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Study of Genetic Effects of Sulphur Mustard Gas on Former Workers of Ohkunojima Poison Gas Factory and Their Offspring

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ABSTRACT

General health examination and one-dimensional electrophoretic examination to detect mutations at the protein level were conducted in order to elucidate potential genetic effects of sulphur mustard gas on children of the former workers of Ohkunojima Poison Gas Factory.

In the general examination, no disease which was definitely considered to be caused by genetic effects was observed, and no examination values obtained for the children proved to be significantly abnormal compared with those for their parents.

Blood samples from 456 children were electrophoretically examined for 30 protein systems. A total of 36 protein variants were detected in 10 protein systems, and the frequency of variants was 2.63 per 1,000 tests. Family study was completed for 32 of these variants, all of which were confirmed to be genetic variants. In 29,868 locus tests, mutation occurred in germ cells of parents could not be detected.

Among 36 variants, two PGM2 variants and one GPI variant were detected for the first time in this study.

Today, radiation and chemicals are matters of worldwide interest as mutagens, and people all over the world are concerned that increase of mutations due to mutagens in our environment might give rise to the problem of genetic effects. According to the study conducted for the past 33 years by the Second Department of Internal Medicine, Hiroshima University School of Medicine, respiratory tract cancers have occurred frequently among former workers of Ohkunojima Poison Gas Factory[3,7,14,15], and the incidence once reached as high as about 37 times the incidence of respiratory tract cancers in all Japan. Recently, the incidence of cancer of the digestive system[11] and skin cancer has been noted to be elevated remarkably, and an elevated incidence has also observed in primary liver cancer, which is considered to be attributable to absorption of sulphur mustard gas in the blood.

Because it is known that some carcinogens can be mutagens and that some mutagens are carcinogens, there is a possibility that the former workers have been affected genetically by sulphur mustard gas. Thorough elucidation of this possibility is necessary for the future of mankind. It will serve as an aid in dispelling the apprehensions entertained by the former workers and their offspring about genetic effects, and also provide an answer with regard to the apprehensions generally entertained about environmental mutagens.

This study aims to detect mutation caused in genes of germ cells at the protein level and determine whether or not exposure to sulphur

mustard gas has affected the mutation rate. Variation caused in DNA, the chemical entity of genes, can be reflected as variant proteins. By electrophoresis, which is used widely as an easy method for detecting variant proteins, changes in the electric charge of protein molecule represent changes in mobility. It is considered that about one-fourth of the variations occurring in genes can be detected by electrophoresis as variant proteins with different mobility. Efforts have been made to conduct examinations using the electrophoretic method and examine the health status of the former workers and their families (spouses and children).

MATERIALS AND METHODS

Subjects

Subjects of the present study were classified into the following three groups according to their working places. Subjects in Group A are former workers who had engaged primarily in the production of yperite and lewisite and are considered to have been exposed to gas of high concentration and their families. Subjects in Group B are former workers who had engaged primarily in engineering, repair, and incineration and are considered to have been exposed to poison gas of medium concentration and their families. Subjects in Group C are former workers who had engaged in the handling of tear gas and sneezing gas, office work, medical work, transportation, and postwar affairs and are considered to have been exposed to poison gas of low concentration and their families.

1. General health examination

Examination was conducted on 325 former workers including 65 in Group A, 52 in Group B, and 208 in Group C. A total of 226 spouses were examined, that is, 60, 40, and 126 in the respective groups. Further, health examination was conducted on 483 children, that is, 95, 80, and 308 in the respective groups (Table 1).

2. Electrophoretic examination

Subjects of electrophoretic examination are children born to the former workers while they were employed and after they had terminated employment. A total of 5,871 children are registered both of whose parents were confirmed to be alive as of 1977, comprising 1,260 in Group A, 773 in Group B, and 3,838 in Group C (Table 2).

Of this number, examinations have already been conducted on 456 children in total (228 males and 228 females), comprising 87 in Group A (44 males and 43 females), 75 in Group B (40 males and 37 females), and 294 in Group C (146 males and 148 females). (See Table 1.)

