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Protein Polymorphisms and Variations of Wild House Rat (*Rattus norvegicus*) Population in Korea

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The protein po;ymorphisms and allele frequencies of wild house rat (*Rattus norvegicus*) population in Korea were studied. The studied proteins and enzymes were transferrin (Tf), albumin (Alb), fumarate hydratase (FH), phosphogoucomutase (PGM), lactate dehydrogenase A (LDHA) and lactate dehydrogenase B (LDHB).

There were two transferrin alleles, Tf^1 and Tf^2 in Korean wild house rat population. The Tf^2 allele was found for the first time by a starch gel, and confirmed by a polyacrylamide gel isoelectric focusing and immunoblotting. The allele frequencies of Tf^1 and Tf^2 were 0.985 and 0.015, respectively.

Two common alleles fumarate hydratase, FH^a and FH^b were found, and frequencies of FH^a and FH^b were calculated to be 0.714 and 0.286, respectively. The frequency of FH^a in Korean wild house rat was higher than that of Finnish and Czechoslovakian population.

Alb, PGM, LDHA and LDHB are only one phenotype each and all. Therefore, these proteins seem to be monomorphic in Korean wild house rat population.

KEY WORDS: Protein polymorphism

Electrophoretic analysis of proteins and enzymes has been widely used for assessment of the degree of genetic variation in populations. The observation of this phenomenon were made in human (Smithies, 1955,1957), mouse (Cohen, 1960; Shreffler, 1960), and laboratory rats independently in a number of laboratories(Beaton *et al.*, 1961; Augustinsson and Henricson, 1966; Eriksson *et al.*, 1976; Festing and Bender, 1984; Matsumoto and Yamada, 1988).

The two transferrin bands of *Rattus norvegicus* (Wistar strain) were firstly described by Beaton *et al.* (1961), and analyzed chemically by Gordon and Louis(1963). Many electrophoretic surveys of *Rattus norvegicus* did not reveal serum transferrin variant in any of the inbred and wild rats. Since the electrophoretic variant of hemoglobin in the laboratory rats have been reported by French and Roberts (1965), the additional loci have been found to vary among inbred strains(Kim and Lee, 1989). They are 6-phosphogluconate dehydrogenase (Carter and Parr,1969), group specific component (Moutier *et al.*, 1973), fumarate hydratase(Eriksson *et al.*, 1976; Carleer and Ansay, 1976), plasma alkaline phosphatase (Jimenz-Martin, 1974), α-amylase 1 (Mizuno and Susuki, 1978), peptidase-3 (Womack and Cramer, 1980), β-galactosidase (Douglas *et al.*, 1982), pepsinogen-1 (Cramer,1981), major urinary

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protein-1 (van Zutphen, 1981), aconitase-1, aldehydedehydrogenase-2 and alkaline phosphatase (Adams *et al.*, 1984), and acid phosphatase 2 and methylglyoxal dehydrogenase (Bender *et al.*, 1984, 1985). Additionally, some enzymes, phosphoglucomutase (Koga *et al.*, 1972), α-glycerophosphate dehydrogenase and xanthine dehydrogenase have been reported to be polymorphic in wild house rat populations (Eriksson *et al.*, 1976). However, Serov (1972) found no evidence of genetic polymorphism at the approximately 20 loci in Siberian wild rat and one laboratory strain, indicating that wild and laboratory populations of *Rattus norvegicus* seem to be completely devoid of polymorphisms. Eriksson *et al.*(1976) found that 8 out of 25 loci examined were found to be polymorphic, and the degree of average heterozygosity per locus per individual of wild house rat was 0.07 from Finnish population. Bender *et al.*(1985) recently reported that 5 out of the 9 loci were found to be polymorphic in Czechoslovakian wild house rat population. However, there are not so much data available on the Korean wild house rat.

Therefore, this study attempts to determine the distribution of phenotype polymorphisms and genetic structure by using 6 loci, such as, transferrin, albumin, fumarate hydratase, phosphoglucomutase, lactate dehydrogenase A, and lactatedehydrogenase B, in Korean wild rat population.

Materials and Methods

Preparation of samples

A total of 97 samples of *Rattus norvegicus* were trapped from Seoul, Korea (Table 1).

After anesthetizing the rats with ether, several organs, such as kidney, heart, lung, testis and ovary were removed. The blood samples were collected by heartpuncture in syringe with heparin and centrifuged at 3,000 g for 10 minutes to obtain the plasma. The removed tissues were washed with 0.15 M NaCl and homogenized with three folds of 0.05M Tris/ HCl buffer (pH 8.0) and centrifuged at 12,000 g for 1 hour at 4° C. The supernatant was taken as sample and were stored at –60° C until use.

