Int J Leg Med (1991) 104:121–122

International Journal of
Legal Medicine
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Original articles

Genetic polymorphism of human glutamate oxaloacetate transaminase (GOT1) detected using isoelectric focusing and a sensitive and positive staining method

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Received September 19, 1990 / Received in revised form November 19, 1990

Summary. Genetic polymorphism of glutamate oxaloacetate transaminase (GOT1) was demonstrated in human erythrocytes by isoelectric focusing in thin layer polyacrylamide gels and a sensitive and positive detection method. Using this technique, five phenotypes, GOT1 1, 2, 2–1, 3–1 and 3–2 were determined and the estimated gene frequencies of *GOT1*1, GOT1*2* and *GOT1*3* in the Japanese population were 0.9740, 0.0173 and 0.0087, respectively.

Key words: Glutamate oxaloacetate transaminase (GOT1) – Isoelectric focusing – Activity staining – Gene frequency – Japanese population

Zusammenfassung. Der genetische Polymorphismus der Glutamat-Oxalacetat-Transaminase (GOT1) wurde an menschlichen Erythrozyten mit Hilfe der isoelektrischen Fokussierung in Dünnschichtpolyamid-Gelen und einer empfindlichen und positiven Nachweismethode dargestellt. Mit Hilfe dieser Technik gelang der Nachweis der 5 Phänotypen GOT1 1, 2, 2–1, 3–1, 3–2. Die Genfrequenzen der Allele *GOT1*1, GOT1*2* und *GOT1*3* wurden in der japanischen Bevölkerung mit 0,9740, 0,0173 und 0,0087 errechnet.

Schlüsselwörter: Glutamat-Oxalacetat-Transaminase (GOT1) – Isoelektrische Fokussierung – Färbung durch Aktivitätsnachweis – Genfrequenzen – Japanische Bevölkerung

Introduction

GOT1 (E.C. 2.6.1.1), also known as aspartate aminotransferase or 2-oxoglutarate aminotransferase, catalyzes the following reaction: L-aspartate + α-ketoglutarate ⇄ oxaloacetate + L-glutamate, and it is widely distributed in high concentrations among animals, plants and microorganisms. Three variants of GOT1 (types 1, 2–1 and 3–1) in human erythrocyte lysates were found in Asiatic population at an appreciable gene frequency (about 0.01) employing starch gel electrophoresis [1, 2]. In the present study, human erythrocyte GOT1 was examined by isoelectric focusing in flat bed polyacrylamide gels by a new positive staining method.

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Materials and methods

Erythrocyte samples were collected in heparinized blood obtained from 462 unrelated Japanese donors living in the Fukui Prefecture. Hemolysates were prepared by diluting 1 vol washed and packed cells with 3 vol 0.05 *M* dithiothreitol.

Isoelectric focusing (IEF) was carried out by the method described previously [3, 4]. Polyacrylamide gels (0.5 × 90 × 120 mm) were prepared as follows: 1.4 ml acrylamide-bis (19.4% w/v, 0.6% w/v), 2.3 ml sucrose-glycerin (20% w/v, 10% v/v), 1 ml distilled water, 250 µl Pharmalyte 5–6 and 30 µl Pharmalyte 5–8 (Pharmacia, Uppsala, Sweden). 5 µl TEMED and 40 µl ammonium persulfate (1.2%). Wicks were formed from strips of filter paper and soaked in the elctrode solution, with 0.04 *M* glutamic acid at the anode and 1.0 *M* NaOH at the cathode. Hemolysates (5 µl) were applied 20 mm from the cathode on filter paper (Whatman 3MM, 3 × 5 mm). IEF was performed for 210 min at a constant power of 5 W (V_{max} 1150 V, I_{max} 20 mA) at 12°C.

As a positive GOT staining solution, the San-assay TA-N kit (Sanko Junyaku, Tokyo, Japan) for clinical examination of serum GOT activity was employed after preparation according to the protocol recommended by the manufacturer, and pyridoxine hydrochloride was added at a final concentration of 10 m*M*. A cellulose acetate membrane (57 × 70 mm, Sartorius, Göttingen, FRG) soaked in the staining solution was applied to the gel and incubated in a moist chamber at 37°C for 15 min.

