STUDIES ON THE GENETIC VARIATION OF PLASMA PROTEINS IN CHEJU POPULATION OF KOREA

MOON YOU OH, SE JAE KIM* , SEONG SOO HONG
AND CHUNG CHOO LEE**

Department of Biology, Cheju National University, Cheju 590
**Department of Zoology, Seoul National University*
Seoul 151, Korea

Received November 14, 1987

ABSTRACT

Genetic variants have been studied in plasma samples from 50 Cheju children and their parents by means of two-dimensional gel electrophoresis followed by coomassie blue and silver staining of the gels. Twenty-two polypeptides chosen without respect to variability were considered suitable for scoring. Genetic variations were encountered in 8 of these polypeptides. Eighty-four of 1,110 polypeptides exhibited the combination of a normal and a variant polypeptide. The index of heterozygosity estimated in this study was 7.6±1.05%. The heterozygosity in Korean (Cheju) population was compared with those in other populations.

Key words : Two-dimensional gel electrophoresis, genetic variation, heterozygosity index.

INTRODUCTION

The level of genetic variation in human and natural populations of animals have been intensely studied since the introduction of electrophoretic techniques to population genetics. Researchers have continually sought to extend the range of proteins examined. One-dimensional electrophoretic (1-DE) studies of proteins that are products of 104 genetic loci, those proteins for the most part functioning as enzymes, have yielded an index of heterozygosity (average heterozygosity per locus) of 6.3% in Caucasoid humans (Harris, 1980). Because the electrophoretic technique fails to detect amino acid substitutions that do not alter molecular charge and mutants charaterized by the absence of enzyme activity, 6.3% was a minimal estimated of human heterozygosity at these loci. This reservation, along with the sampling bias, makes it difficult to extrapolate total genetic variability from the results of routine allozyme surveys.

An alternative approach utilizes the two-dimensional electrophoresis

This study was supported by Korea Research Foundation, 1986.

technique(2-DE) introduced by O'Farel (1975). Wanner *et al.* (1981) found that 90% of the known protein variants examined were also detected by 2-DE. The extent of protein charge genetic variations was originally reported to be rather low when 2-DE was first applied to human cellular proteins. Average per locus heterozygosities of less than 1% were estimated in preparations of fibroblast cell lines, brain, kidney, and lymphocytes (McConkey *et al.*, 1979; Walton *et al.*, 1979; Smith *et al.*, 1980; Coming, 1982; Hamaguchi *et al.*, 1981). However, several recent studies have revised upward the estimates of average protein heterozygosity by 2-DE. Goldman and Merril (1983) reported 2.4% heterozygosity on lymphocytes. Rosenblum *et al.* (1983, 1984) estimated that the average heterozygosity of serum protein loci detected by 2-DE was 6.2%, and that the average heterozygosity of erythrocyte loci was 3.1%.

The present study was carried out in order to investigate the genetic variation in plasma proteins of Korean (Cheju) population, and also the heterozygosity estimated in this study was compared with those of other populations.

MATERIALS AND METHODS

Sample Preparation

To prepare the gels in trios derived from a children and his/her father and mother was convenient because this not only permits the immediate verification of an apparent genetic variant in children, but also reveals the presence of null variants which would otherwise be very difficult to detect. Therefore the blood samples from 50 children and their parents were obtained from normal volunteers in Cheju-do. The plasma was removed following centrifugation and stored at -80˚C until used. For 2-DE of plasma samples, 10μl of plasma was added to 20μl of sample buffer, consisting of 9.4M urea, 5% mercaptoethanol, and 1.6%, pH 5-7, and 0.4%, pH 3.5-10, Ampholine(LKB). The sample buffer was made fresh prior to use, mixed with sample, and applied directly to isoelectric focusing (IEF) gels.

Electrophoresis and Staining

In general, the electrophoresis was carried out following the O'Farrell technique (1975), wherein the first dimension of the two-dimensional polyacrylamide gel system involved isoelectric focusing of the protein mixture, and the second dimension, electrophoresis on a slab gel containing SDS. Ten to 20μl of samples were focused in first-dimension gels cast with the mixture of pH 3.5-10 and pH 5-7 Ampholine(LKB). Following the isoelectric focusing, the gels were extracted from the tubes and equilibrated. Gels were generally stored at -80°C until the second dimension run. Second-dimension SDS gels were prepared with 12.5% acrylamide.

