Genetic Variation of Soluble Glutamic-Oxaloacetic Transaminase in Man

SHI-HAN CHEN[1] AND ELOISE R. GIBLETT[1]

Glutamic-oxaloacetic transaminase (GOT), also known as aspartate aminotransferase (E.C.2.6.1.1.) catalyzes the reversible reaction: L-aspartate + α-ketoglutarate $\leftrightharpoons$ oxaloacetate + L-glutamate. The enzyme is widely distributed in most animal and human tissues and has been intensively studied in heart and liver [1–5]. The GOT level in serum is widely used for assessing myocardial or hepatocellular damage.

Two molecular forms of GOT were described independently by Moore and Lee [6] and Fleisher, Potter, and Wakim [7], and were subsequently confirmed by others [8, 9]. One of these enzymes is mitochondrial in origin; its electrophoretic migration is cathodal at neutral *p*H. The other enzyme is found in the soluble fraction of cells; its electrophoretic migration is anodal. Mature erythrocytes contain only the soluble form of GOT, while reticulocytes and white cells have both soluble (S-GOT) and mitochondrial (M-GOT) forms [10, 11]. The two forms of GOT differ not only in their site of origin and electrophoretic mobility but also in their *p*H optimum, kinetic properties, immunological specificities, amino-acid composition, N-terminal amino acids, and tryptic peptide maps [4, 5, 12]. Thus, they are distinctly different proteins.

Davidson et al. [13] have reported genetic polymorphism of human mitochondrial GOT (M-GOT). In a survey of 860 unselected human placental extracts (mainly Caucasian and Afro-American infants), they found three M-GOT variants which were common enough to be considered representative of polymorphism. Genetic variations of mouse mitochondrial GOT have also been reported [14]. Davidson et al. [13] detected one electrophoretic variant of human soluble GOT (S-GOT) in a "white female," but family studies could not be performed. In this paper we describe a genetic polymorphism of S-GOT found in Mongoloid populations.

MATERIALS AND METHODS

Blood specimens were collected in ACD solution and hemolysates were prepared as described previously [15]. For preparation of white-cell extracts, 10 ml specimens of blood, collected in siliconized tubes in ACD solution, were centrifuged at about 1,000 g for seven minutes and the plasma was removed. The sedimented cells were suspended in three

Received December 18, 1970.

This work was supported by PHS grant AM 09745.

[1] King County Central Blood Bank, Seattle, Washington 98104.

volumes of 3% dextran in physiological saline, carefully mixed, and allowed to sediment for about 30 minutes at room temperature. The supernatant fraction containing the white cells was recovered and the remaining red cells removed by the method of Silber et al. [16]. The white cells were then disrupted by sonication and the particulate material discarded after centrifugation at 17,400 g for 20 minutes.

Vertical starch gel electrophoresis of the hemolysates and white-cell extracts was performed at 4° C for 18 hours at 8 v/centimeter in 0.1 M tris-citrate buffer, *p*H 7.5. The gel was sliced and stained for GOT activity by a modification of the method described by Boyd [5]. The staining mixture consists of 67 mM sodium phosphate buffer, *p*H 7.4, 62 mM L-aspartate (K salt), 8.7 mM α-ketoglutarate (Na salt), 1.1 mM $NADH_2$, and approximately eight units per milliliter of malate dehydrogenase (MDH). During a 40-minute incubation period at 37° C, the reaction proceeds as shown in figure 1. The

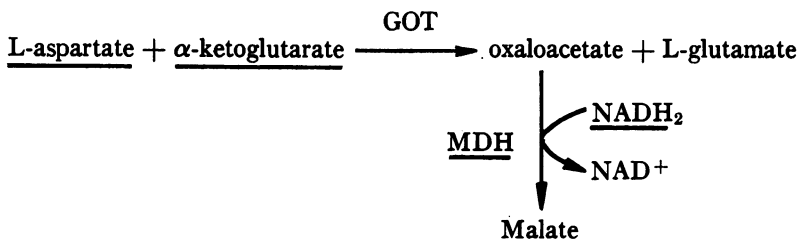

FIG. 1.—Diagram of the reaction sequence for the detection of GOT isozymes. The underlined compounds are contained in the reaction mixture.

oxaloacetate formed at the sites of GOT activity is reduced by the MDH, and in the coupled reaction the fluorescent $NADH_2$ is oxidized to nonfluorescent NAD^+. The enzymatic sites, which appear as dark bands against a fluorescent background under ultraviolet light, are photographed as previously described [15].

