Blood Groups and Serum Protein Polymorphism in Pigs and Their Application as Genetic Markers

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Within many animal species genetic variation is observed in qualitative traits. This variation is called genetic polymorphism. Blood groups and biochemical polymorphisms are the remarkable genetic polymorphism that are found out by laboratory methods. Blood groups can be usually detected by serological techniques and biochemical polymorphism by electrophoretical methods. A widespread variation has been observed in man and other vertebrate and invertebrate species. In farm animals, blood groups and biochemical polymorphisms are frequently used as genetic markers in parentage control, breed structure analyses and so forth. In pigs, many blood group and blood protein systems have been reported and they are being applied in the research works of genetics and breeding. The study about pig blood groups and serum protein polymorphisms done in Japan are described in this report, including the quotation of one investigation reported by foreign worker.

Variation of blood groups and serum protein polymorphism in pigs

Agglutination test, hemolytic test and Coombs' test were carried out for detecting pig erythrocyte antigens. By means of these tests, the twenty-three antibodies to erythrocyte antigens were isolated from isoimmune sera, heteroimmune sera, normal pig sera, normal cattle sera and the sera of the sows which farrowed the piglets suffering from hemolytic disease. They were sixteen agglutinins, five hemolysins and two incomplete agglutinins. Then, the experiments were made to determine the genetic systems of antigens detected with these antibodies and identify these antibodies with the reference reagents standardized internationally. As the results, the following blood group factors belonging to the eight systems were determined: A, O (A system), Ea, Eb, Ed, Ee, Ef, Eg (E system), Fa, Fb (F system), Ga (G system), Ha, Hb (H system), Ka, Kb (K system), Lh, Lk (L system), Oa (O system). Table 1 shows the alleles determined by detecting the blood group factors in each system. The detailed data obtained in this research work have been described in other papers (Abe et al.; 1968, Oishi et al.; 1974a).

By means of starch gel electrophoresis, genetic variation of several serum proteins was studied in order to use them as genetic markers. At first, the most effective conditions for the electrophoretic analysis were searched to make sure the best separation of the following serum proteins: transferrin (Tf), pre-albumin (Pa), hemopexin (Hp), ceruloplasmin (Cp) and amylase (Am). Consequently, the five serum protein polymorphisms were detected at the same running electrophoresis using a selected buffer system and by cutting each gel horizontally into three

Table 1. Blood group and serum protein systems used for study

	System	Reagent	Allele
Blood groups	A	A, O	A^A, a^O (S, s)
	E	Ea, Eb, Ed, Ee, Ef, Eg	E^{aeg}, E^{bdg}, E^{edg}, E^{efd}, E^{bfd}
	F	Fa, Fb	F^a, F^b
	G	Ga	G^a, G^-
	H	Ha, Hb	H^a, H^b, H^{ab}, H^-
	K	Ka, Kb	K^a, K^b, K^-
	L	Lh, Lk	L^{hk}, L^h, L^-
	O	Oa	O^a, O^-
Serum proteins	Tf	detected by starch gel electrophoresis	Tf^A, Tf^B, Tf^D Chiba
	Pa		Pa^A, Pa^B
	Hp		Hp^0, Hp^{1F}, Hp^1, Hp^2, Hp^3
	Cp		Cp^a, Cp^b, Cp^{Omi}
	Am		Am^A, Am^B, Am^C, Am^X

slices. The buffer system used in this electrophoresis was the discontinuous buffer system in which boric—NaOH buffer (pH 8.7) were used for electrolyte and Tris-HCl buffer (pH 7.4) for gel. Secondarily, the variability of transferrin, pre—albumin, hemopexin, ceruloplasmin and amylase types in Japanese pig populations were investigated and the genetic control of these proteins was proved from the family data. In these five serum proteins, the considerable variations were observed among the pigs originated from European breeds. There were variants in each protein as follows: Tf-A, Tf-B and Tf-D_{Chiba} in transferrin, Pa-A and Pa-B in prealbumin, Hp-0, Hp-1F, Hp-1, Hp-2 and Hp-3 in hemopexin, Cp-a and Cp-b in ceruloplasmin and Am-A, Am-B, Am-C and Am-X in amylase. The family data showed that these variants were controlled by the codominant alleles belonging to the five different loci, respectively (Oishi et al.; 1970a). It was supposed, however, that Tf-D_{Chiba} variant in transferrin locus was probably the product by a mutant gene. The alleles detected in each protein system are shown in Table 1.