The examined children ranged in age from 13

Table 1. Number examined on this study

Parental category	Former workers	Spouse of former workers	Children			Total
			Born before exposure	Born during exposure	Born after exposure	
Male						
A	53	4	6	11	33	50
B	43	3	3	3	35	41
C	74	60	6	16	130	152
Female						
A	12	56	2	6	37	45
B	9	37	2	6	31	39
C	134	66	8	13	135	156
Subtotal						
A	65	60	8	17	70	95
B	52	40	5	9	66	80
C	208	126	14	29	265	308
Total	325	226	27	55	401	483

Table 2. The availability of subjects for a study of children born to persons occupationally exposed to sulphur mustard fumes during WW II

Parental category	Born during exposure	Born after exposure	Total
A. Workers manufacturing sulphur mustard	214	1046	1260
B. Workers maintaining the equipment for manufacturing sulphur mustard	118	655	773
C. Other workers at plant	332	3506	3838
Total	664	5207	5871

The tabulation is restricted to children both of whose parents were alive in 1977.

Table 3. Age distribution of children examined electrophoresis

Parental category	Age				Total	Average age
	<20	20—29	30—39	40+		
A	0	24	53	10	87	32.8±5.9
B	2	24	44	5	75	30.7±5.4
C	11	103	167	13	294	30.4±5.8
Total	13	151	264	28	456	30.9±5.8

to 43, the average age being 30 ± 5.8, and there was no difference in age among Groups A, B and C (Table 3).

Methods

1. General health examination

Examinations conducted were anamnesis, physical examination, urinalysis, stool, peripheral blood test (erythrocytes, leukocytes, platelets, leukocyte differential count), HB antigens, blood chemistry (GOT, GPT, AL-P, LDH, γ-GTP, total cholesterol, total bilirubin, total protein, A/G ratio, ZTT), serum protein quantitation (I_gG, I_gA, I_gM, α_1-AT, α_1-AG, α_2-HS, α_2-Macro, C_3, C_4, TF, HP), chest X-ray and ECG.

2. Electrophoretic examination

About 8 ml of blood samples were obtained from children using heparin as anticoagulant and separated into plasma and erythrocyte layers by centrifugation at 1,500 × *g*. After washing the erythrocyte layer three times with 0.85% NaCl solution, 0.5-1.0 ml each of packed red cells and plasma were separately dispensed into small vials and preserved in liquid nitrogen. They were taken out after 1-3 months, and the primary screening, mainly by starch gel electrophoresis, was performed. When variants with abnormal mobility were detected, comparison of variants was made with variants detected in the children of the atomic bomb survivors and their controls examined at the Radiation Effects Research Foundation (RERF)[1,8,12,13] using the same methodology. For the variants identified to be "rare" variants (gene frequency < 0.01), same examinations were conducted on both parents as family studies to determine whether they were genetic variants or variants caused by mutation. The well known polymorphic variants are by definition not the type of variant under consideration. The rationale for concentrating on the rare variants is that they are, relatively speaking, much more apt to be recent mutational origin than polymorphic variants.

1) Plasma proteins

Five proteins were examined, i.e., albumin

(ALB), haptoglobin (HP), transferrin (TF), ceruloplasmin (CP), and α_1-antitrypsin (protein inhibitor, PI). Primary screening was performed by conducting polyacrylamide thin layer gel isoelectric focusing for PI and, for other proteins, by starch gel electrophoresis using the technique of Ferrell et al[1].

2) Erythrocyte proteins

Twenty-five proteins were examined, i.e., hemoglobin A1 (HBA1), hemoglobin A2 (HBA2), adenosine deaminase (ADA), 6-phosphogluconate dehydrogenase (6-PGD), adenylate kinase-1 (AK1), phosphoglucomutase-1, 2 and 3 (PGM1, PGM2, PGM3), acid phosphatase-1 (ACP1), triose phosphate isomerase (TPI), nucleoside phosphorylase (NP), esterase A, B and D (ESA, ESB, ESD), peptidase A and B (PEPA, PEPB), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (ICD), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), carbonic anhydrase-1 and 2 (CA1, CA2), glutamate-oxalacetate transaminase 1 (GOT1), glutamate-pyruvate transaminase (GPT), phosphoglycerate kinase (PGK). Here also, primary screening was conducted using starch gel electrophoresis[8,12,13].

3) Family studies

For "rare" variants, same electrophoretic examination was conducted for both parents. when a variant with the same mobility as that of a child was detected in either of the parents, the variant was considered to be a genetic variant transmitted from the parent to the child. When neither parent exhibited the variant, blood types (ABO, Rh, MNSs, Kk, and Fy) and HLA typing at A, B, and C loci were made to determine the possible discrepancies between stated and biological parentage. In addition, phenotypes of the following 10 polymorphic proteins included in the 30 protein systems routinely examined by electrophoresis were also determined for this purpose: HP, PI, ADA, ACP1, ESD, 6-PGD, GOT1, GPT, PGM1 and PGM3.

