A STUDY ON THE GENETIC VARIATIONS IN ERYTHROCYTE LYSATES BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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ABSTRACT

Two-dimensional gel electrophoresis followed by silver staining has been employed to study genetic variation of 59 red cell lysates in Korean (Cheju) population. Forty-nine polypepetides selected without respect to variability were considered suitable for scoring. Genetic variations were encounted in 6 of these polypeptides. Ninety-eight of total 2,891 polypeptides exhibited the combination of a normal and a variant polypepetide. The index of heterozygosity estimated in this study was 3.3 ± 0.23%. The heterozygosity in Korean population was compared with those in other populations.

Key words: Two-dimensional gelelectrophoresis, genetic variation, heterozygosity

INTRODUCTION

The study of genetic variation and high-resolution genetic mapping depends on the analysis of variants whose phenotypes can be reliably scored. Studies of protein electrophoretic polymorphisms revealed that human genetic molecular variation may be high. Harris (1980) found that 24 of 104 human enzyme loci were polymorphic in an European Causcasian population, with an average heterozygosity of 6.3%. Because the variants detected by one-dimensional electrophoresis (1-DE) involve charge alteration, in general most of these are also detectable by two-dimensional electrophoresis (2-DE), which resolve charge-altered protein in the isoelectric focusing step (Anderson and Anderson, 1977; Wanner *et al.*, 1981). The extent of 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, brains, kidneys, and lymphocytes (McConkey *et al.*, 1979; Walton *et al.*, 1981; Smith *et al.*, 1980; Coming, 1982; Hamaguchi *et al.*, *1981).*

However, several recent studies have revised upward estimates of average

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protein heterozygosity by 2-DE. Goldman and Merril (1983) reported 2.4% heterozygosity on human lymphocytes. Also, Rosenblum *et al.*, (1983, 1984) reported that the average heterozygosity of serum and erythrocyte loci was 6.2% and 3.1%, respectively. In a previous study of plasma proteins, we (Oh *et al.*, 1987) estimated an index of heterozygosity of 7.6 ± 1.05% in plasma loci. In this study, authors extended the previous observation to a study of genetic variation in red cell lysate proteins of Korean (Cheju) population, and the heterozygosity estimated in this study was compared with those of other populations.

MATERIALS AND METHODS

Sample preparation

Blood was obtained from 59 children and their parent in Cheju-do. Plasma was removed following centrifugation. Nonpolymorphonuclear leukocytes were removed on a Ficoll Paque (Pharmacia) gradient. The remaining polymorphonuclear neutrophils were removed as a buffy coat after centrifugation of the red cells in saline. The wahsed red cells were stored as packed red cells at -70°C. Cells were lysed by adding phosphate buffer, pH 8.0, to 1 vol of packed red cells. Small volumes of lysate were centrifuged for 5 min at 13,000g to the red cell membrane pellet. The lysate was removed and re-centrifuged at 13,000g for 5 min.

Prior to electrophoresis, lysates were diluted with an equal volume of solubilization solution containing 9M urea, 2% Nonidet P-40, 2% β-mercaptoethanol, and 2% pH3.5-10, Ampholine (LKB) [Edwards et al., 1979]. Samples were contrifuged at 13,000g for 2 min before application to the first-dimension isoelectric focusing gels.

Electrophoresis and Staining

Two-dimension gel electrophoresis with some modification has carried out according to O'Farrell (1975). Ten to 20μl of samples were focused in first-dimension gel cast with pH3.5-10 Ampholine. Following focusing, the gels were extracted from the tubes and equilibrated. Gels were generally stored at -70°C for the second dimension run. Second dimension SDS ges were prepared with 12.5% acrylamide (Rosenblum *et al., 1982).* The protein patterns in the gels were visualized by staining with sensitive silver technique of Merril *et al.*, (1982).

Choice of polypeptide for analysis

Forty-nine polypeptides were selected for analysis, with no knowledge of their identity, on the basis of their appearance on a silver-stained gel. For analysis of genetic variation, the gel pattern was subdivided into convenient regions (A, B, C, D, E, and F). As our previous study of plasma proteins (Oh *et al.*, 1987), three criteria were applied to the selection of polypeptides; reproducibility, intensity, and relative isolation on the gel.

