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THE GENETIC STUDIES OF FAMILIAL AMYLOID POLYNEUROPATHY IN THE ARAO DISTRICT OF JAPAN: II. STUDIES OF GENETIC MARKERS IN BLOOD

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Summary Blood samples from 21 patients with familial amyloid polyneuropathy (FAP) and 81 normal family members among 7 affected families in Arao were tested for 9 blood group systems, 8 serum polymorphic proteins, 12 red cell polymorphic enzymes, and HLA. One of the most important findings was the existence of two relatively rare variants, *i.e.*, group specific component Gc*1A2 and phosphoglucomutase PGM1*7 in 3 families. This observation suggests that the three genealogically independent families may have a common ancester. Phenotype AB in the ABO blood group system, phenotype 1 in the haptoglobin system, and M_2 gene in the protease inhibitor system were not seen, and phenotype Jk(a+b−) in the Kidd groups was found in only one patient. Whether these observations reflect the characteristics of FAP in the Arao district or that of FAP itself can not be determined from the present study. No phenotype attributable to Caucasians was found, hence the study provides no support for the hypothesis that the gene for FAP was introduced into Japan by the Portuguese.

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INTRODUCTION

Familial amyloid polyneuropathy (FAP) is a late-onset hereditary disease of unknown etiology (Glenner *et al.*, 1978). The diagnosis of FAP is based on characteristic clinical manifestations and demonstration of amyloid deposition in peripheral nerves. The disease has a curious geographic distribution: there are large foci in Portugal (Andrade *et al.*, 1969), Sweden (Andersson, 1976), and Japan (Araki *et al.*, 1968; Kito *et al.*, 1973), but the disease is uncommon elsewhere. Whether FAP in Japan has a Portuguese ancestry or not is an interesting enigma (Andrade *et al.*, 1970). In a previous paper (Sakoda *et al.*, 1983), we reported genealogical studies of FAP in the Arao district of Kumamoto prefecture. They revealed that there were 9 families in the focus, but did not provide any information about its origin.

The study of genetic markers seems useful for solving such clinical and genetic problems related to the disease. In this paper, we present an analysis of frequencies of thirty polymorphic genetic markers in blood from patients with FAP and their relatives in the Arao district with special reference to red cell phosphoglucomutase-1 and serum group specific component.

MATERIALS AND METHODS

Twenty-one patients with FAP (11 males and 10 females) and 81 normal family members from 7 affected families were donors of blood for examination (Table 1). The diagnosis of FAP was made on the basis of clinical and bioptic studies. Each subject was typed for 9 blood groups, 8 serum protein markers including Gm haplotypes, 12 red cell enzymes, and HLA.

Table 1. Number of patients with familial amyloid polyneuropathy in Arao tested for genetic markers and their relation to 7 families.

Family	Patients	
	Male	Female
U	6	2
Sa	3	1
S	1	2
Tn	0	1
Tu	0	2
Ni	1	1
H	0	1
Total	11	10

Blood groups (ABO, MN, P, Rh, Kell, Kidd, Duffy, Lutheran, Diego), haptoglobin (Hp), transferrin (Tf), acid phosphatase (AcP), 6-phosphogluconate dehydrogenase (6-PGD), esterase D (EsD), glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), phosphohexose isomerase (PHI), lactate dehydrogenase (LDH), adenosine deaminase (ADA), uridin-5-monophosphate kinase (UMPK), and glyoxalase 1 (Glo-1) were determined by standard methods (Giblett, 1969; Harris and Hopkinson, 1976). Group specific component (Gc) (Constans and Viau, 1977), protease inhibitor (Pi) (Kueppers and Christopherson, 1978), phosphoglucomutase-1 (PGM1) (Sutton and Burgess, 1978), and second component of complement (C2) (Tokunaga *et al.*, 1980) were determined by isoelectric focusing. Factor B (Bf) was determined by agarose gel electrophoresis (Alper *et al.*, 1972). Gm and Km were determined by hemagglutination inhibition tests (Giblett, 1969; Steinberg, 1962). Abnormal hemoglobin was determined by isoelectric focusing of globin (unpublished). 28 HLA-A, 69 HLA-B, and 12 HLA-C locus antigens were determined by lymphocyte microcytotoxity assay using the NIH standardized methods. By checking the clinical charts of Arao City Hospital, we were able to add ABO phenotypes of 19 patients, some of whom had already died, to those of the patients examined.