RESULTS

1. General health examination (Table 4)

In peripheral blood examination, 5.5% of the former workers and 4.9% of the spouses showed abnormality, while abnormality was observed in 10.8% of the children (about two-fold higher frequency). In blood chemistry examination, 5.0% of the children showed abnormality, while abnormality was observed at about two-fold higher frequency in the former workers (10.2%) and in the spouses (8.8%). In serum protein quantitation, abnormality was observed in about 6% of each group. In chest X-ray, ECG, and urinalysis, the former workers and spouses showed abnormality at higher frequencies than the children. Also, though not shown in the Table, eight former workers (2.5%), four spouses (1.8%), and four children (0.2%) showed positive occult blood in stool, while seven (2.1%), six

Table 4. Summary of laboratory data

Item		Former workers	Spouse of former workers	Children			Total
				A	B	C	
		n:325	n:226	n:95	n:80	n:308	n:483
CBC	Abnormal case	18 (5.5)	11 (4.9)	13 (13.6)	7 (8.6)	32 (10.4)	52 (10.8)
Blood chemistry	Abnormal case	33 (10.2)	20 (8.8)	2 (2.1)	3 (3.8)	19 (6.2)	24 (5.0)
Serum proteins	Abnormal case	21 (6.4)	16 (7.1)	6 (6.3)	5 (6.3)	17 (5.5)	28 (5.8)
Chest X-P	Abnormal case	15 (4.6)	10 (4.4)	2 (2.1)	1 (1.3)	6 (1.9)	10 (2.1)
ECG	Abnormal case	20 (6.2)	17 (7.5)	5 (5.3)	1 (1.3)	3 (1.0)	8 (1.7)
Urine	Abnormal case	15 (4.6)	17 (7.5)	3 (3.2)	2 (2.5)	8 (2.6)	13 (2.7)

(%)

(2.7%), and 16 (3.3%) showed positive HB antigen reaction, respectively. For the above two examination items, no explicit difference was observed in the frequency of abnormalities among children of Groups A, B and C. Malignant tumors detected in this health examination were lung cancer (adenocarcinoma) in a female aged 34 and chronic lymphocytic leukemia in a female aged 36, both of whom belonged to the children group.

Thus, in the general health examination, no diseases which could definitely be ascribed to genetic effects were observed, and no examination values obtained from the children group were considered to be significantly abnormal compared with those of the parents groups.

2. Electrophoretic examination

1) Plasma proteins (Table 5)

In HP, three variants showing a set of bands migrating cathodal to the normal set of HP2 bands and one variant showing bands migrating anodal to the HP2 bands, i.e., a total of two kinds and four cases of variants, were detected. In TF, two kinds of variants in five individuals were encountered. The first variant showed a band migrating anodal to the normal TF C band and the second variant in four individuals migrated anodal to the first variant. In PI, one variant showing a band migrating cathodal to the normal PI M1 band and one variant showing a band anodal to the normal PI M2 band, i.e., a total of two kinds and two cases of variants, were detected.

Thus, with regard to plasma proteins, six kinds of "rare" variants were detected in 11 individuals and, except for the two variants of PI, these variants showed the same mobility as those of variants already detected at RERF in the populations of Hiroshima and Nagasaki.

2) Erythrocyte proteins (Table 6)

In PGM1, two variants showing bands migrating cathodal to the normal PGM1 1 bands were detected. In PGM2, one variant showing bands migrating cathodal to the normal PGM2 1 bands and one variant migrating cathodal to the first variants, i.e., a total of two kinds and two cases of variants, were detected. In NP, three variants showing a set of bands migrating anodal to the normal set of NP 1 bands were detected. In GPI examination, one variant showing bands migrating anodal to the normal GPI 1 bands (normal type) and two variants migrating anodal to the first variant, i.e., a total of two kinds and three cases of variants, were detected. In CA1, one variant showing a band migrating cathodal to the normal CA1 band was detected. In GOT1, two variants showing bands migrating cathodal to the normal GOT1 1 band were detected. In GPT, one variant showing a band migrating anodal to the normal of GPT 1 band, seven variants with migration still anodally, and four variants showing a band with anodal migration than those of the above two kinds, i.e., a total of three kinds and 12 cases of variants, were detected.