In addition to the blood group systems shown in Table 1, seven blood group systems (B, C, D, I, J, M, N) have been reported internationally in pigs up to now. In red cell enzymes, seven protein systems have been proved to be polymorphic. Moreover, besides Tf, Pa, Hp, Cp and Am, several polymorphic loci in serum proteins have been detected by using electrophoretic and serological techniques.

The gene frequencies were calculated in order to make clear genetic variations in the blood group and serum protein systems in Japanese pig populations. The breeds investigated in this research work included five European large pig breeds (Middle Yorkshire, Landrace, Berkshire, Hampshire and Large White) and the two miniature pig strains (Pitman-Moore and Ohmini miniature pigs). The gene frequency in each breed is shown in Table 2. The gene frequency differences were observed among seven pig breed populations. Cp^a and Am^x alleles were detected only in Landrace breed, and Cp^{Omi} allele only in Ohmini pigs. E^{bfd}, L^{hk}, Hp^0 and Am^C alleles were found as rare allele in several breeds. These data are described minutely in other papers (Oishi et al.; 1970b, Oishi and Abe; 1974b, Oishi and Tomita; 1976). The gene frequency data shown in Table 2 were used as the basic materials in order to investigate the practical use of blood groups and serum protein polymorphism as genetic marker.

Table 2. The frequency of blood group and serum protein genes in Japanese pig populations

System	Allele	Gene frequency						
		Middle Yorkshire	Landrace	Berkshire	Hampshire	Large White	Pitman-Moore pigs	Ohmini pigs
A[1]	A	0.304	0.421	0.303	0.469	0.429	0.160	0.780
	O	0.143	0.338	0.050	0.359	0.303	0.720	0.220
	—	0.553	0.241	0.647	0.172	0.268	0.120	0
E	E^{aeg}	0.384	0.060	0.543	0.347	0.355	0.150	0.940
	E^{bdg}	0.114	0.496	0.031	0.508	0.273	0.410	0.030
	E^{edg}	0.160	0.220	0.404	0.129	0.227	0.440	0
	E^{efd}	0.338	0.224	0.022	0.008	0.145	0	0.030
	E^{bfd}	0.004	0	0	0.008	0	0	0
F	F^{a}	0.022	0.066	0.627	0.484	0	0.800	0
	F^{b}	0.978	0.934	0.373	0.516	1.000	0.200	1.000
G	G^{a}	0.661	0.490	0.445	0.687	0.195	0.510	0.041
	G^{-}	0.339	0.510	0.555	0.313	0.805	0.490	0.959
H[2]	H^{a}	0.414	0.369	0.079	0.415	0.182	0	0.174
	H^{b}	0.148	0.041	0.069	0.040	0.078	0.322	0
	H^{ab}	—	—	—	—	—	0	0.163
	H^{-}	0.438	0.590	0.852	0.545	0.740	0.678	0.663
K	K^{a}	0.232	0.186	0.123	0.163	0.087	0	0.860
	K^{b}	0.605	0.372	0.815	0.837	0.913	1.000	0.140
	K^{-}	0.163	0.442	0.062	0	0	0	0
L	L^{hk}	0.141	0.027	0	0	0	0	0
	L^{h}	0.009	0.048	0.052	0.271	0.036	0.531	0.800
	L^{-}	0.850	0.925	0.948	0.729	0.964	0.469	0.200
O	O^{a}	0.411	0.038	0.047	0.008	0.144	0	0.352
	O^{-}	0.589	0.962	0.953	0.992	0.856	1.000	0.648
Tf[3]	Tf^{A}	0.114	0	0.275	0.238	0.204	0	0.010
	Tf^{B}	0.886	1.000	0.725	0.762	0.796	1.000	0.990
Pa	Pa^{A}	0.820	0.373	0.768	0.111	0.704	0.470	0.990
	Pa^{B}	0.180	0.627	0.232	0.889	0.296	0.530	0.010
Hp	Hp^{0}	0	0.057	0	0.111	0.010	0	0
	Hp^{1F}	0.127	0.134	0.026	0.008	0.296	0	0.290
	Hp^{1}	0.837	0.363	0.969	0.730	0.509	0.650	0.030
	Hp^{2}	0	0.060	0.005	0.119	0	0.350	0.520
	Hp^{3}	0.036	0.386	0	0.032	0.185	0	0.160
Cp	Cp^{a}	0	0.037	0	0	0	0	0
	Cp^{b}	1.000	0.963	1.000	1.000	1.000	1.000	0.940
	Cp^{Omi}	0	0	0	0	0	0	0.060
Am	Am^{A}	0.093	0.156	0	0.071	0.078	0	0.480
	Am^{B}	0.907	0.830	0.990	0.929	0.911	1.000	0.520
	Am^{C}	0	0.007	0.010	0	0.011	0	0
	Am^{X}	0	0.007	0	0	0	0	0

