* type (1, 3 band pattern). Haplotype without bands in the I region was only "CII" as of yet, and Hb- α types involving this haplotype were recognized in two kinds of *BI/CII* and *BII/CII* types. Hb- α types with haplotype C series are very rare variants, but it is clear that these new variants are not merely abnormal Hb- α types because they are also recognized in several horse breeds not just one. Then, since the nomenclature for this haplotype is not internationally identified as yet, haplotype C series was supposed as third haplotype next to the already identified haplotypes A and B series, and was named as CII according to international nomenclature rule.

Although the frequency of appearance of Hb variants with haplotype CII is low, it is estimated that these can be use as available marker genes for characterizing each horse population. For further clarification on this point it will be necessary to research the haplotype CII variants in additional horse breeds, and also, observe variants with haplotype CI which is not recognized yet.

3. Problems on servicing Hb- α types

The first point necessary to take into consideration when typing equine Hb- α types is the blood chimera case. No. 1 individual in Fig. 4 is a chimera type with the *BI/BII* type and *BII/BII* type being mixed. Also, the chimera type between homozygote and heterozygote is easy to identified, because the overlapped bands (Nos. 1 and 3 bands in Photo A in Fig. 4) is observed to be stronger than the normal heterozygotic bands line No. 2 individual in Poto A. However, the chimera case mixed between homozygotes like *BI/BI* type and *BII/BII* type is difficult to distinguish from each

Fig. 4. A chimera of equine Hb- α type (Photo A) and Hb types in donkey and hinny.

Photo A: 1(chimera type, *BI/BII*+*BII/BII*) and (normal type, *BI/BII*). Photo B: 1 (*BII/BII* in horse), 2(*D/D* in donkey) 3(*A/D* in hinny) and 4(*BII/D* in hinny).

other.

Also, the second point is the case of minor bands just like Nos. 5 and 6 in Photo A and Nos. 1 and 2 in photo in Fig. 2. showed by the arrows. These minor bands which are Hb ghost components in the I region are separated in the II region, and are detected strongly more and more if blood samples were preserved for a long time. Consequently, their bands are apt to be confused with ordinary Nos. 3 and 4 bands in the II region. Fortunately, since the mobilities between these minor bands and both the normal bands in the II region are different from a practical sense, it can be identified clearly by comparing each of them with the corresponding bands between the minor ones and those of the *BI/BII* type used as the control.

Moreover, when the Hb solution is adjusted for IEF, the uniform lysates were obtained more effective by the 0.1% K CN solution than by distilled water, because the former can be solved highly red cell stroma.

4. Comparison with Hb types between donkeys and horses

Hb type patterns in horses, donkeys and hinnys are shown in Photo B in Fig. 4. The Hb band of a donkey (No. 2) was located as a wide one between the I and II regions of the equine Hb- α types, donkey had a very different Hb type pattern com-

pared with horses. Accordingly, the Hb band of the donkey was temporally named as "D". As Nos. 3 and 4 are hybrid types between horses (♂) and donkeys (♀), the former was the *A/D* type inherited haplotype A from the sire horse, and latter was the *BII/D* type received haplotype BII from the sire.

REFERENCES

- 1) Yokohama, M. and Mogi, K.: Jpn. J. Zootech. Sci., **54**: 794, 1983.
- 2) Yokohama, M. et al.: Jpn. J. Zootech. Sci., **56**: 624, 1985.
- 3) Yokohama, M. et al.: ABRI., No. 8: 11, 1980.
- 4) Braend, M. et al.: Acta Vet. Scand., **24**: 384, 1983.

- 5) Patterson, S.D. and Bell, K.: Anim. Blood Groups Biochem., **16**: 239, 1986.

要 旨

ウマ Hb- α 型は、国際的に認定された A, AII, BI および BII の四つのハプロタイプにより 10 種類の型に分類されてきた。しかし、これらのハプロタイプでは説明できない Hb- α 型パターンが北海道和種馬で検出され、このような変異型は複数の外来馬種にも存在していた。またこれらの Hb- α 型は、いずれも同じハプロタイプによる変異であることが明らかになったので、この新たなハプロタイプを "CII" と命名し、さらにはこれに対応するハプロタイプとして "CI" も想定した。同時に、これらのハプロタイプにより分類できる各 Hb- α 型パターンとそれらの分類上の問題点も考察した。