The protein patterns in the gels were initially analysed by staining with 0.25% coomassie blue R-250 in methanol: acetic acid: water (40:10:50 by volume). Gels were destained with different methanol: acetic acid: water mixture, and finally with water. Gels were further stained with the sensitive silver technique of Oakley *et al.* (1980) in order to study the less-abundant plasma proteins.

Choice of Polypeptide for Scoring

There were two groups of polypeptides. The first set was selected for study by inspecting silver-stained gels, on the basis of certain desirable characteristics; reproducibility, relative isolation on the gel, and a density such that were a variant to occur (with approximately half the normal density). The second set was a group of generally more abundant polypeptides, readily visualized with the coomassie blue staining. The identity of scored polypeptides was recognised by comparing with the plasma protein map of Anderson *et al.* (1984). The polypeptides whose identities were unknown were arbitrarily designated.

RESULTS

Fig. 1 and Fig. 2 identify the position of the 22 polypeptides. Phenotype and genotype frequencies for the polypeptides found to be variable in this study (Table 1). Table 1 also presents the results of tests for agreement with Hardy-Weinberg equilibrium for systems where homozygotes for variants were observed. Eight of the 22 polypeptides yielded genetic variants, all of which were observed in one parent or the other. Fig. 3 illustrates the eight variants observed. Of a total of 1,110 polypeptides scored, 84 exhibited the combination of a normal and a variant polypeptide; this correspond to an index of heterozygosity of 7.6±1.05% (Table 2). The finding for the eight variable proteins may be briefly summarized.

Transferrin. The ability of 2-D PAGE to distinguish transferrin variants have been demonstrated by Anderson and Anderson (1977). Among 50 individuals surveyed one anodally migrating variant was detected as heterozygote (Fig. 3A).

Gc-globulin. Tracy *et al.* (1982) demonstrated that 2-D PAGE would distinguish between the common allelic form of Gc, namely Gc 1 and Gc 2. In this study, these alleles were encounted, but subtyping of Gc 1 and Gc 2 phenotypes were not attempted (Fig. 3B). New variants reported by Asakawa *et al.* (1985) did not detected in this survey.

Apolipoprotein E. The ability of 2-D PAGE to detect variant of this system have been demonstrated by Zannis *et al.* (1981) and Utermann *et al.* (1982). In Korean (Cheju), both an anodally (Apo E 2) and cathodally (Apo E 4) migrating variant were observed (Fig. 3C).

Apolipoprotein A-I. The ability of 2-D PAGE to detect variant of this system has been demonstrated by Schaman *et al.* (1983). The common APO A-I appears as two protein spots, with one major and one minor isoform. In this study, a Apo A-I variant was found (Fig. 3D).

Haptoglobin. The ability of 2-D PAGE to distinguish haptoglobin phenotypes has been demonstrated by Anderson and Anderson (1977). In this study, the common allelic form of Hp was distinguished, but subtypings of Hp 1 and Hp 2 phenotypes were not attempted (Fig. 3E).

Alpha-2 HS Glycoprotein. Anderson and Anderson (1977) demonstrated that 2-D PAGE would distinguish between the common allelic forms of Alpha-2 HS, namely L and N types. In this study, these alleles were detected (Fig. 3F).

Table 1
Occurence of genetic variation in 22 proteins scored in 50 persons (Cheju population)