Thus, with regard to erythrocyte proteins, 11

Table 5. Result of the electrophoresis data on plasma proteins

Systems (symbols)	Total determination	Variants	
		No. of types	Total
ALB	912	0	0
HP	912	2	4
TF	912	2	5
CP	912	0	0
PI	912	2	2
Total	4560	6	11

Table 6. Result of the electrophoresis data on erythrocyte proteins

Systems (symbols)	E.C.No.	Total determination	Variants	
			No. of types	Total
HBA1		2736	0	0
HBA2		912	0	0
ADA	3.5.4.4.	912	0	0
6-PGD	1.1.1.44	912	0	0
AK1	2.7.4.3	912	0	0
PGM1	2.7.5.1	912	1	2
PGM2	2.7.5.1	912	2	2
PGM3	2.7.5.1	912	0	0
ACP1	3.1.3.2	912	0	0
TPI	5.3.1.1	912	0	0
NP	2.4.2.1	912	1	3
ESA	3.1.1.1	912	0	0
ESB	3.1.1.1	912	0	0
ESD	3.1.1.1	912	0	0
PEPA	3.4.11.—	912	0	0
PEPB	3.4.11.—	912	0	0
GPI	5.3.1.9	912	2	3
ICD	1.1.1.42	912	0	0
LDH	1.1.1.27	1824	0	0
MDH	1.1.1.37	912	0	0
CA1	4.2.1.1	912	1	1
CA2	4.2.1.1	912	0	0
COT1	2.6.1.1	912	1	2
GPT	2.6.1.2	912	3	12
PGK	2.7.2.3	684	0	0
Total		25308	11	25

kinds of "rare" variants were detected in 25 individuals and, except for the two variants of PGM2 and one variant of GPI, variant having the identical mobilities with these variants were already detected in the populations of Hiroshima and Nagasaki.

The first new variants of PGM2 found in a child with ID number of PG0587 showed two variant bands, one being slightly anodal to the b band and one slightly cathodal to the d band. Though it was difficult to distinguish these variant bands from the b and d bands of PGM1 when stained on the basis of PGM activities, they were clearly identified as PGM2 bands by staining based on phosphopentomutase (PPM) activity. The second variant encountered in a child (PG0683) showed a variant band of PMG2 between the c and d bands (Fig. 1). The variant of GPI (PG0483) was composed of 3 bands, normal homodimer, normal-variant heterodimer and variant homodimer band, and the latter two bands were on the anodal side of the normal homodimer band, which were definitely different from those of GPI 1-4HR1 already reported[12] which have been encountered in the population of Hiroshima and Nagasaki. Two variant bands of the new GPI variant migrated to the positions anodal to the bands of GPI 1-2NG1 and cathodal to those of GPI 1-3HR1, respectively (Fig. 2).

A total of 36 "rare" variants with different electrophoretic mobility were encountered among children of former workers of the poison gas factory (Table 7). They were comprised of 10 variants in Group A, eight in Group B, and 18 Group C. The frequency of these variants was 2.63 per 1,000 tests (3.83 in Group A, 3.56 in Group B, and 2.04 in Group C). A significant difference ($p<0.05$) was observed between this value and value 1.89 per 1,000 tests obtained at RERF[10].