1) In this system, the frequencies of phenotypes are shown.
2) In the five European large pig breeds, H^{ab} allele is excluded from the calculation of gene frequencies.
3) The unexpected allele found in one offspring from BB×BB mating, Tf^{D} Chiba, is excluded from this table.

Usefulness of blood groups and serum protien polymorphism for parentage control

The probability that two individuals chosen at random will have different genotypes was calculated by using the gene frequencies shown in Table 2. The pig breeds selected for the calculation of probability were Middle Yorkshire, Landrace, Berkshire, Hampshire and Large White. Consequently, the calculated probabilities were higher at A, E, H. K, Pa and Hp systems than the others. By using these data, the usefulness of each locus as genetic marker and the difference of genetic variability among five breed populations were discussed (Oishi et al.; 1970b). Moreover, the probability calculated from all the loci combined was almost 100% in the five breeds. This result showed that all the blood group and serum protein systems combined were very useful as genetic marker.

The parentage test of pig has scarcely been enforced in Japan up to now, but the establishment of its technique is important for the prevention of erroneous paternity on the excellent boars. So, the possibility of parentage control by using blood group and serum protein systems was investigated in the five breed populations reared in Japan. The formulae showing probability of proving non-paternity in general paternity case (2 sires, 1 dam, 1 offspring) was made in the cases of the systems having from two to five alleles, and its general formula was presumed. Moreover, the modified formulae adapted to each locus were made. By putting the gene frequencies shown in Table 2 into the formulae, the efficiency of the 12 loci for parentage test in the five breeds was investigated. A system was excluded from the calculation of probability because the genotype could not fully be determined. Table 3 shows the results obtained from the estimation of the average probability of making exclusions of one of the two possible boars in paternity cases with the aid of different 12 loci. The values of probability calculated were above 15% at E, H and K locus in Middle Yorkshire, at Hp, E, K, Pa and H locus in Landrace, at E, F and Tf locus in Berkshire, at E, Hp, F and H locus in Hampshire and at E, Hp, H and Pa locus in Large White, and their loci were proved

Table 3. Estimation of the average probability of making exclusions of one of the two possible boars in parentage cases with the aid of different 12 loci

System	No. of alleles	Probability of proving non-paternity				
		Middle Yorkshire	Landrace	Berkshire	Hampshire	Large White
E	5	0.435	0.393	0.250	0.326	0.384
F	2	0.021	0.058	0.179	0.187	0
G	2	0.059	0.096	0.103	0.053	0.107
H	3	0.220	0.158	0.126	0.151	0.180
K	3	0.182	0.239	0.105	0.118	0.073
L	3	0.100	0.066	0.044	0.116	0.032
O	2	0.108	0.034	0.041	0.008	0.093
Tf	2	0.091	0	0.160	0.149	0.136
Pa	2	0.126	0.179	0.146	0.089	0.165
Hp	5	0.139	0.444	0.030	0.249	0.342
Cp	2	0	0.034	0	0	0
Am	4	0.077	0.131	0.010	0.062	0.079
All loci		0.832	0.886	0.727	0.814	0.840

Note: In G, H, K, L and O systems, the probability was calculated for the case that the genotypes of sires were clarified.