Table 1. Analyzed proteins and sample numbers of wild house rat (*Rattus norvegicus*) in study

Proteins	Tf	Alb	FH	PGM	LDH
Numbers	97	97	96	97	97

Electrophoresis and staining

Transferrin (Tf)

Polyacrylamide gel isoelectric focusing: Thin layer polyacrylamide gel isoelectric focusing (PAGIF) was performed by the method described by Oh (1986)with a slight modification.

Immunoblotting: Immunoblotting of Tf was carried out according to the method of Reinhart and Malamud(1982), Tamaki *et al.* (1985), Yuasa *et al.* (1985) and Kim (1989) with slight modifications.

Albumin (Alb)

The method is the same as that of transferrin (Tf) using starch gel electrophoresis. Briefly, starch gel was 11.0%, the samples were run at 150 V for 30 minutes and then, 350 V for 4 hours at 4° C.

Fumarate hydratase (FH)

A horizontal starch gel electrophoresis was carried out using the system described by Eriksson *et al.* (1976) except heart tissues and at 80 V for 17 hours.

Phosphoglucomutase (PGM)

Horizontal agarose gel electrophoresis was carried out using the system described by Bender and Gunther (1978) and, Teisberg (1970) with a slight modification.

Lactate dehydrogenase A(LDHA) and Lactate dehydrogenase B (LDHB)

Horizontal starch gel electrophoresis was carried out using the system described in FH with slight modifications.

Results

Phenotype distributions and allele frequencies

Transferrin (Tf)

Transferrin showed the immunospecific Tf banding patterns detected by a polyacrylamide gel isoelectric focusing (PAGIF) at pH range of 4.0-7.0 followed by immunoblotting with polyclonal monospecific rabbit antirat Tf (Figure 1).

The homozygous phenotype, Tf 1-1 was characterized by two major bands. The homozygous phenotype of rare Tf^2 allele was not found in the present study, but heterozygous phenotype, Tf 2-1 corresponded to a mixture of the two homozygous phenotypes.

The phenotype distribution and allele frequencies of Tf in Korean wild house rat population were represented in Table 2.

Ninety seven samples of type 1-1 (96.91%), 3 of type 2-1 (3.09%) were observed. The allele frequencies of Tf^1 and Tf^2 were 0.985 and 0.015, respectively.

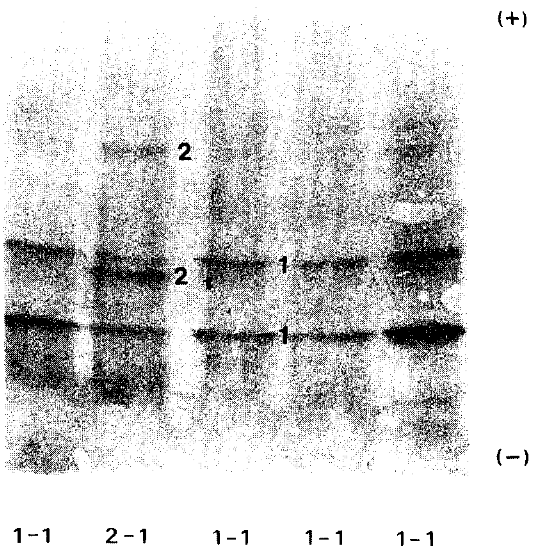

Fig. 1. Transferrin (Tf) phenotypes of *Rattus norvegicus* by immunoblotting after polyacrylamide gel isoelectric focusing. Tf 2-1 type was confirmed by immunoblotting as a heterozygous form. Two different phenotypes (1-1, 2-1) of Tf were shown.

Albumin (Alb)

Only one phenotype was detected. The band pattern of albumin was composed of one major band and two minor bands. The phenotype was referred to Alb C type.

Fumarate hydratase (FH)

The electrophoretic patterns of FH isozyme in heart muscle were shown in Figure 2.

The phenotypes of the homozygous FH A and FH B were characterized by a single major band. The heterozygous phenotype FH AB corresponded to a mixture of the two homozygous phenotypes. Two major alleles, FH^a and FH^b were found.

Table 3 shows the phenotypes and allele frequencies of FH in Korean wildhouse rat population.

Fifty four samples of FH A type (56.25 %), 29 samples of FH AB type (30.21%) and 13 samples of FH B type (13.54 %) were observed. The allele frequencies of FH^a and FH^b were estimated to be 0.714 and 0.286, respectively.