RESULTS

The positions for 49 polypeptides selected for analysis of genetic variation were pointed out as numbers in Fig. 1. Table 1 summarizes the data on the

Table 1
Genetic variations observed for six red cell lysate polypeptides among 59 individuals (Cheju population)

	B-001	B-011	C-006	D-014	D-012	E-007
N	48	33	52	36	44	28
NV	11	26	7	20	15	19
V				3		12
T	59	59	59	59	59	59
P	0.907	0.780	0.941	0.780	0.873	0.636
Q	0.093	0.220	0.059	0.220	0.127	0.364
x^2*	0.624	4.727	0.350	0.004	1.250	5.450

Note: A total of 49 spots were scored of which only these six polypeptides showed genetic vatiation. Criteria for selection were described in text. The genetic nature of the variation was in all instances confirmed by the presence of the same variant in the parents.
*Test for agreement with Hardy-Weinberg equilibrium proportions.
N; Homozygote of normal spot
NV; Heterozygote of normal and variant spot
V; Homozygote of variant spot
T; Total
P; The gene frequency of normal spot
Q; The gene frequency of variant spot.

frequency of the variant that were encounted in preparation of the 59 red cell lysates. Six of the 49 polypeptides each yielded a single type of genetic Variant. These were shown in Figs. 2 and 3. Of a total 2,891 polypeptides scored, 98 exhibited the combination of a normal and a variant polypeptides; this corresponded to an index of heterozygosity with the standard error of 3.3 ± 0.25% (Table 2). The finding for the six variable proteins (B-001, B-001, C-006, D-012, D-014, and E-007) may be summarized as follows;

B-001

This was a polypepetide of approximately 60,000 daltons. A variant seen in eleven individuals migrated approximately 1mm to the basic side of a normal spot (Fig. 2a). The normal spot is one of a row of three spots, the other two migrating to the acidic side of spot B-001. While all three spots may show coordinate variation, the observed electrophoretic shift was of the magnitude that would result in spot superimposition, and so it was not certain if the other two spots are related.

Table 2
Summary of polymorphisms identified using 2-D gel electrophoresis

Tissue	No. of loci surveyed	No. of polymorphic loci	Heterozygosity
Erythrocyte	49	6	N/V combination 98 Total polypeptides 2891
			Average heterozygosity = 3.3±0.23%