3) Family studies

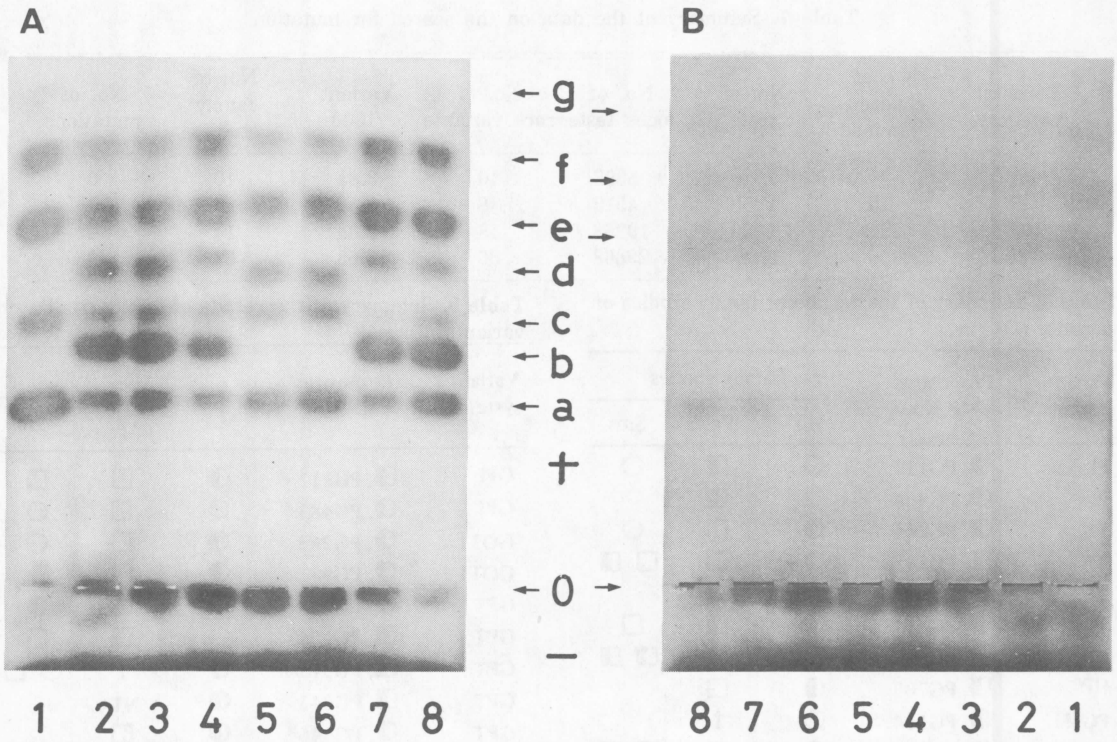

Fig. 1. Starch gel electrophoresis of PGM2 variants encountered in this study
A: staining based on PGM activity
B: staining based on PPM activity
well 1, PGM1 1, PGM2 1; well 2, PGM1 1-2, PGM2 1-Variant (mother of PG 0587); well 3, PGM1 1-2, PGM2 1-Variant (PG0587); well 4, PGM1 1-2, PGM2 1 (father of PG0587); well 5, PGM1 1, PGM2, 1-Variant (mother of PG0683); well 6, PGM1 1, PGM2 1-Variant (PG0683); well 7, PGM1 1-2, PGM2 1 (father of PG0683); well 8, PGM1 1-2, PGM2 1

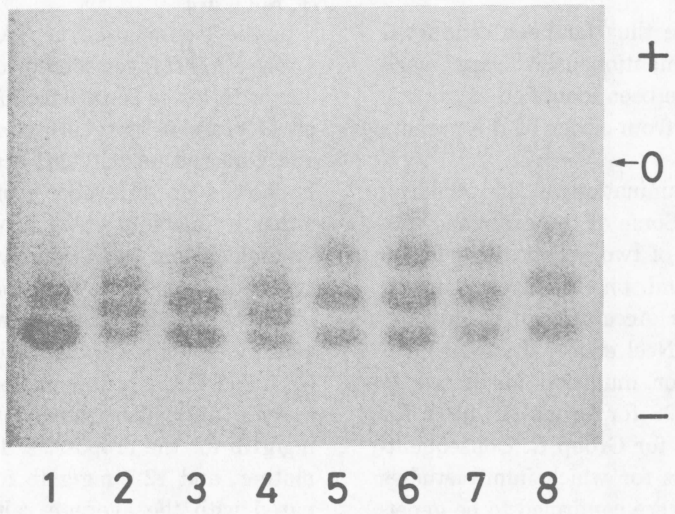

Fig. 2. Comparison of some GPI variants encountered in this study
well 1, GPI 1; wells 2 & 4, 1-2NG1; well 3, 1-Variant (PG0483); wells 5, 6, & 7, 1-3HR1; well 8, 1-4HR1

Table 7. Summary of the data on the search for mutation

Parental category	No. of children examined	No. of tests	No. of locus tests	No. of rare variants	Variant /1000	No. of family studies	No. of mutations
A	87	2610	5698	10	3.83	10	0
B	75	2250	4912	8	3.56	8	0
C	294	8820	19258	18	2.04	14	0
Total	456	13680	29868	36	2.63	32	0

Table 8. Summary of the results of family studies on variants (1)