RESULTS

The common pattern of the red-cell GOT, designated S-GOT 1, consists of an anodal band accompanied by a slightly faster minor band (fig. 2). This pattern was observed in most of the red-cell hemolysates prepared from blood donors in Seattle. However, among 227 Oriental donors (mostly Japanese), seven were found to have an exceptional pattern, designated S-GOT 2-1 in figure 2. It consists of a band corresponding to the major component of S-GOT 1 and two additional bands with more rapid migration, the most anodal being the weakest. A third type, S-GOT 3-1, was found in the red cells of some North and South American Indians. Its two additional components migrate more slowly.

Two of the Oriental blood donors with S-GOT 2-1 had family members available for study. The pedigrees of these families (both of Japanese origin) are shown in figure 3. In the A Family, two daughters of the propositus have the S-GOT 2-1 phenotype, while his wife and son have the usual type, S-GOT 1. In the Y Family, the S-GOT 2-1 type occurs in the father and his two sons; the wife and daughter have S-GOT 1.

The S-GOT 1 and 2-1 patterns observed in the hemolysates were also found in the white-cell extracts (fig. 4), and in each family member the white-cell S-GOT

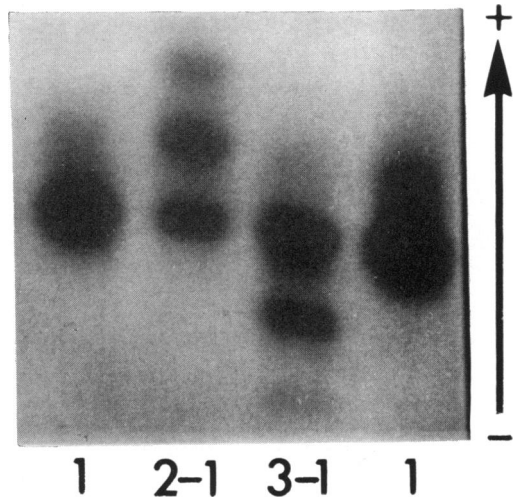

FIG. 2.—Photograph of starch gel, showing three phenotypes (S-GOT 1, S-GOT 2-1, and S-GOT 3-1) in red-cell hemolysates. The site of sample insertion is not shown because it lies 11 cm cathodal to the major S-GOT 1 band.

matched that of the red cells. On the other hand, no variation was found in the white-cell mitochondrial GOT (M-GOT) regardless of the soluble GOT phenotype.

A number of old hemolysates from previous studies were also examined for S-GOT phenotype (see table 1). Some of the samples had been frozen for as long as five years. The fact that the electrophoretic pattern of these specimens did not differ from that of fresh samples is an indication of the enzyme's stability. The phenotype designated S-GOT 3-1 in figure 2 was found among two different Indian tribes of North and South America. Unfortunately, families were not available for study of the inheritance of S-GOT 3-1. Its frequency, as well as that of S-GOT 1 and S-GOT 2-1, is shown in table 1, which includes data from fresh specimens (blood donors) and stored hemolysates.

In order to distinguish a possible difference between the S-GOT 2-1 found in Japanese and Indians, several specimens from each ethnic group were electrophoresed in three different buffer systems (0.1 M tris-citrate, 0.01 M tris-citrate for gel, *p*H 7.5; 0.1 M sodium phosphate, 0.005 M sodium phosphate for gel, *p*H

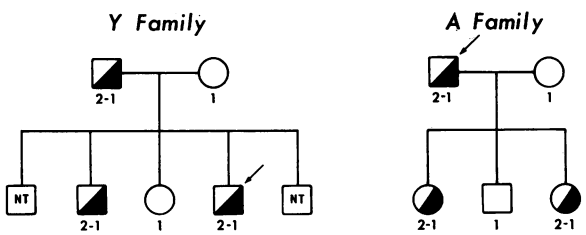

FIG. 3.—S-GOT phenotypes in two families. *NT* = not tested.