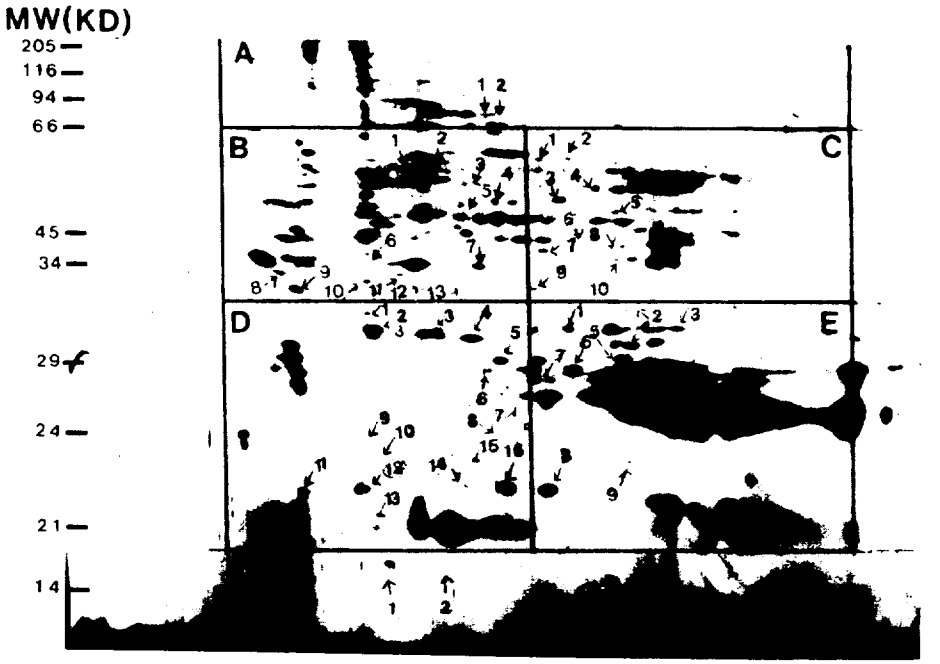

Fig. 1. Pattern of red cell lysate polypeptides separated by two-dimensional gel electrophoresis. The pattern is subdivided into convenient regions, and the polypeptides scored for genetic variation are designated. The pattern was oriented with acidic side to the left and basic side to the right.

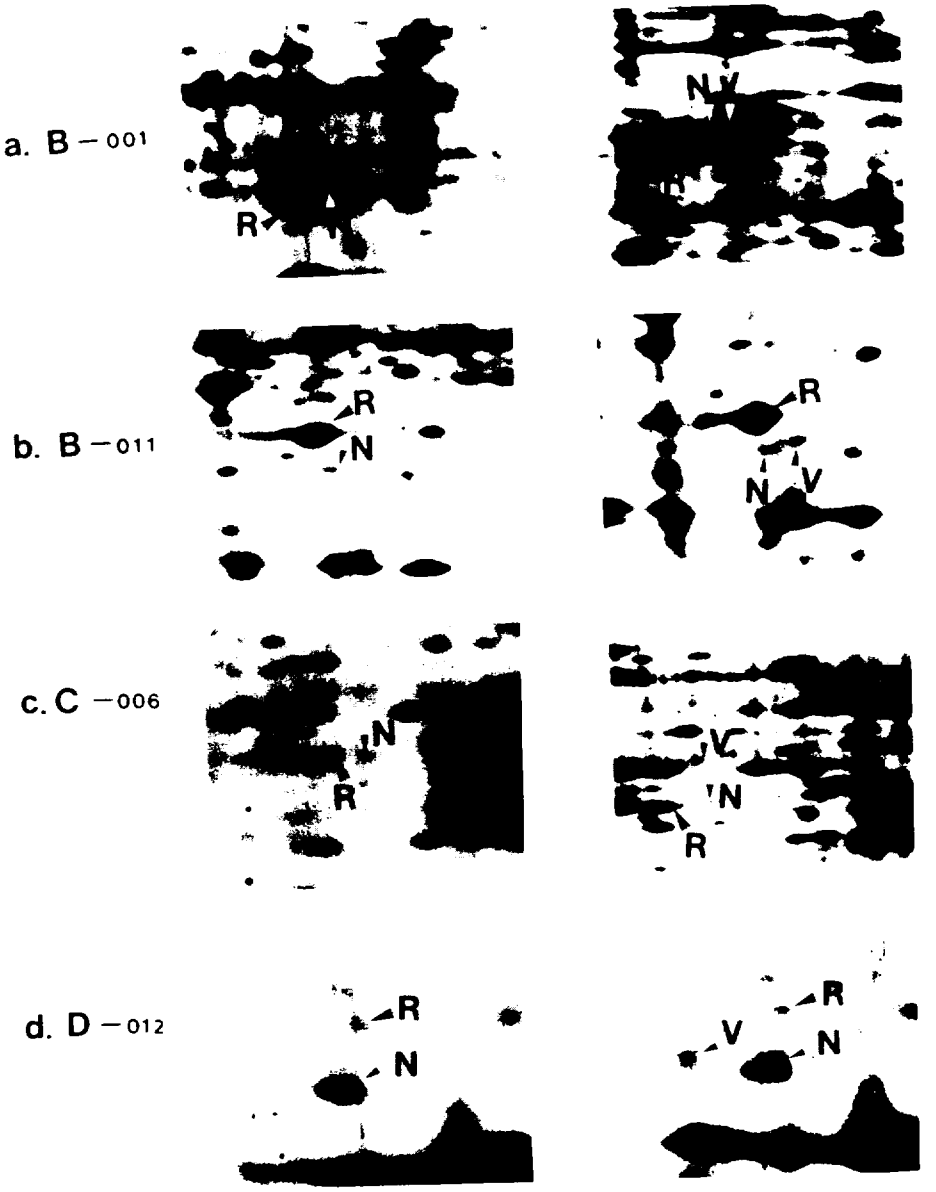

Fig. 2. Section of the two-dimensional gels showing the variants (V) and the corresponding normal (N) polypeptides. In all photos, a convenient reference spot (R) has been designated.