Protein	Phenotypic classification		Allelic frequency
Transferrin	N	49	$P^L = 0.99$
	N/V	1	$q^V = 0.01$
Gc-Globulin	Gc 1	24	$p^1 = 0.67$
	Gc 1-2	19	$q^2 = 0.33$
	Gc 2	7	$x^2 = 1.746$ (df=1,0.2>p>0.1)
	T	50	
Apolipoprotein E	3	40	$p^3 = 0.9$
	2-3	9	$q^2 = 0.09$
	3-4	1	$r^4 = 0.01$
	T	50	$x^2 = 0.616$(df=2, 0.8>p>0.7)
Apolipoprotein A-I	N	49	$p^N = 0.99$
	N/V	1	$q^V = 0.01$
	T	50	
Haptoglobin	1	33	$p^1 = 0.29$
	2-1	17	$q^2 = 0.71$
	2	27	$x^2 = 1.18$(df=1, 0.3>p>0.2)
	T	50	
α_2-HS Glycoprotein	L	23	$p^L = 0.73$
	N/L	27	$q^N = 0.27$
	T	50	
Protein 2(?)	N	44	$p^N = 0.92$
	N/V	6	$q^V = 0.08$
	T	50	
Protein 3(?)	N	47	$p^N = 0.97$
	N/V	3	$q^V = 0.03$
	T	50	
Hemopexin	N	50	$p^N = 1$
α_1-Antitrypsin	N	50	$p^N = 1$
Apolipoprotein A-IV	N	50	$p^N = 1$
Prealbumin	N	50	$p^N = 1$
Antithrombin III	N	50	$p^N = 1$
Apoplipoprotein A-II	N	50	$p^N = 1$
Plasmonogen	N	50	$p^N = 1$
Alpha-1 acid glycoprotein		50	$p^N = 1$
Alpha-fibrinogen	N	50	$p^N = 1$
Beta-fibrinogen	N	50	$p^N = 1$
Gamma-fibrinogen	N	50	$p^N = 1$
C4	N	50	$p^N = 1$
Protein 1(?)	N	50	$p^N = 1$
Protein 4(?)	N	50	$p^N = 1$

^{N}/v: Normal/Varient Polypeptides.
^{N}/$_{L}$: N type/$_{L}$ type of α_2 HS.

Table 2
Summary of polymorphisms identified using 2-D gel

Tissue	No. of loci surveyed	No. of loci polymorphic	Heterozygosity
Plasma	22	8	N/V combination 84 Total polypeptide 1110 Average heterozygosity = 7.6 ± 1.05%

V and N represent the variant polypeptide and the corresponding normal polypeptide, respectively.

Table 3
Heterozygosity indices from population surveys using 2-D gel analysis in plasma samples

Population	No. of individuals surveyed	No. of loci surveyed	Average heterozygosity	Reference
Caucasoids	60	20	6.2 ±0.7%	Rosenblum *et al.*, 1983
Caucasoids	25	40	5.6%	Goldman *et al.*, 1985
Caucasoids	62	11	8.0 ±1.1%	Asakawa *et al.*, 1985
Amerindians	95	11	4.6 ±0.6%	Asakawa *et al.*, 1985
Japanese	110	11	5.7 ±0.7%	Asakawa *et al.*, 1985
Korean	50	22	7.6 ±1.05%	Present study

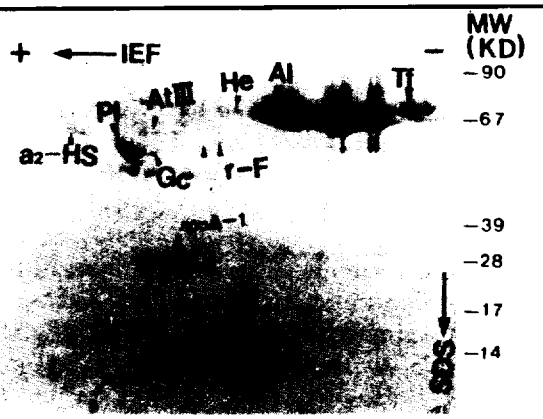

Fig. 1. Coomassie blue R-250 stained 2-D PAGE pattern of human plasma proteins. Each arrow head indicates the scored polypeptides whose identies are known.

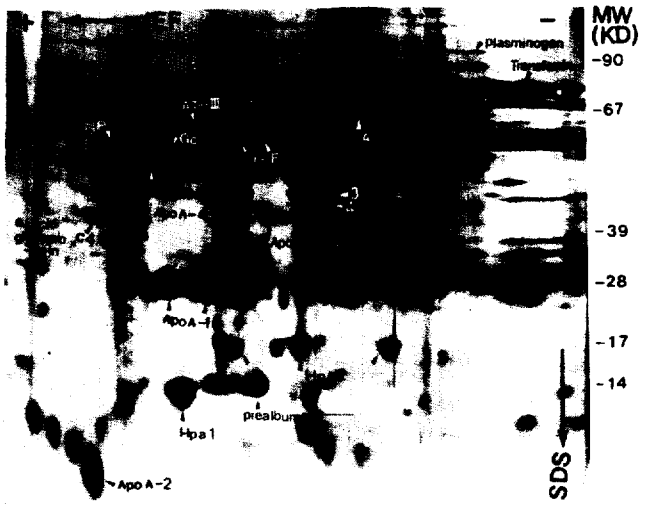

Fig. 2. Silver-stained 2-D PAGE pattern of human plasma proteins. Each arrow head indicates the scored polypeptides whose identies are known. Number 1,2,3,4 indicate the scored polypeptides whose identies are unknown.