Phosphoglucomutase (PGM)

The PGM phenotype consisted of two major bands and one minor band on agarose gel. This common phosphoglucomutase pattern corresponded to the PGM A typereported previously (Bender and Gunther, 1978). Ninety seven samples were analyzedfor PGM typing. Interestingly enough, only PGM A phenotype was found in Korean wild house rat population. This protein seems to be monomorphic in Korea.

Table 2. Transferrin (Tf) phenotypes and allele frequencies of wild house rat (*Rattus norvegicus*) in Korea

Phenotypes	1-1	2-1	2-2
Number of samples	94 (94.11)	3 (2.87)	0 (0.02)

Allele frequencies: Tf^1 = 0.895 ± 0.009, Tf^2 = 0.015 ± 0.009. X^2 = 0.001, df = 1, P > 0.95. Expected numbers are in parentheses.

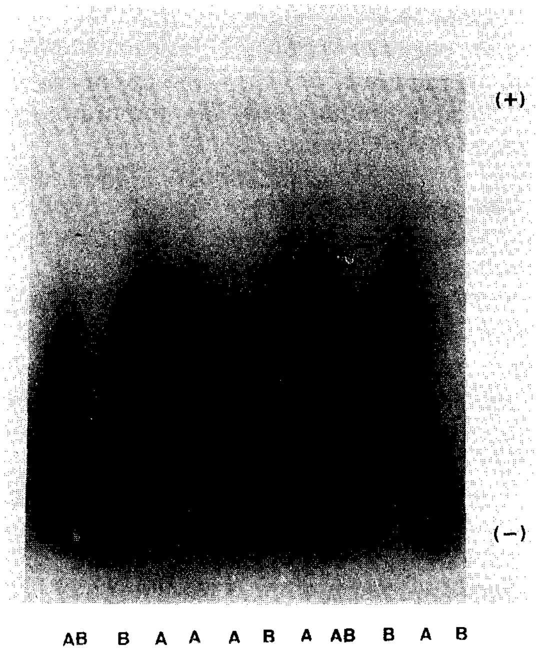

Fig. 2. Fumarate hydratase (FH) phenotypes of *Rattus norvegicus* by 11% starch gel electrophoresis. Three different phenotypes (A, AB, B) were shown. The homozygous types (A, B) of FH were consisted of a single band. The heterozygous type (AB) of FH was consisted of two bands.

Table 3. Fumarate hydratase (FH) phenotypes and allele frequencies of wild house rat (*Rattus norvegicus*) in Korea

Phenotypes	A	AB	B
Number of samples	54 (48.94)	29 (39.21)	13 (7.85)

Allele frequencies: FH^a = 0.714 ± 0.033, FH^b = 0. 286 ± 0.033. X^2 = 6.555, df = 1, 0.01 < P < 0.05*.
*Significant: The differences are partly due to higher expected number of FH AB type than observed number. Expected number are in parentheses.

Lactate dehydrogenase A (LDHA) and Lactate dehydrogenase B (LDHB)

The LDH isozymes were tetramers and contained polypeptides derived from two loci. The isozyme patterns seen in rat tissues suggest that LDH A and LDH Bpolypeptides are being formed in roughly amounts (Mankert and Ursprung, 1962;Markert, 1968). In total, 97 samples were analyzed for LDH phenotyping. But novariant phenotype was found in Korea.

Discussion

Since Smithies (1957) found that the transferrins are a group of genetically determined plasma proteins, many investigators are followed to examined the electrophoretic properties of these substances (Beaton *et al.*, 1961; Gordon and Louis, 1963; Putnam, 1975; Schreiber *et al.*, 1979).

The two band types of transferrin present in the plasma were separated by starch gel electrophoresis (Beston *et al.*, 1961; Gordon and Louis,1963). However, variant phenotype were found in Korean wild house rat population by starch gelelectrophoresis (Kim and Lee, 1989). The variant phenotype was confirmed by immunoblotting techniques (Figure 2). Tf 2-1 type was consisted of four bands, especially, pH5.65 region has one additional band. It is assumed that Tf is controlled by 2 codominant alleles, Tf^1 and Tf^2. However, the new allele (Tf^2)should be confirmed by breeding tests.

Electrophoretic band patterns of albumin on starch gels consist of 3 bands, which are composed of one type of subunit. The phenotype is designated as Alb C in the rat. Nagase *et al.* (1979) reported the Alb variant strain, Sprague-Dawley, in laboratory rats. The analbuminemia of rats is inherited as an autosomal recessive traits (Nagase *et al.*, 1979, 1980). Esumi *et al.* (1983) showed that thegene from the analbuminemia rat has a 7 base deletion in an intron.