RESULTS

Gc and PGM1

Relatively rare variant alleles of Gc and PGM1 were found in 3 of 7 families affected with FAP. Gc*1A2 whose frequency is 0.6–5.1% in Japanese population (Omoto and Miyake, 1978; Matsumoto *et al.*, 1979) was seen in the Sa (III-1 in Fig. 1), U (III-4, IV-10, IV-11, and IV-14 in Fig. 2), and Tu (II-3 in Fig. 3) families. PGM-1*7 whose frequency is approximately 2.1% in the Japanese population (Satoh *et al.*, 1977) was seen in the Sa (III-7, III-9, III-11, IV-2, IV-3, and IV-5 in Fig. 1), and U (III-4, III-13, IV-2, and IV-3 in Fig. 2) families.

Other genetic markers

There was no patient with phenotype AB in the ABO blood groups, phenotype Hp1, M_2 gene in the Pi system, and phenotype AcPA. There was only one patient with Jk(a+b−) in the Kidd groups. In HLA, 11 of the 21 patients had the HLA Bw52 antigen; this is a relatively high proportion (0.52) for a normal Japanese population (Baur and Danilovs, 1980), and is almost the same as the figures reported by Araki *et al.* (1982). There was no significant difference between the FAP patient group and normal Japanese population with respect to the frequencies of other genetic markers. Phenotypes attributable to Caucasians, for example K+ in the Kell blood groups, were not found in this study.

Location of the permanent addresses of 7 afflicted families in Arao City

From Fig. 4, it is clear that the permanent addresses of 7 afflicted families in-

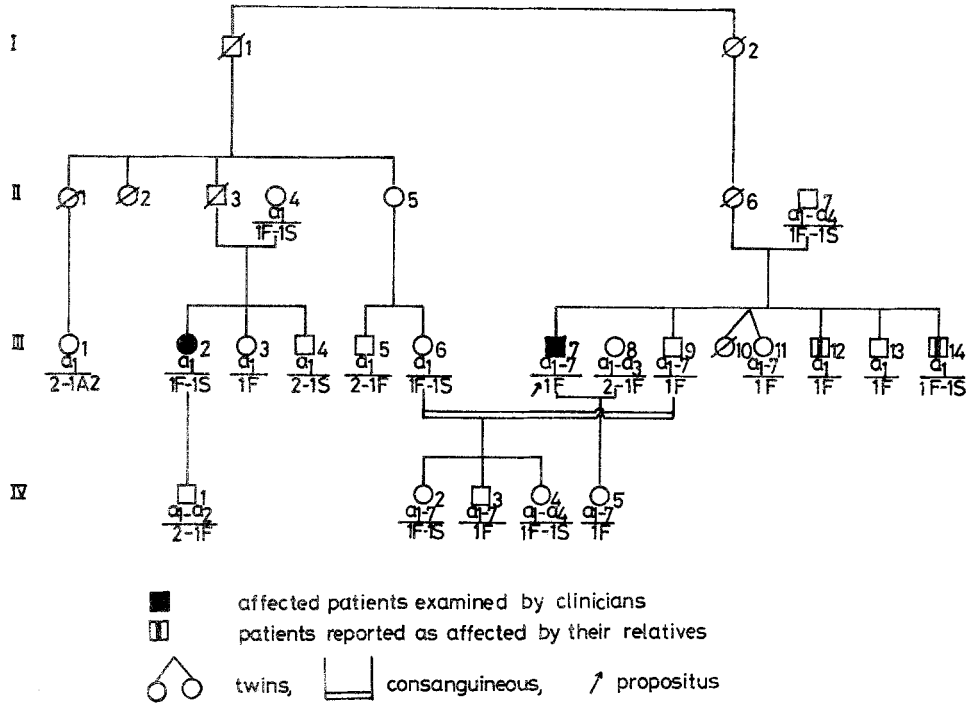

Fig. 1. The PGM1 (upper) and Gc (lower) phenotypes of the members of the Sa family.

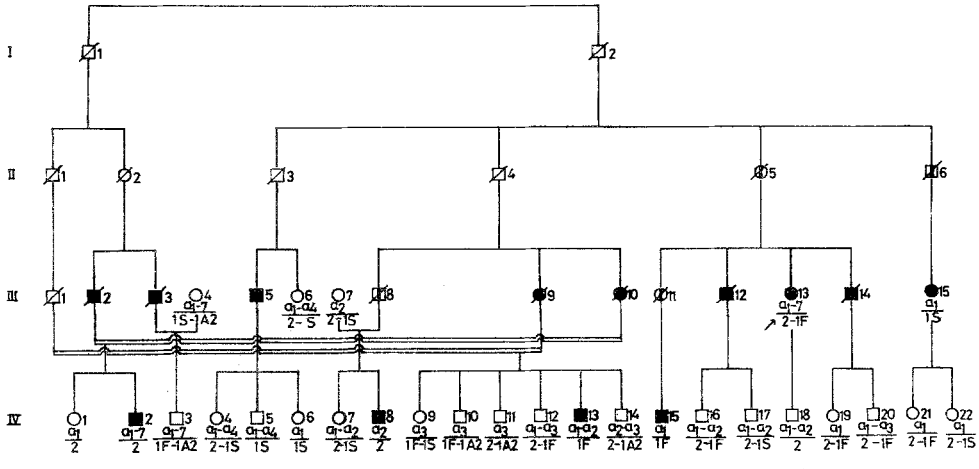

Fig. 2. The PGM1 (upper) and Gc (lower) phenotypes of the members of the U family.