Protein 2 and Protein 3. These polypeptides whose identities unknown were arbitrarily designated (Fig. 2). Six individuals in protein 2 and 3 individuals in protein 3 were detected as heterozygotes showing normal and variant polypeptide (Fig. 3G and H).

DISCUSSION

The discussion will be limited to the heterozygosity issue. With respect to the 22 polypeptides when they were selected for study, it was found that 8 to be polymorphic, with heterozygosity index for the group of 7.6±1.05%. One dimensional electrophoretic studies have provided an estimate for the index of heterozygosity of 6.3% (Harris, 1980). McConkey *et al.* (1979) examined the hetrozygosity revealed in four human diploid fibroblast lines by double label autoradiography. They reported that the average heterozygosity appears to be less than 1% for changes involving charged amino acids. Only about 1.2% of the proteins of normal human fibroblasts were found to differ in their electrophoretic mobility. This corresponds to an average heterozygosity of approximately 0.6% (Walton *et al.*, 1979). Smith *et al.* (1980) found no genetic variation in a survey of 25 human kidneys with respect to 83 proteins. Hamaguchi *et al.* (1981) suggested a heterozygosity index of 0.5±0.3% in phytohemagglutinin-stimnlated peripheral blood lymphocyte preparations. Comings (1982), using 145 brains obtained from patients who died of a variety of neurological diseases, observed polymor-