Variant systems	Propositus MF No.	Family studies		
		Mo.	Fa.	Sibs
PI	◑, PG111	○	◨	○
PI	◑, PG434	○	◨	
TF	◑, PG264	◑	□	○
TF	◨, PG582	◑	□	□ ◨
TF	◑, PG631	○	◨	
TF	◑, PG887	○	◨	□
HP	◨, PG582	○	◨	◨ ◨
HP	◨, PG703	◑	□	
PGM1	◑, PG184	○	†	
PGM1	◑, PG677	◑	□	□ □
PGM2	◑, PG587	◑	□	□
PGM2	◑, PG683	◑	□	
NP	◨, PG067	○	◨	
NP	◑, PG640	○	◨	
NP	◑, PG678	◑	□	

○ □, Normal; ◑ ◨, Heterozygote; †, Deceased

Table 9. Summary of the results of family studies on variants (2)

Variant systems	Propositus MF No.	Family studies		
		Mo.	Fa.	Sibs
GPI	◨, PG212	◑	□	◨
GPI	◑, PG483	◑	□	○
GOT1	◑, PG263	◑	□	○
GOT1	◑, PG981	◑	□	
GPT	◑, PG198	○	◨	◑
GPT	◨, PG206	○	◨	□
GPT	◨, PG315	◑	†	○ □
GPT	◨, PG343	◑	NT	
GPT	◑, PG446	◑	□	
GPT	◨, PG746	◑	□	◑ □
GPT	◨, PG885	◑	□	○ □
GPT	◑, PG892	◑	□	○
GPT	◑, PG950	○	†	
GPT	◨, PG1023	○	◨	
CA1	◑, PG947	○	◨	

○ □, Normal; ◑ ◨, Heterozygote; †, Deceased; NT, Not tested

Family studies have thus far been conducted for 32 of the above-mentioned 36 "rare" variants, all of which have been identified as genetic variants transmitted from either of the parents (Tables 8 and 9).

Electrophoretic examination on 456 children has been conducted. Some of the examined proteins were comprised of two genetically independent polypeptides and one of them showed sex-linked inheritance. According to the method of Harris et al[2] and Neel et al[6], the total number of loci tested for mutation amounted to 29,868 including, 5,698 for Group A, 4,912 for Group B, and 19,258 for Group C. Consequently, all the 32 variants for which family studies had been conducted were confirmed to be genetic variants, and no variant attributable to mutation was detected (Table 7).

4) Other variant

In the examination of CA1, we encountered a case (PG0074) considered to be an activity variant with lower sensitivity in the staining based on esterase activity using α-naphythyl acetate and 4-methyl umbelliferyl acetate as substrates. It showed no difference from the normal type either by mobility or by protein staining based on amideblack. In the family study (Fig. 3), enzymes of the parents and siblings were observed to be normal by protein staining and esterase activity staining. Quantitation of isozymes of CA1 and CA2 by immunological method using specific antibodies showed CA1 value of 10.9 mg/gHb for the propositus, 13.3 mg/gHb for the mother, and 12.7 mg/gHb for the father. Compared with the average value of 12.2 mg/gHb of healthy adults (n:45, SD = 2.9 mg/gHb), no difference in protein quantity of the propositus was observed, and the same tendency was

