

## DEMONSTRATION OF A GLUTAMATE-PYRUVATE TRANSAMINASE SILENT ALLELE IN A JAPANESE FAMILY

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*Summary* Electrophoretic examination of glutamate-pyruvate transaminase (GPT) in a Japanese mother-child pair showed an atypical segregation of the GPT allele. In further investigation of the mother-grandmother pair, the phenotypes of the GPT also showed the atypical segregation. All the other genetic blood markers examined in this family were transmitted according to Mendelian inheritance. Any variant GPT could not be detected in these samples by the polyacrylamide gel electrophoresis at acidic pH. These results suggest that the silent allele for GPT exists in this Japanese family.

### INTRODUCTION

Glutamate-pyruvate transaminase (GPT) or alanine aminotransferase [EC 2.6.1.2] catalyzes the reversible interconversion of L-alanine and  $\alpha$ -ketoglutarate to L-glutamate and pyruvate. This enzyme exists in both soluble (cytoplasmic) form and mitochondrial form.

The genetic polymorphism of soluble GPT in human red blood cells was first described by Chen and Giblett (1971). They presented evidence that the two common allele products, GPT\*1 and GPT\*2, are detectable by starch gel electrophoresis and enzyme specific staining procedures. Electrophoretic variants of rare GPT alleles have also been detected in various human populations of different ethnic origins: GPT\*3-GPT\*6 (Chen *et al.*, 1972), GPT\*1m (Kömpf *et al.*, 1974), GPT\*7 (Olaisen, 1973a), GPT\*8 (Santachiara Benerecetti *et al.*, 1975) and GPT\*9 (Kömpf and Ritter, 1979). Furthermore, the occurrence of GPT silent allele has been described by Spielman *et al.* (1973), Olaisen (1973b), Speiser and Pausch (1977), Kömpf and Ritter (1979), Mithal *et al.* (1980) and Maryellen *et al.* (1983).

In this paper, we report a GPT silent allele discovered in a Japanese family.

## MATERIALS AND METHODS

*Sample.* Blood samples were washed three times with saline, and the packed red blood cells were lysed by freezing and thawing for three times.

*Starch gel electrophoresis.* The GPT phenotypes were detected by starch gel electrophoresis according to the method of Kömpf and Ritter (1979). Electrophoresis was carried out for 18 hr using a Tris-histidine-HCl buffer system. The gel buffer was a 1 : 12 dilution of bridge buffer (0.2 M Tris and 0.17 M histidine, pH 7.6). Horizontal electrophoresis was performed at 5 V/cm with cooling at 4°C.

*Staining method.* The enzyme specific staining was carried out according to the method of Harris and Hopkinson (1976). The staining mixture consisted of 360 mg L-alanine, 25 mg  $\alpha$ -ketoglutaric acid, 14 mg NADH, 8 U LDH and 25 ml staining buffer (0.1 M Tris-HCl, pH 8.0). Whatman no. 1 filter paper saturated with staining solution was laid on the bottom half of the sliced gel, which was then incubated at 37°C for 4 hr. Under the UV light, zones of GPT activity appeared as dark blue spots.

## RESULTS AND DISCUSSION

In paternity examinations, atypical segregation of the GPT phenotype was observed between the mother(II-2) and the child(III-1) in one case: The mother's phenotype was GPT 1 and the child's phenotype was GPT 2, as shown in Fig. 1. To examine the maternity, we analyzed the other genetic blood markers of the

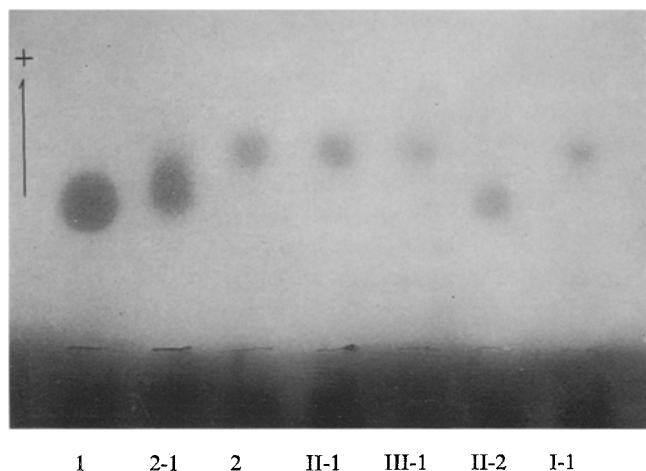


Fig. 1. Electrophoretic patterns of GPT isozymes in starch gel electrophoresis. 1, 2-1, and 2: standard samples for GPT 1, 2-1 and 2, respectively. II-1, III-1, II-2, and I-1: samples from the father, child, mother, and grandmother, respectively.