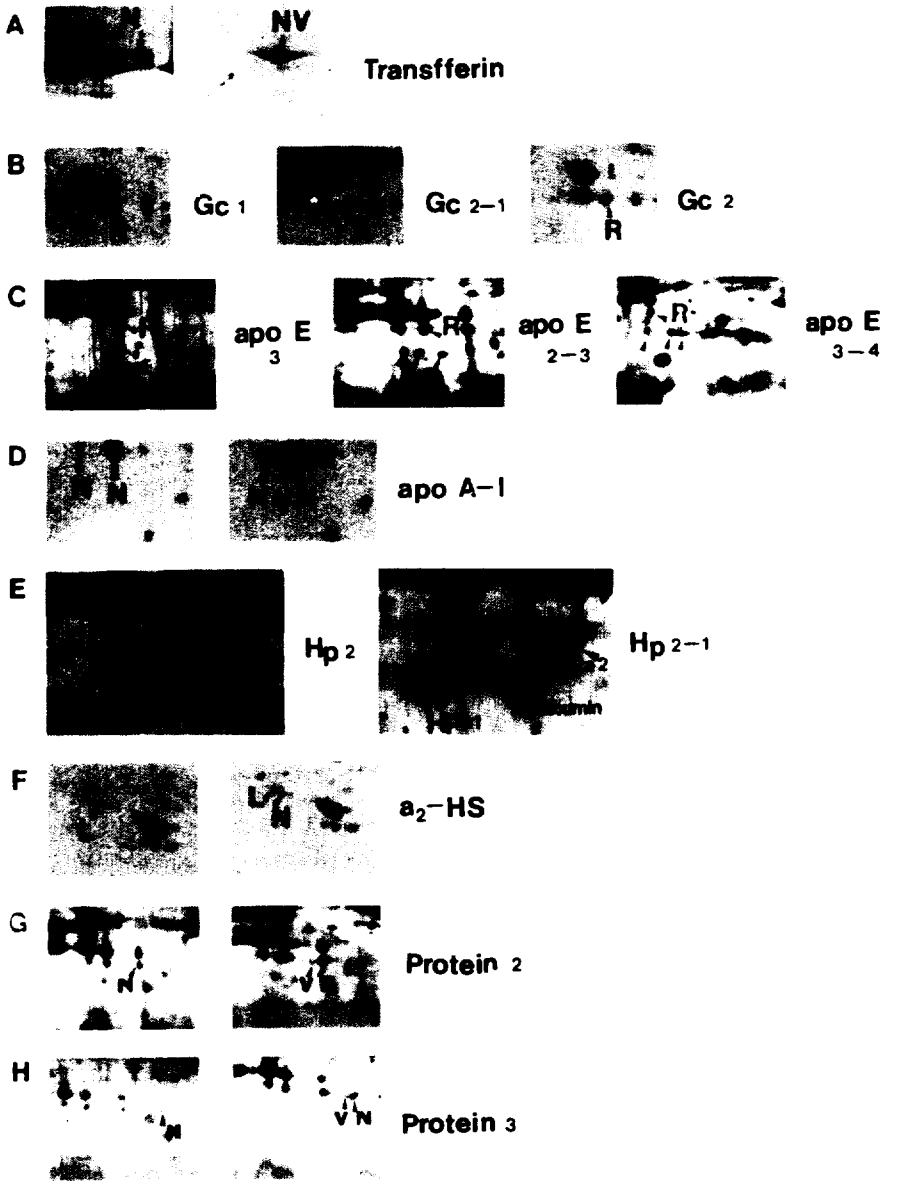

Fig. 3. Section of the two-dimensional gels showing the phenotypes. In photos, the variants(V) and the corresponding normal(N) polypeptides, a convenient spot(R) have been designated.
A, Transferrin; B, Gc-globulin; C, Apolipoprotein E; D, Apolipoprotein A-I; E, Haptoglobin; F, Alpha-2 HS Glycoprotein; G, Protein 2; and H, Protein 3, respectively.

phism in only 2 of the 176 polypeptides. Eleven presumed heterozygotes were encountered among 24,600 polypeptides, resulting in heterozygosity index of 0.04%. Goldman and Merril (1983) using human lymphocytes, observed 186 proteins scored for variation in 28 persons, 19 exhibited variation, for an average heterozygosity of 2.4±0.2%. Rosenblum *et al.* (1984) reported heterozygosity index, using the 2-D PAGE technique, of 3.1±0.5% in randomly selected polypeptides of erythrocyte lysates.

However, the heterozygosity index, using the 2-D PAGE technique, of plasma samples were higher than those of other preparations (Table 3). There are several possible explanations for this apparent variation in the index of heterozygosity yielded by 2-D PAGE preparations of various cells, tissues, and/or body fluids (Rosenblum *et al.*, 1984). First, it is clear that in a small series of scored polypeptides, the addition of a single polymorphic polypeptide or deletion of a polypeptide, because it is related to another scored polypeptide, can increase or decrease the index of heterozygosity by 1% or 2%. Second, it may be that the difference is due to the use of different staining for the identification of polypeptides. Finally, it is possible that the difference is due to criteria that investigators have employed in the selection of spot for analysis.

The interethnic comparisons of heterozygosity indices were demonstrated following, in decreasing order: Caucasoid, 8.0± 1.1%; Korean, 7.6±1.05%; Japanese, 5.7±0.7%; Amerindian, 4.5±0.6% (Table 3).

Neel (1978) reported that for a series of 28 erythrocyte enzymes and serum proteins scored for genetic variability by 1-DE in Amerindians, Japanese, and Caucasians, the indices of heterozygosity were 5.4%, 7.7%, and 7.8%, respectively. The results of the present study confirmed that the indices of heterozygosity of Mongoloids were demonstrated to be intermediate between Caucasoids and Amerindians. Also these results support in general finding of more genetic variability in plasma proteins than reported by other investigators for different types of preparations. However, further studies for more individuals and tissues are required to estimate the true genetic variations in Koreans, and to be compared with other ethnic groups.

REFERENCES

Anderson, L. and N. C. anderson, 1977. High resolution two dimensional electrophoresis of human plasma protein. Proc. Natl. Acad. Sci. USA *74:* 5421-5425.

Anderson, N. L., P. Russel, P. Tracy and N. G. Anderson, 1984. High-resolution two-dimensional electrophoretic mapping of plasma proteins. In: *Plasma Proteins.* Vol. IV. (F.W. Putnam, editor), Academic Press, Inc., New York. pp. 222-268.