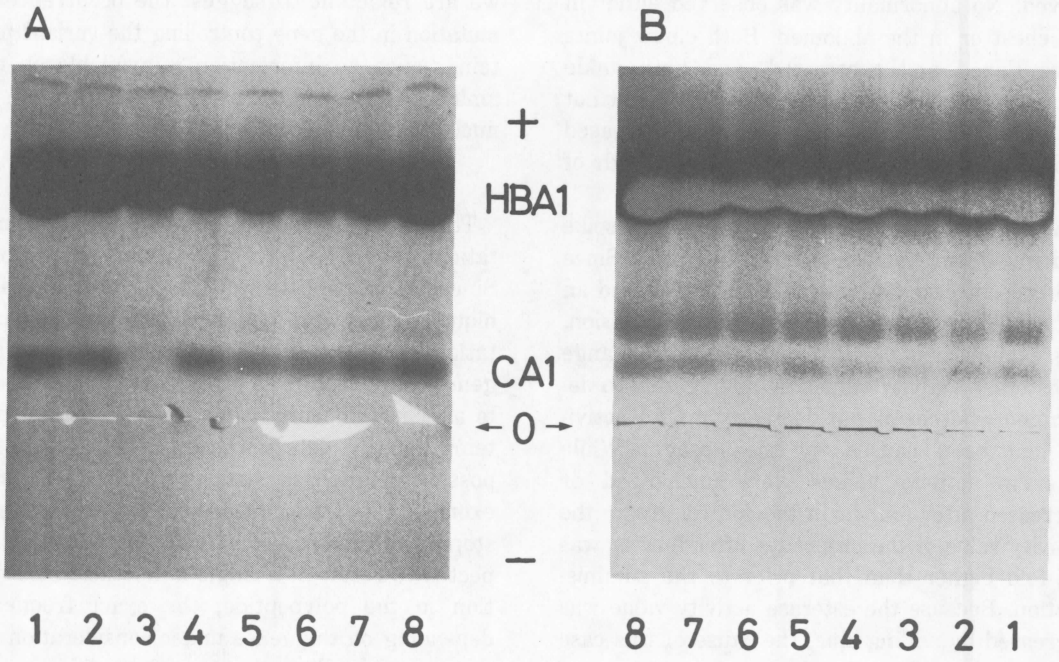

Fig. 3. Family study of CA1 (PG0074)
A: esterase stain B: protein stain wells 1 & 8, normal control; well 2, nother of PG 0074; well 3, PG0074; well 4, sester of PG 0074; well 5, brother 1 of PG0074, well 6, brother 2 of PG0074; well 7, father of PG0074

observed for CA2 (Table 10). Enzyme activities generally depend on the quantity of enzyme proteins, but this is a rare case showing normal protein quantity and decreased activity value. Serological studies and examination of protein types conducted for confirmation of biological parentage revealed no contradiction, and the banding pattern of chromosomes was observed to be normal by the G-band technique.

The following is a brief description of the propositus:

Patient: Y.H. 23 years old. Female.

Chief complaint: Mental retardation

Family history: The mother served at the former Ohkunojima Poison Gas Factory from May to August 1945. There is nothing noteworthy for the father.

Previous history: The patient was born as the second child 13 years after her mother's retirement and contracted erysipelas one month later.

History of present illness: Body weight at birth, 3,112 g; height, 50 cm. Cleft soft palate and hypomyotonia were observed. Development in infancy (stability of neck, commencement of walking, etc.) was retarded and diagnosis of infantile cerebral paralysis was made. Functional training of the extremities was begun at the age of about 6 years, but joint contracture of the extremities improved only slightly. About that time, convulsion, which is trembling of extremities, was occasionally observed. At the age of about 18, the attacks of convulsion became frequent (once a day), and she was diagnosed as "epilepsy" by EEG and administered an anticonvulsant. The attacks of convulsion have not occurred thereafter. Present illness: Body weight, 35 kg; height, 142 cm; blood pressure, 116-68 mmHg; pulse, 78/min., regular. External squint of the left eye and cleft soft palate were ob-

Table 10. Erythrocyte CA1 and CA2 concentration

	CA1 (mg/gHb)	CA2 (mg/gHb)
Daily mean (n=45)	12.2±2.9	2.33±0.4
Mother of PG0074	13.3	2.71
PG0074	10.9	2.93
Father of PG0074	12.7	2.56
Sister of PG0074	14.8	2.80
Brother 1 of PG0074	10.8	2.52
Brother 2 of PG0074	11.0	2.52

served. No abnormality was observed either in the chest or in the abdomen. Both elbow joints were flexed and contracted, and both ankle joints were markedly contracted and could not be flexed. All tendon reflexes were increased and pathologic reflex was observed in both of the lower extremities.

As a major laboratory finding (Table 11), spike and wave complex was observed on EEG. Since the present case had long been administered an anticonvulsant to stop her attacks of convulsion, the examination *in vitro* was made on change in esterase activity values by adding zinc to determine whether or not decrease in CA1 activity had been caused by the dosage. While esterase activity values were unchanged or decreased after four hr in the control group, the activity value of the propositus after four hr was 1.5-fold higher than that prior to the administration. Because the esterase activity value was increased by adding zinc, the cause of this case which was considered to have a variant type of CA1 activity was probably attributable to the difference in the primary structure of the adhesion site of zinc rather than to activity inhibition by the anticonvulsant. However, at present we are reluctant to suggest the occurrence of mutation in the gene controlling the variant proteins, since no information is available on the amino acid sequence of the variant protein or nucleotide sequence of the gene.