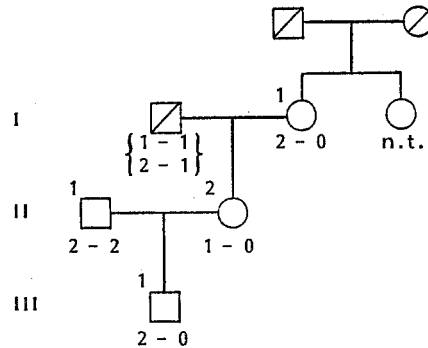


Fig. 2. Pedigree chart of the family showing the atypical segregation of the red blood cell GPT phenotype.

Table 1. Genetic blood markers in the family members.

	ABO	MNSs	Rh	Fy	Jk	Le	Kell	P	AcP
I-1	B	Nss	CcDee	a+b-	a-b+	a+b-	K-k+	P <sub>1</sub> -	BA
II-1	A	MNss	CcDEe	a+b-	a+b+	a-b+	K-k+	P <sub>1</sub> -	B
II-2	O	MNss	ccDEe	a-b-	a-b+	a+b-	K-k+	P <sub>1</sub> -	B
III-1	O	Nss	CcDEe	a+b-	a+b+	a-b+	K-k+	P <sub>1</sub> -	B

	PGM	PGD	GPT	EsD	GLO	ADA	FUC	GDH	Gm	Km
I-1	2A	A	2	1	2	1	2-1	1	1, -2, -12	-1
II-1	1A	A	2	2-1	2-1	1	2-1	1	1, -2, -12	1
II-2	1A2A	A	1	2-1	2-1	1	2-1	2-1	1, -2, -12	-1
III-1	1A2A	A	2	2	2-1	1	2-1	1	1, -2, -12	1

	Hp	Gc	F-XIIIA	F-XIIIB	Tf	Pi
I-1	2	1F-1S	1	1-3	C1	M1M1
II-1	2	2	1	3	C1	M1M2
II-2	2	1F-1S	1	3	C1	M1M2
III-1	2	2-1F	1	3	C1	M2M2

HLA system					
	A2	Aw33	Bw60	Bw44	Cw-
I-1	A2	Aw33	Bw60	Bw44	Cw-
II-1	A2	Aw31	Bw51	Bw-	Cw3
II-2	Aw26	Aw33	Bw39	Bw44	Cw-
III-1	A2	Aw33	Bw51	Bw44	Cw3

mother(II-2) and the child(III-1), including those of the accused father(II-1) (Fig. 2). These included ABO, MNSs, Rh, Duffy, Kidd, Lewis, Kell, and P in red blood cell antigens, AcP, PGM, PGD, GPT, EsD, GLO, and ADA in red blood cell isozymes, FUC and GDH in white blood cell isozymes, Gm, Km, Hp, Gc, Factors XIII A and B, Tf, and Pi in plasma proteins, and the A, B and C antigens of the HLA system. In all these genetic markers, contradictable combinations were not found between the mother(II-2) and the child(III-1), and also between the accused father(II-1) and the child(III-1), indicating that both the mother(II-2) and the accused father(II-1) are his real parents (Table 1). Since these findings suggested that a GPT silent allele exists in the mother(II-2) and her son(III-1), we further examined the genetic blood markers of the grandmother(I-1).

As shown in Fig. 1, the GPT staining intensity of the child(III-1), the mother(II-2) and the grandmother(I-1) were very weak compared with controls, suggesting a decrease in the activity of the GPT isozymes in these family members. In addition, the atypical segregation of the GPT phenotype was also observed between the mother and the grandmother, though in the other genetic markers, contradictable genetic combinations were not found (Fig. 1 and Table 1). McLellan (1982) showed that the GPT 2 type consists of the GPT 2A and the GPT 2B using the polyacrylamide gel electrophoresis at acidic pH. We examined the GPT types of these samples using the same electrophoretic method, but the variant of GPT could not be detected.

These results indicate that the child, mother and grandmother in this Japanese family have a GPT silent allele. It is very likely that a decrease in the GPT activity in the child, mother and grandmother is due to a gene dosage effect.

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