The zymogram of the common albumin type is composed of one broad main band and two minor faint bands. Variant phenotype was not found in wild populations of the rat(Serov, 1973; Bender *et al.*, 1979,1984; Adams *et al.*, 1984; Matsumoto and Yamada, 1988). There was, also, no unusual band pattern in Korean wild houserat population.

Eriksson *et al.*(1976) was the first to report the

FH polymorphism of wild house rat population from Finland. And, the allele frequencies of FH^a and FH^b was 0.315 and 0.685, respectively. Also, the allele frequencies of FH^a and FH^b in Czechoslovakian population were 0.32 and 0.68, respectively. The allele frequencies of the two populations were very similar. However, the allele frequencies of FH^a and FH^b in Korean population were observed to be 0.7135 and 0.2865, respectively. The allele frequencies of FH^a in Korean wild house rat population was considerably higher than in Finnish and Czechoslovakian population(Table 4).

Previously, Reyns *et al.* (1975) did not succeed in demonstrating the FH polymorphisms by isoelectric focusing. This study failed to phenotype the FH in the rat by cellulose acetate membrane and PAGIF, either. Tolly and Chaig (1975)have suggested another source of multiplicity. The enzyme appears to be 2 molecular forms on electrophoresis; one from mitochondrial, the other from cytoplasmic origin. No major difference was found between these two subcellular fractions in the tissue homogenates of the wild house rats (Figure 2).

Investigations of PGM isozyme in wild house rat populations are very few. Carter and Parr (1969) and, Serov(1973) failed to detect individual variation of PGM in wild house rat. Koga *et al.* (1972) found the individual variation of PGM isozyme of the wild rat population in Japan. The polymorphism of PGM system is composed of two common allele which determine electrophoretically distinct variants, PGM^A and PGM^B with allele frequencies of about 0.656 and 0.344, respectively. But, the phenotype of PGM in Korean wild house rat population was monomorphic on agarose gels (Table 5).

Slender and Yang (1969) reported that there were two "prominant" PGM system in hemolysate zymogram of the mouse (*Mus musculus*), the more anodal one being monomorphic and the other polymorphic in many wild populations in U. S.A. And the phenotypes of PGM zymogram in wild house rats are observed to be very similar to those of human red blood cell (Spencer et *al.*, 1964; Koga *et al.*, 1972).

Electrophoretic pattern of LDH on starch gels consisted of 5 bands, which are composed of two types of subunits (LDHA and LDHB). Lactate dehydrogenase-1contains only one kind of monomer and lactate dehydrogenase-5 the other kind. If,in the subunit A mutant heterozygote, LDH-1 band appears in normal position and LDH-2,3,4,5 have some additional bands (Markert, 1963; Zinkham, 1968; Markert *et al.*, 1975). However, the polymorphisms of LDH isozyme in the house rat were not found in inbred and wild rat populations (Eriksson *et al.*, 1976; Bender and Gunther, 1978; Adams *et al.*, 1984;

Table 4. Distribution of the fumarate hydratase (FH) allele frequencies in different wild house rat (*Rattus norvegicus*) populations of the world

Populations	Number of samples	Allele frequencies		Referencies
		FH^a	FH^b	
Finnish	86	0.325	0.675	Eriksson *et al.*, 1976
Czechoslovakian	47	0.32	0.68	Bender *et al.*, 1985
Korean	96	0.714	0.286	*present study*

Table 5. Differences of the phosphoglucomutase (PGM) allele frequencies between Japanese and Korean wild house rat (*Rattus norvegicus*) populations

Populations	Number of samples	Allele frequencies		Referencies
		PGM^A	PGM^B	
Japanese	61	0.656	0.344	Koga *et al.*, 1972
Korean	97	1.000		*present study*

Bender *et al.*, 1984).

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한국산 야생집쥐(***Rattus norvegicus***) 집단의 단백질 다형과 유전적 변이

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한국산 야생집쥐(*Rattus norvegicus*) 집단에서 단백질(transferrin: Tf, albumin: Alb, fumarate hydratase: FH, phosphoglucomutase: PGM, lactate dehydrogenase A:LDHA, 및 lactate dehydrogenase B: LDHB)의 다형과 유전적 변이를 분석하였다.

Tf의 대립유전자는 Tf^1과 Tf^2로 밝혀졌으며 Tf^2유전자는 처음으로 발견되었다. Tf^1과 Tf^2 유전자 빈도는 0.985와 0.015로 각각 산출되었다. 그리고 FH의 유전자는 FH^a와 FH^b의 빈도가 0.714와 0.286으로 계산되었는데, FH^a의 빈도는 Czechoslovakia와 Finland 집단보다 높았다.

그 외의 단백질들은 모두 한가지 표현형을 나타냈으므로 monomorphic한 것으로 나타났다.