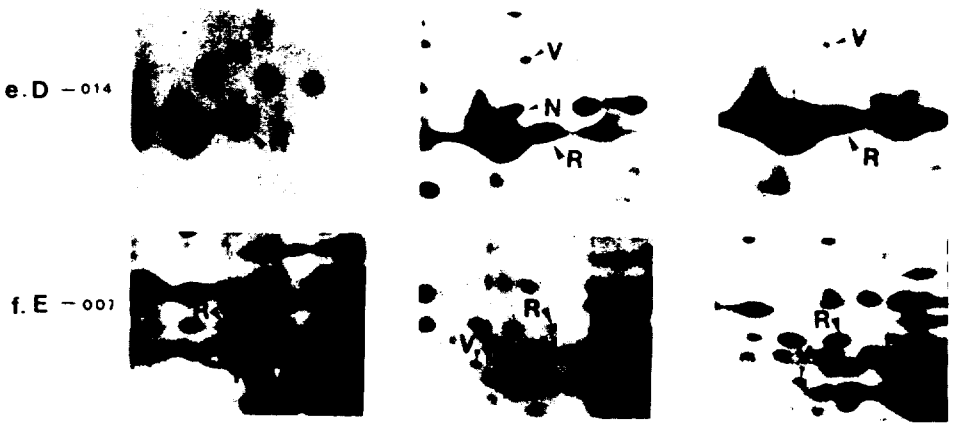

Fig. 3. **Section of the two-dimensional gels showing the variants (V) and the corresponding normal (N) polypeptides. In all photos, a convenient reference spot (R) has been designated.**

B-011

This was a polypepitde of approximately 40,000 daltons (Fig. 2b). An apparently identical variant was observed in 26 individuals (Table 1). The variant was characterized by a spot migrating approximately 2.5mm more basically than the normal spot. Gene frequencies of 0.780 and 0.220 were observed for the common and variant alleles, respectively (Table 1).

C-006

This was a polypeptide of approximately 45,000 daltons. The variant, detected in seven individuals, was observed as a shift in the molecular weight axis (Fig. 2c). The variant polypeptide migrated appoximately 3mm above the normal polypeptides. Gene frequencies of the normal and variant alleles were 0.941 and 0.059, respectively (Table 1).

D-012

This was a polypeptide of approximately 20,000 daltons. Gene frequencies of the normal and variant alleles were 0.873 and 0.127, respetively (Table 1). The variant polypeptide migrated approximately 5mm to the acidic side of a normal spot (Fig. 2d).

D-014

This was a polypeptide of approximately 20,000 daltons. The variant allele was observed as an alteration in the apparent molecular wight, the variant being visualized 3mm above the normal position (Fig. 2e). The gene

frequencies for the normal and variant alleles were 0.780 and 0.220, respectively (Table 1).

E-007

This was a polypeptide of approximately 30,000 daltons. The variant spot migrated approximately 10mm to the acidic side of the normal spot (Fig. 2f). In our system of subdivided region designations, the variant spot was located in the D region. The variant spot differed only slightly in the position from a spot in the D region (D-006) that was invariant in this study (Fig. 1). The gene frequencies for the normal and variant alleles were 0.636 and 0.364, respectively (Table 1).

DISCUSSION

In this study, 49 polypeptides were selected for analysis without bias in regard to their variability. Of the six polypeptides each with a single type of variant, two (C-006 and D-014) were observed as alterations in the apparent molecular weight of the variant polypeptide with respect to the presumed normal spot. Though the nature of the alteration in the polypeptide structure responsible for these variant is unclear, the subsititution of a single amino acid altering molecular charge and molecular weight of a polypeptide (Leavitt *et al.*, 1982; Wilson *et al.*, 1981).