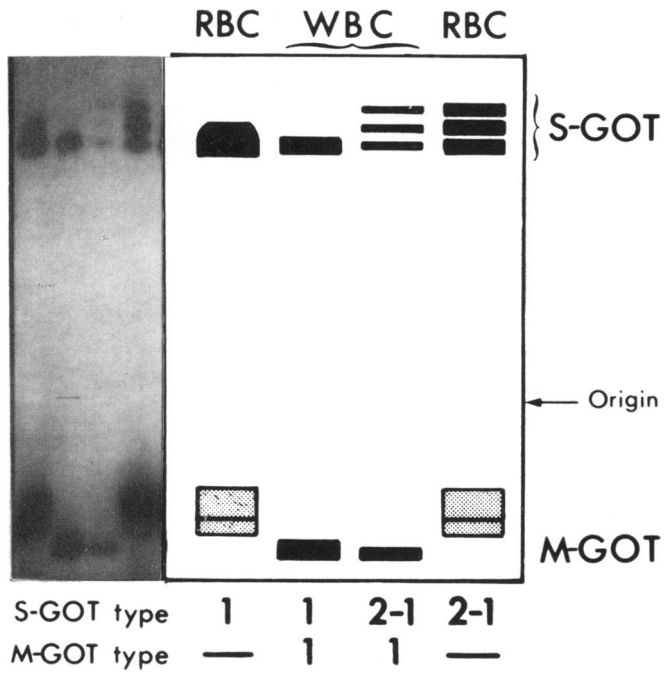

FIG. 4.—Photograph and diagram of starch gel showing S-GOT in red cells and white cells and M-GOT in white cells of two subjects. The specimens in channels 1 and 2 were obtained from a subject with S-GOT 1; the specimens in channels 3 and 4, from a subject with S-GOT 2-1. The cross-hatched regions represent hemoglobin in the red-cell hemolysates.

7.0; and 0.41 M citrate, 0.005 M histidine for gel, *p*H 7.0). Although the S-GOT 2-1 pattern did vary slightly according to the buffer used, the individual patterns on each gel did not differ.

TABLE 1

INCIDENCE OF S-GOT PHENOTYPES IN DIFFERENT POPULATIONS

Ethnic Group	No.	S-GOT 1	S-GOT 2-1	S-GOT 3-1
Caucasian	97	97	0	0
Afro-American	145	145	0	0
Congolese	91	91	0	0
Japanese-American	227	220	7	0
Chinese (Taiwan)	120	119	1	0
Filipino	165	162	3	0
New Guinea	182	182	0	0
Canadian Indian	188	167	21	0
Yakima Indian	84	79	2	3
Peruvian Indian	79	70	5	4

DISCUSSION

The autosomal codominant inheritance of S-GOT is demonstrated by the two family studies; in one there was father-to-son transmission of the variant. Individuals with S-GOT 1 are homozygous for a common allele, designated *S-Got*^{1}, while those with S-GOT 2-1 are heterozygous for the common allele and a rare allele designated *S-Got*^{2}. Although we were not able to study the family of a person with S-GOT 3-1, the phenotypic pattern probably represents heterozygosity for *S-Got*^{1} and a third allele, *S-Got*^{3}, at the same locus.

The frequency of the *S-Got*^{2} allele is about .016 in the Seattle Japanese, .059 in the Canadian Indians, .013 in the Yakima Indians, and .034 in the Peruvian Indians. The frequency of *S-Got*^{3} in the Yakima and Peruvian Indians is about .018 and .028, respectively. These frequencies are within the range generally considered to represent genetic polymorphism. The apparent restriction of S-GOT variants to Mongoloid populations is reminiscent of the blood group antigen, Di^a. However, there is no available clue in either case to the presumed selective effect which maintains them.

The presence of three main components in the electrophoretic pattern of the heterozygous individuals is compatible with the dimer structure of this enzyme suggested by others on the basis of the molecular weight, number of coenzyme binding sites, amino-acid analysis, and tryptic peptide maps [4, 12]. The main component of S-GOT 1 is probably a dimer composed of two identical or very similar subunits. In the heterozygote, the slow and fast components represent two different dimers, while the intermediate component is a hybrid dimer containing both subunits. The consistently observed decrease in staining intensity of the fast band of S-GOT 2-1 and the slow band of S-GOT 3-1 as compared with the other two bands could reflect either a decrease in the amount of variant enzyme protein synthesized, a lower specific activity, or molecular instability.