Asakawa, J., N. Takahashi, B. B. Rosenblum and J. V. Neel, 1985. Two-dimensional gel studies of genetic variation in the plasma proteins of Amerindians and Japanese. Hum. Genet. *70:* 222-230.

Coming, D. E., 1982. Two-dimensional gel electrophoresis of human brain proteins. III. Genetic and non-genetic variation in 145 brains. Clin. Chem. *28:* 798-804.

Goldma., D. and C. R. Merril, 1983. Human lymphocyte polymorphisms detected by quantitative two-dimensional electrophoresis. Am. J. Hum. Genet. *35:* 827-837.

Goldman, D., L. R. Goldin, P. Rathnagiri, S. J. O'Brien, J. A. Egeland and C. R. Merril, 1985. Twenty-seven protein polymorphisms by two-dimensional electrophoresis of serum, erythrocytes, and fibroblasts in two pedgrees. Am. J. Hum. Genet. *37:* 898-911.

Harris, H., 1980. The Principles of human biochemical genetics, 3rd revised Ed. Amsterdam, Elsevier/North-Holland, pp. XV and 554.

Hamaguti, H., A. Ohta, R. Mukau, T. Yabe and M. Yamada, 1981. Genetic analysis of human lymphocyte protein by two-dimensional gel electrophoresis. I. Detection of genetic variant polypeptides in PHA-stimulated peripheral blood lymphocytes. Hum. Genet. *59:* 215-220.

McConkey, E.H., B.J. Taylor and D. Phan, 1979. Human heterozygosity; a new estimate. Pro. Natl. Acad. Sci. USA *76:* 6500-6504.

Neel, J. V., 1978. Rare variants, private polymorphism, and locus heterozygosity in Amerindian population. Am. J. Hum. Genet. *30:* 465-490.

Neel, J. V., B. B. Rosenblum, C. F. Sing, M. M. Skoinick, S. M. Hanshi and S. Sternberg, 1984. Adapting two-dimensional gel electrophoresis to the study of human germ-line mutation rates. In: *Methods and Applications of two Dimensional Gel Electrophoresis of Proteins.* (J. E. Celis and R. Bravo, editors). Academic Press, New York, pp. 259-306.

Oakley, B. B., D. R. Kirsch and N. R. Morris, 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal. Biochem. *105:* 361-363.

Rosenblum, B. B., J. V. Neel and S. M. Hanash, 1983. Two-dimensional electrophoresis of plasma polypeptides reveals "high" heterozygosity indices. Pro. Natl. Acad. Sci. USA *80:* 5002-5006.

Rosenblum, B. B., J. V. Neel, S. M. Hanash, J. L. Joseph and N. Yew, 1984. Identification of genetic variants in erythrocyte lysate by two-dimensional gel electrophoresis. Am. J. Hum. Genet. *36:* 601-612.

Schamaun, O., B. Olaisen, T. Gedde-Dahl, Jr and P. Teisberg, 1983. Genetic studies of an apo A-I lipoprotein variant. Hum. Genet. *64:* 380-383.

Smith, S. C., R. R. Racine and C. H. Langley, 1980. Lack of genetic variation in the abundant proteins of human kidney. Genetics *96:* 967-974.

Tracy, R. P., R. R. Racine and C. H. Langley, 1982. Two-dimensional gel electrophoresis of serum specimens from a normal population. Clin. Chem. *28:* 890-899.

Utermann, G. A., A. Seinmetz and W. Weber, 1982. Genetic control of human apolipoprotein E polymorphism; Comparison of one-and two-dimensional technique of isoprotein analysis. Hum. Genet. *60:* 344-351.

Walton, K. E., D. Styer and E. I. Gruenstein, 1979. Genetic polymorphism in normal human fibroblasts as analysed by two-dimensional polyacrylamide gel electrophoresis. J. Biol. Chem. *254:* 7951-7960.

Wanner, L. A., J. V. Neel and M. H. Meisler, 1981. Separation of allelic variants by two-dimensional electrophoresis. Hum. Genet. *59:* 215-220.

Zannis, V. I., P. W. Just and J. L. Breslow, 1981. Human apolipoprotein E isoprotein subclass are genetically determined. Am. J. Hum. Genet. *33:* 11-24.