to be more effective than the others. Especially, the values of probability at Hp locus in Landrace and at E locus in Middle Yorkshire were above 40%. The probability by all the 12 loci combined would be 83.2% in Middle Yorkshire, 88.6% in Landrace, 72.7% in Berkshire, 81.4% in Hampshire and 84.0% in Large White if the genotypes of sires were clarified in all the systems. This fact shows that 88.6% of parentage test cases in Landrace, for example, will be solved. It was recognized that parentage control by using the 12 loci could be carried out practically in Japanese pig populations (Oishi et al.; 1970c). Moreover, the investigation of parentage control in many kinds of parentage test cases need to be carried out in the near future.

Study of genetic similarities among several pig populations of European and Asian origins

Genetic similarity between the two pig populations was determined by following formula:

$$D_m=\sqrt{\sum_{i=1}^{n}(X_{ij}-X_{ik})^2}$$

where D_m shows the genetic distance between the j-th and k-th populations at the m-th locus, and X_{ij} and X_{ik} are the frequencies of the i-th allele in the two populations, respectively. n is the number of alleles at the locus. As X varies from zero to 1, D can take a value between zero and $\sqrt{2}$. This formula is described by Sokal & Sneath (1963) for taxonomical research. The overall genetic distance ($\bar{D}$) between the two populations was given by the mean of genetic distances measured for all the different loci, as $\bar{D}=\frac{1}{l}\sum_{m=1}^{l}D_m$, where l is the number of loci tested. Comparison of the genetic structure of the nine pig breed populations based on the gene frequencies of eight blood group and five serum protein loci was carried out by using the above-mentioned formula and cluster analysis. The pig populations used for the analysis of genetic similarity were Thai and Philippine native pigs, and the seven breeds shown in Table 2. The gene frequencies in five European large pig breeds and the two miniature pig strains were based on the data shown in Table 2 and those in Thai and Philippine native pigs on the data reported by Tanaka et al. (1974).

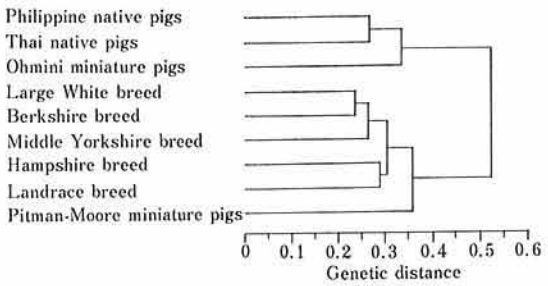

Fig. 1. Dendrogram showing genetic similarities among nine pig populations

Fig. 1 is the dendrogram showing genetic similarities among nine pig populations, based on the weighted pair-group clustering method. This dendrogram was made by using a computer program from the overall genetic distance coefficients ($\bar{D}$) calculated between the two populations taken from the nine populations. Fig. 1 shows that Ohmini miniature pigs, Thai and Philippine natives constitute one group and they are different from European breeds and Pitman-Moore miniature pigs. This result can be understood easily from the geographical relation that Thailand and the Philippine Islands are located in the near region, and from the historical fact that the Ohmini miniature pig strain was developed originally from the small pigs from Manchuria by Ohmi, a Japanese breeder. The genetic distances among European breeds were closer than those between them and the other populations. Pitman-Moore pigs is situated closer to European breeds than Ohmini pigs and East Asian natives. Also, it can be understood from the fact that this miniature pigs was originally developed from feral hogs from the southern area (Florida Peninsula) of the U.S.A. It will be possible in the near future that the variations in the blood groups and

serum protein polymorphism of other East Asian native pigs and wild boars are made clear and genetic similarities among many more pig populations are investigated. These research works are described originally in another paper (Oishi and Tomita; 1976).

Other investigations

Some investigations on the blood group systems related to hemolytic disease of newborn pigs were carried out. In the sera of the several sows giving birth to piglets affected with the hemolytic disease, some blood group antibodies were detected. The antibodies isolated from these sera were anti-Fb, anti-Ea, anti-Fa, anti-Ef, anti-Lh and anti-Oa. Himeno et al. (1969) reported that anti-Oa was the blood group antibody involved in three cases of hemolytic disease. However, it could not been made clear whether the other antibodies were related to hemolytic disease or not. In future, it will have to be made clear which blood group system is related mostly to hemolytic disease besides O system.