In their study of the M-GOT polymorphism, Davidson et al. [13] found three different variants which were inherited as autosomal codominants. The electrophoretic pattern of soluble GOT (S-GOT) was unaffected in the individuals with variant M-GOT. In the present study, the M-GOT pattern was similarly uninfluenced in the individuals with a variant S-GOT. Thus it seems almost certain that the two chemically and immunologically distinct enzymes have separate structural gene loci.

SUMMARY

Two electrophoretic variants of soluble glutamic-oxaloacetic transaminase (S-GOT) are described in this paper. Studies of two Japanese-American families showed that one of these variants is inherited as an autosomal codominant and that the locus for S-GOT is separate from that of mitochondrial glutamic-oxaloacetic transaminase (M-GOT). Families were not available for study of the inheritance of the second variant. However, like the first variant, it occurred only

among Mongoloid individuals. The electrophoretic patterns of the heterozygotes were consistent with a dimeric structure of the enzyme.

ACKNOWLEDGMENTS

For supplying us with many of the blood specimens from various populations, we wish to express our sincere thanks to Drs. N. Carter, G. Modiano, A. Motulsky, T. Reed, and H. van den Berghe. Also we acknowledge our appreciation to Miss Jeanne Anderson for her expert technical assistance.

REFERENCES

1. JENKINS WT, YPHANTIS DA, SIZER IW: Glutamic aspartic transaminase. I. Assay, purification, and general properties. *J Biol Chem* 234:51–57, 1959
2. SIZER IW, JENKINS WT: Glutamic aspartic transaminase from pig ventricles, in *Methods in Enzymology,* vol 5, edited by COLOWICK SP, KAPLAN NO, New York, Academic, 1962, pp 677–684
3. DE JIMÉNEZ ES, BRUNNER LA, SOBERÓN G: Characteristics of aspartate aminotransferase isozymes of rat and chicken livers. *Arch Biochem* 120:175–185, 1967
4. NISSELBAUM JS, BODANSKY O: Kinetics and electrophoretic properties of the isozymes of aspartate aminotransferase from pig heart. *J Biol Chem* 241:2661–2664, 1966
5. BOYD JW: The extraction and purification of two isozymes of L-aspartate:2-oxoglutarate aminotransferase. *Biochim Biophys Acta* 113:302–311, 1966
6. MOORE BW, LEE RH: Chromatography of rat liver soluble proteins and localization of enzyme activities. *J Biol Chem* 235:1359–1364, 1960
7. FLEISHER GA, POTTER CS, WAKIM KG: Separation of 2 glutamic-oxalacetic transaminases by paper electrophoresis. *Proc Soc Exp Biol Med* 103:229–231, 1960
8. BOYD JW: The intracellular distribution, latency and electrophoretic mobility of L-glutamate-oxaloacetate transaminase from rat liver. *Biochem J* 81:434–441, 1961
9. BORST P, PEETERS EM: The intracellular localization of glutamate-oxaloacetate transaminases in heart. *Biochim Biophys Acta* 54:188–189, 1961
10. NISSELBAUM JS: Erythrocyte glutamic-oxaloacetic transaminase. *Fed Proc* 24:356, 1965
11. NISSELBAUM JS, BODANSKY O: Glutamic-oxaloacetic transaminase in reticulocytes and erythrocytes. *Science* 149:195–197, 1965
12. MARTINEZ-CARRION M, TIEMEIER D: Mitochondrial glutamate-aspartate transaminase. I. Structural comparison with the supernatant isozyme. *Biochemistry* 6:1715–1722, 1967
13. DAVIDSON RG, CORTNER JA, RATTAZZI MC, et al: Genetic polymorphisms of human mitochondrial glutamic oxaloacetic transaminase. *Science* 169:391–392, 1970
14. DELORENZO RJ, RUDDLE FH: Glutamate oxalate transaminase (GOT) genetics in *Mus musculus*: linkage, polymorphism and phenotypes of the *GOT-2* and *GOT-1* loci. *Biochem Genet* 4:259–273, 1970
15. CHEN SH, MALCOLM LA, YOSHIDA A, et al: Phosphoglycerate kinase: an X-linked polymorphism in man. *Amer J Hum Genet* 23:87–91, 1971
16. SILBER R, GABRIO BW, HUENNEKENS FM: Studies on normal and leukemic leukocytes. III. Pyridine nucleotides. *J Clin Invest* 41:230–234, 1962