cluding the U, Tu, and Sa families in which a genetic relationship was suggested by the distribution of two variants, PGM1*7 and Gc*1A2, are located along the seashore.

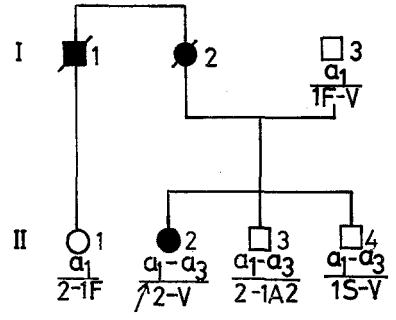

Fig. 3. The PGM1 (upper) and Gc (lower) phenotypes of the members of the Tu family. GcV is a variant phenotype.

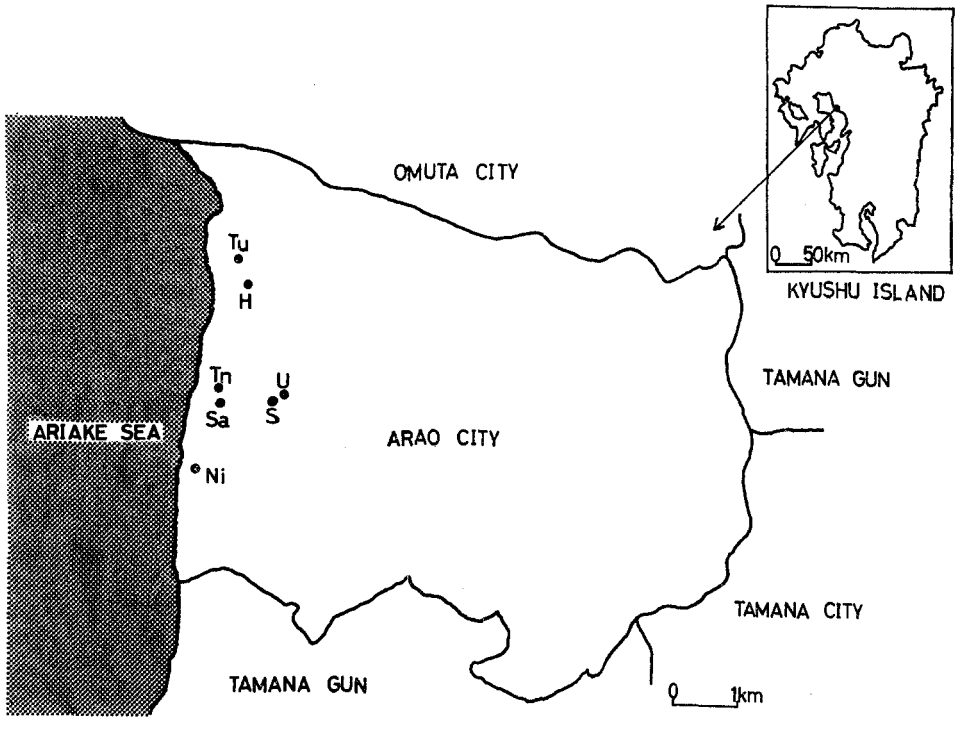

Fig. 4. The geographical distribution of the 7 afflicted families in Arao City. Gc*1A2 was found in the U, Tu, and Sa families, and PGM1*7 was found in the U and Sa families.

DISCUSSION

The present study is the first to investigate various genetic markers in patients with FAP in order to elucidate the genetic origin of seven genealogically independent

families affected with FAP in Arao (Sakoda *et al.*, 1983). The finding that the relatively rare variants of two genetic markers in Japan, Gc*1A2 and PGM1*7, are distributed over the three genealogically independent families suggests that the families (Sa, U and Tu) may have a common ancester. Phenotypes attributable to Caucasians, for example K+ in the Kell blood group, were not found in the present study and hence no evidence was found for the hypothesis that the FAP gene in Arao was derived from the Portuguese (Andrade *et al.*, 1970). Whether a few rather unusual findings in the ABO blood groups, the Kidd groups, the Hp and the Pi systems, reflect only the characteristics of FAP in the Arao district or that of FAP itself remains to be solved. Finally, it should be stressed that the more extended studies of genetic markers are promising in elucidating the genetic characteristics of the focus and further solving the origin of the mutant gene in Arao.