DISCUSSION

This study is directed at detecting gene mutations as opposed to chromosomal mutations. Since electrophoresis was employed as a technique to detect protein variants, only point mutations which occurred in exons of structural genes and resulted in amino acid substitutions to alter electrophoretic properties of variant proteins could be detected in this study. Of all the possible single base changes that could occur in exons, 1/4 — 1/3 would result in synonymous or stop codons and approximately 3/4 would be expected to result in a single amino acid substitution in the polypeptide, the exact frequency depending on the genes under consideration. Of those that result in single amino acid substitutions, 1/2 — 1/3 would be detected electrophoretically.

Other studies to determine the rate of mutation at human loci encoding protein structure us-

Table 11. Laboratory data of propositus (PG0074)

Urine		Blood chemistry		Serum protein (mg/dl)	
Sugar	(—)	T. Cholesterol (mg/dl)	205	IgG	2,214
Protein	(—)	T. Protein (mg/dl)	7.6	IgA	491
Urobilinogen	(±)	Classification (%)		IgM	423
Occult blood	(—)			α_1-AT	407
		ALB	58.1	α_1-AG	36
CBC		α_1-G	4.1	α_2-HS	86
		α_2-G	7.9	α_2-Macro	300
Leukocyte (/mm^3)	6,300	β-G	9.7	C_3	77
		γ-G	20.0	C_4	22
Classification (%)				TF	291
Neutrocyte	46	T. Bilirubin (mg/dl)	0.3	HP	73
Lymphocyte	45	ZTT (u)	12.8		
Eosinocyte	3	GOT (ku/ml)	20	HB antigen	(—)
Basocyte	1	GPT (ku/ml)	8		
Monocyte	5	AL-P (BLu/ml)	8.8	Chest X-P	Normal
		LDH (u/ml)	213		
Erythrocyte (10^4/mm^3)	453	γ-GTP (mu/ml)	65	ECG	Normal
Hb (g/dl)	11.5				
Ht (%)	39			EEG	Spike & wave complex at left temporal and parietal lesion
Thrombocyte (10^4/mm^3)	24.2				

ing electrophoresis have been conducted by Harris et al[2], Neel et al[5,6], and Satoh et al[10]. Our electrophoretic technique and method of calculating equivalent locus tests are identical to theirs. Harris et al examined 133,478 equivalent locus tests of Caucasian from England, while Neel et al[6] examined 94,796 and 105,649 locus tests of Amerindians from Central and South America and newborn of mainly Caucasian origin from Michigan, USA, respectively. No fresh mutations were detected in any of these populations.

In an attempt to assess the potential genetic effects of the atomic bombs in Hiroshima and Nagasaki, studies to detect mutations affecting protein structure and function have been in progress at RERF (Satoh et al[10] and Neel et al[5]). A total of 12,242 children born to proximally exposed survivors (children both or one of whose parents were exposed within 2,000 m of the hypocenter) and 10,154 children born to distally exposed survivors (children both or one of whose parents were exposed beyond 2,500 m form the hypocenter) were examined using electrophoresis for a maximum of 30 protein systems. Since distally exposed parents are presumed to have received either no radiation negligible amount of radiation (less than 1 rad), their children serve as controls and the frequency of mutants detected among these children is regarded to be the spontaneous mutation rate. Employing the same method of calculation as that of Harris et al[2] and Neel et al[6], the number of equivalent locus tests was estimated to be 582,268 for the exposed and 453,476 for the control group. Since three fresh mutants were observed in each group, the calculated mutation rates were 0.52×10^{-5} and 0.66×10^{-5} per locus per generation for the former and the latter, respectively[10]. Thus far no measurable genetic effects due to A-bomb exposure of the parents could be demonstrated.

To discuss whether or not exposure to sulphur mustard gas genetically affects offspring at the protein level, it is necessary to examine a larger number of gene loci. For this purpose, it is necessary to conduct examination over a long period and perform screening for activity variants with decreased activity whose electrophoretic mobility is unchanged. There are no data at present from which to estimate the frequency in humans of mutations affecting enzyme activity. The only data having sufficient number to permit estimation of rate of mutation resulting in loss-of-activity are those of Mukai and Cockerham on Drosphila melanogaster[4], in which mutations resulting in loss-of-activity were five times more frequent than mutations altering electrophoretic mobility. Satoh et al[9] reported that two or three activity variants had been encountered per 1,000 tests, and these variants can be fully used for the search of mutation. However, it is necessary to remember that we do not consider this study or any of the other genetic studies on the children born to those afflicted by poison gas as a test of the hypothesis that mutations were produced by rather as an effort to provide a responsible estimate of the magnitude of the genetic effects which must be presumed to have occurred.

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