In a selection experiment for performance of porcine meat production blood groups and serum protein polymorphism are being used as genetic marker in order to investigate the change of genetic structure from generation to generation. Breeding for meat production and quality of Landrace pig breed in Japan is being carried out in the two experimental stations of northern and southern places. The change of genetic structure from the first to fifth generation has been reflected well in the change of gene frequencies in 7 blood group and 3 serum protein loci. The original gene frequencies of two pig populations shared one by one from many same litter pairs were similar, but they are differing greatly from each other with the progress of the generations. There are several loci showing significant change with the progress of the generations. This study will be continued till the seventh generation and also the relation between genetic marker genes and productive traits will be discussed in future papers.

Recently, the investigation about H and A blood group systems in pigs as predictors of stress susceptibility was reported. At least two genotypes in the H system of blood groups in pigs are responsible for blood types associated with the porcine stress syndrome (PSS), and at least three genotypes are responsible for blood types associated with freedom from PSS. Two blood types, each of which apparently may result from more than one genotype, are associated with PSS in some pigs and not in others. The detailed data are shown in the paper reported by Rasmusen and Christian (1976). The relation between H system and PSS is important because PSS is frequently associated with pale, soft, exudative (PSE) pork. Blood typing may be used for the further research work of PSS and PSE pork in Japan.

Moreover, the information about blood group systems may be available for the practice of blood transfusion in pigs. Also the parentage control technique by using blood groups and serum protein polymorphism may make possible the double sire mating systems for the study of pig breeding and reproduction.

References

1) Abe., T. et al.: Studies on blood groups of pigs. II. Genetic relationship among blood antigens detected by isolated antibodies from various sources, and identification of the antibodies with reference reagents. *Bull. Nat. Inst. Anim. Indust.*, 18, 7–13 (1968) [In Japanese with English summary].

2) Himeno, K. et al.: Studies on the hemolytic disease of newborn pigs. VII. Genetic study on the red cell antigen involved in hemolytic disease. *Jap. J. Zootech. Sci.*, 40, 212–219 (1969). [In Japanese with English summary].

3) Oishi, T., Abe, T. & Mogi, K.: Studies on blood groups of pigs. IV. Genetic determination of the serum transferrin, pre-albumin, hemopexin, ceruloplasmin and amylase variants in pigs. *Jap. J. Zootech. Sci.*, 41, 364–371 (1970a) [In Japanese

with English summary].

4) Oishi, T., Abe, T. & Mogi, K.: Studies on blood groups of pigs. V. Gene frequencies of blood groups and serum protein types, and their usefulness as marker gene. *Jap. J. Zootech. Sci.*, 41, 495–500 (1970b) [In Japanese with English summary].

5) Oishi, T. & Abe, T.: Studies on blood groups of pigs. VI. Usefulness of blood groups and serum protein types for paretage test. *Jap. J. Zootech. Sci.*, 41, 501–506 (1970c) [In Japanese with English summary].

6) Oishi, T., Abe, N. & Abe, T.: Determination of blood group systems of not-identified antisera by international comparison test of pig blood groups. *Jap. J. Zootech. Sci.*, 45, 564–566 (1974a) [In Japanese].

7) Oishi, T. and Abe, T.: The gene frequencies of blood groups and serum protein types in Hampshire and Large white breeds reared in Japan. *Jap. J. Swine Research*, 11(1), 23–25 (1974b) [In Japanese with English summary].

8) Oishi, T. & Tomita, T.: Blood groups and serum protein polymorphisms in the Pitman-Moore and Ohmini strains of miniature pigs. *Anim. Blood Grps. Biochem. Genet.*, 7, 27–32 (1976).

9) Rasmusen, B. A. & Christian, L. L.: H blood types in pigs as predictors of stress susceptibility. *Science*, 191, 947–948 (1976).

10) Tanaka, K.: The morphological and serological investigation of Thai native swine. *Rep. Res. Grp. Native Farm Anim.*, 6, 98–109 (1974) [In Japanese].