Results and discussion

IEF in thin layer polyacrylamide gels gave good separation of the five phenotypes, GOT1 (440 samples), 2–1

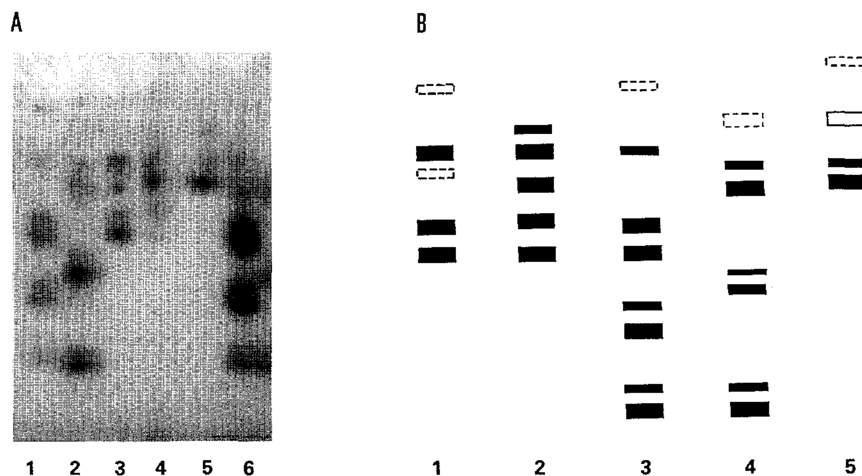

Fig. 1. A Photographic and **B** schematic representations of IEF patterns (Pharmalyte pH 5–8) of erythrocyte GOT1 isozymes detected by activity staining. Anode is at the *top*. The focused gel was incubated at 37°C for 15 min in contact with a cellulose acetate membrane soaked in the staining solution and semi-dried. Other details are given in Materials and methods. **A** *Lanes 1* and *6,* phenotype 3–1; *lane 2,* 3–2; *lane 3,* 1; *lane 4,* 2–1; *lane 5,* 2. **B** *Lane 1,* phenotype 1; *lane 2,* 2–1; *lane 3,* 3–1; *lane 4,* 3–2; *lane 5,* 2

(13), 2 (1), 3–1 (7), and 3–2 (1), as illustrated in Fig. 1. In this study, we confirmed that the isozyme patterns of GOT1 were constant and reproducible. Homozygote GOT1 1 showed four main bands of activity and the homozygote GOT1 2 showed three main bands, with pI values between 5.6 and 6.0, respectively. The heterozygotes GOT1 2–1 showed five main bands GOT1 3–1 showed six or seven main bands and GOT1 3–2 showed four or five main bands. Type 2–1 variants had bands slightly more anodal to the type 1 phenotype, whereas bands of the type 3–1 variants were more cathodal than the type 1. IEF permitted a better separation, resolution and reproduction of the GOT1 isozymes than conventional starch gel electrophoresis performed using several undefined buffer systems. A combination of IEF and the present sensitive staining technique is expected to produce a new subtyping of the GOT1 system.

Erythrocyte samples from 462 unrelated individuals were analyzed, and the gene frequencies of *GOT1*1, GOT1*2,* and *GOT1*3* were estimated to be 0.9740, 0.0173, and 0.0087, respectively. The observed distribution of phenotypes was not statistically different from that expected on the basis of the Hardy-Weinberg equilibrium. *GOT1*2* and *GOT1*3* are very rare in Europeans and Africans, but occur at an appreciable frequency in Japanese populations as previously indicated [2].

Fluorescent staining for the detection of GOT1 on the gel has generally been used; this is based on oxidation of NADH to NAD, which accompanies the conversion of oxaloacetate to malate by malate dehydrogenase [1, 2]. The staining method employed here was simpler, as GOT activity was visualized as sharp purple bands against a white background without the need for a dark room and an ultraviolet light source. Moreover, addition of pyridoxine-HCl to the staining solution gave intense staining for GOT1 isozymes. The detection method described here was much simpler, more rapid and more sensitive and therefore appeared to be more convenient for the analysis of GOT isozyme patterns by IEF.

Acknowledgements. This study was supported in part by a grant from the Research Foundation for Traffic-Preventive Medicine and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. We wish to thank Mrs. F. Nakamura for her secretarial assistance.

References

1. Chen S-H, Giblett ER (1971) Genetic variation of soluble glutamic-oxaloacetic transaminase in men. Am J Hum Genet 23: 419–424
2. Ishimoto G, Kuwata M (1974) Studies on human glutamic-oxaloacetic transaminase variation, including enzyme patterns in some non-human primates. Jpn J Hum Genet 18:364–372
3. Kishi K, Yasuda T, Iida R (1985) Glutamate-pyruvate transaminase (GPT) polymorphism detected in human urine by isoelectric focusing. Proc Jpn Acad 61B:490–493
4. Kishi K, Yasuda T, Ikehara Y, Sawazaki K, Sato W, Iida R (1990) Human serum deoxyribonuclease I (DNase I) polymorphism: pattern similarities among isozymes from serum, urine, kidney, liver, and pancreas. Am J Hum Genet 47:121–126