One-dimensional electrophoretic (1-DE) studies of proteins that are the products of 104 genetic loci, those proteins for the most part functioning as enzymes, have yielded an index of heterozygosity of 6.3% in Caucasoid humans (Harris, 1980). Also, Neel (1978) reported that the indices of heterozygosity for a series of 28 erythrocyte enzymes and serum protein scored for genetic variability by 1-DE in Amerindians, Japanese, and Caucasians, were 5.4%, 7.7%, and 7.8%, respectively.

In contrast, most studies using two-dimensional gel electrophoresis (2-DE) have yielded different values for the index of heterozygosity (Table 3). It falls into three groups: (1) approximately 1% or less in fibroblastic cell lines (McConkey *et al.*, 1979 and Walton *et al.*, 1979), kidney (Smith *et al.*, 1980), brain (Coming, 1982), and lymphocytes (Hamaguchi *et al.*, 1981, 1982a, 1982b, 1982c), (2) 2%-3% in lymphocytes (Goldman and Merril, 1983) and red cell lysates (Rosenblum *et al.*, 1984; this paper), and (3) 6%-8% in plasma (Asakawa *et al.*, 1985; Oh *et al.*, 1987; Rosenblum *et al.*, 1983). There are several possible explanations for this apparent variation in the index of heterozygosity yielded by 2-DE PAGE preaprations of various cells, tissues, and/or body fluids (Rosenblum *et al.*, 1984). First, it is clear that in a small series of scored polypeptide the addition of a single polymorphic polypeptide or the deletion of a polypeptide, because it is related to another scored polypeptide, can increase or decrease the index

Table 3
Heterozygosity indices from population surveys using 2-D gel analysis in various human tissues

Population	Tissue	Average heterozygosity	Reference
Caucasoids	Brains	0.04%	Coming (1982)
Caucasoids	Kidneys	no genetic variation	Smith *et al.* (1979)
Caucasoids	Fibroblasts	1.0%	McConkey *et al.* (1979)
Caucasoids	Fibroblasts	2.0%	Goldman *et al.* (1985)
Japaneses	Lymphocytes	0.5%	Hamaguchi *et al.* (1981)
Caucasoids	Lymphocytes	2.4%	Goldman & Merril (1983)
Caucasiods	Plasmas	6.2%	Rosenblum *et al.* (1983)
Caucasoids	Plasmas	8.0%	Asakawa *et al.* (1985)
Korean	Plasmas	7.6%	Oh *et al.* (1987)
Caucasoids	Erythrocytes	3.1%	Rosenblum *et al.* (1984)
Korean	Erythrocytes	3.3%	*present study*

of heterozygosity by 1% or 2%. Second, it may be that the difference is due to the use of silver staining for the identification of the relatively abundant proteins. Goldman and Merril (1983) have used autoradiography to study lymphocyte polypeptides, and they found the index of heterozygosity of 2.4%. Finally, it is possible that the difference is due to the more rigorous criteria that investigators have employed in the selection of spots for analysis, We are especially skeptical of the ability to score with precision for variants in crowded regions of 2-DE gels.

Compared to our previous study (Oh *et al.*, 1987) on plasma proteins, the level of heterozygosity in red cell lysates is lower (Table 3). This finding that the prominant proteins of plasma are more polymorphic than those of other cells suggests that cellular proteins may be under selective pressures that mitigate against genetic variation. In Korean (Cheju) population, the level of the combined heterozygosity in two loci was estimated to be as 5.45% (Table 4). However we do not consider that this value represents the level of true genetic variation of Korean population. Therefore, further studies on various tissue preparations are required to estimate the true amount of genetic variation in Korean population and to be compared with other ethnic groups.

Table 4
Heterozygosity indices surveyed in Cheju population

Tissue	Method	Heterozygosity	Reference
Plasmas	2-D, Coomassie Blue R-250 and silver staining	7.6%	Oh *et al.* (1987)
Erythrocytes	2-D, silver staining	3.3%	*present study*
		Combined 5.45%	

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