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REFERENCES

Alper, C.A., Boenish, T., and Watson, L. 1972. Genetic polymorphism in human glycine-rich beta-glycoprotein. *J. Exp. Med.* **35**: 68–80.

Andersson, R. 1976. Familial amyloidosis with polyneuropathy. *Acta Med. Scand.* Suppl. **590**: 1–64.

Andrade, C., Canijo, M., Klein, D., and Kaelin, A. 1969. The genetic aspect of the familial amyloidotic polyneuropathy. *Humangenetik* **7**: 163–175.

Andrade, C., Araki, S., Block, W.D., Cohen, A.S., Jackson, C.E., Kuroiwa, Y., McKusick, V.A., Nissim, J., Sohar, E., and Van Allen, M.W. 1970. Hereditary amyloidosis. *Arthr. Rheum.* **13**: 902–915.

Araki, S., Mawatari, S., Ohta, M., Nakajima, A., and Kuroiwa, Y. 1968. Polyneuritic amyloidosis in a Japanese family. *Arch. Neurol.* **18**: 593–602.

Araki, S., Kurihara, T., and Kuribayashi, T. 1982. Familial amyloidotic polyneuropathy and HLA. *Clin. Immunol.* **14** [Suppl. 4]: 96–102 (in Japanese).

Baur, M.P. and Danilovs, J.A. 1980. Population analysis of HLA A, B, C, DR, and other genetic markers. In *Histocompatibility Testing*, Terasaki, Paul I., ed., UCLA Tissue Typing Laboratory, Los Angeles, pp. 955–993.

Constans, J. and Viau, M. 1977. Group specific component: Evidence for two subtypes of the Gc^1 gene. *Science* **198**: 1070–1071.

Giblett, E.R. 1969. *Genetic Markers in Human Blood*, F.A. Davis Co., Phila., Pa.

Glenner, G.G., Ignaczak, T.F., and Page, D.L. 1978. The inherited systemic amyloidosis and localized amyloid deposits. In *The Metabolic Basis of Inherited Disease*, Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S., eds., 4th Ed., McGraw-Hill Book Co., New York, pp. 1308–1339.

Harris, H. and Hopkinson, D.A. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*, North-Holland/American Elsevier, Amsterdam, Oxford, New York.

Kito, S., Fujimori, N., Yamamoto, M., Itoga, E., Toyoizumi, Y., Kakizaki, T., Mitsui, Z., Ichikawa, H., Morinaga, T., Wakatsuki, K., Satoh, S., and Iwasaki, I. 1973. A focus of familial amyloid polyneuropathy. *Nippon Rinsho* **31**: 2326–2338.

Kueppers, F. and Christopherson, M.J. 1978. Alpha$_1$-antitrypsin: further genetic heterogeneity revealed by isoelectric focusing. *Am. J. Hum. Genet.* **30**: 359–365.

Matsumoto, H., Toyomasu, T., Tamaki, Y., Katayama, K., and Matsui, K. 1979. The distribution of Gc subtypes in Japanese and its application in paternity case. *Jpn. J. Legal Med.* **33**: 74–79.

Omoto, K. and Miyake, K. 1978. The distribution of the group specific component (Gc) subtypes in Japanese. *Jpn. J. Human Genet.* **23**: 119–135.

Sakoda, S., Suzuki, T., Higa, S., Ueji, M., Kishimoto, S., Hayashi, A., Yasuda, N., Takaba, Y., and Nakajima, A. 1983. Genetic studies of familial amyloid polyneuropathy in the Arao district of Japan: I. The genealogical survey. *Clin. Genet.* **24**: 334–338.

Satoh, C., Ferrell, R.E., Tanis, R.J., Ueda, N., Kishimoto, S., Neel, J.V., Hamilton, H.B., and Baba, K. 1977. The frequency in Japanese of genetic variants of 22 proteins. *Ann. Hum. Genet. (Lond.)* **41**: 169–183.

Steinberg, A.G. 1962. Progress in the study of genetically determined human gamma globulin types (the Gm and Inv groups). *Progress Med. Genet.* **2**: 1–33.

Sutton, J.G. and Burgess, R. 1978. Genetic evidence for four common alleles at the phosphoglucomutase-1 locus detectable by isoelectric focusing. *Vox Sang.* **34**: 97–103.

Tokunaga, K., Omoto, K., Araki, C., and Juji, T. 1980. Genetic polymorphism of the second component of human complement (C2) in Japanese. *Jpn. J. Human Genet.* **25**: 287–293.