Genetic Polymorphisms of Human Mitochondrial Glutamic Oxaloacetic Transaminase

Abstract. *In a survey of 860 unselected human placental extracts, three variants of mitochondrial glutamic oxaloacetic transaminase were found, all of which were common enough to be considered polymorphisms. Family studies showed that this enzyme is under the control of nuclear rather than mitochondrial DNA.*

Glutamic oxaloacetic transaminase (GOT) catalyzes the reversible conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate; pyridoxal 5-phosphate is coenzyme. GOT is an important enzyme in clinical medicine due to its presence in high concentration in, for example, liver and cardiac muscle cells—overflow of GOT into the serum of patients provides physicians with a sensitive early indicator of damage to the liver or to myocardial muscle. Fleischer *et al.* (*1*) described two distinct forms of the enzyme in mammalian heart muscle. Subsequently, several investigators (*2*) have shown that one form of the enzyme is present in the soluble fraction of the cell and that the other is associated with the mitochondria. Both forms have been purified from a variety of mammalian tissues (*3*) and the two forms of GOT are, in fact, two distinct enzymes. They differ in their electrophoretic and chromatographic behavior, substrate dissociation constants, *p*H activity curves, molecular weights, tryptic peptide maps, and immunologic properties (*2–4*). This report describes three genetic polymorphisms of the mitochondrial form of the enzyme in man.

Placentae from a population of unselected infants born in the Yale New Haven Medical Center were studied. Approximately 0.5 g of placental tissue was minced with scissors, suspended in an equal volume of 0.9 percent sodium chloride solution, and disrupted with high-frequency sound. After centrifugation at 20,000*g*, the supernatant was subjected to vertical starch-gel electrophoresis at 4°C for 16 hours at 4 to 6 volt/cm in a phosphate-citrate buffer system at *p*H 7.0 (*5*). The staining solution was modified from reported techniques (*6*) and made up as indicated (*7*).

The usual pattern consists of a major anodal band and two minor bands, which represent the soluble form of the enzyme; and a major cathodal band with at least two minor bands, which represent the mitochondrial form of GOT (Fig. 1). A total of 860 placental preparations have been examined with this technique—705 from Caucasians, 148 from Negroes, and the remainder primarily from Puerto Ricans. Only one variant of the soluble form of GOT has been found, and the pattern was a characteristic triplet. This occurred in a white female but, unfortunately, family studies were not possible. Two relatively common electrophoretic variants of the mitochondrial form of the enzyme were found initially (Fig. 1A).

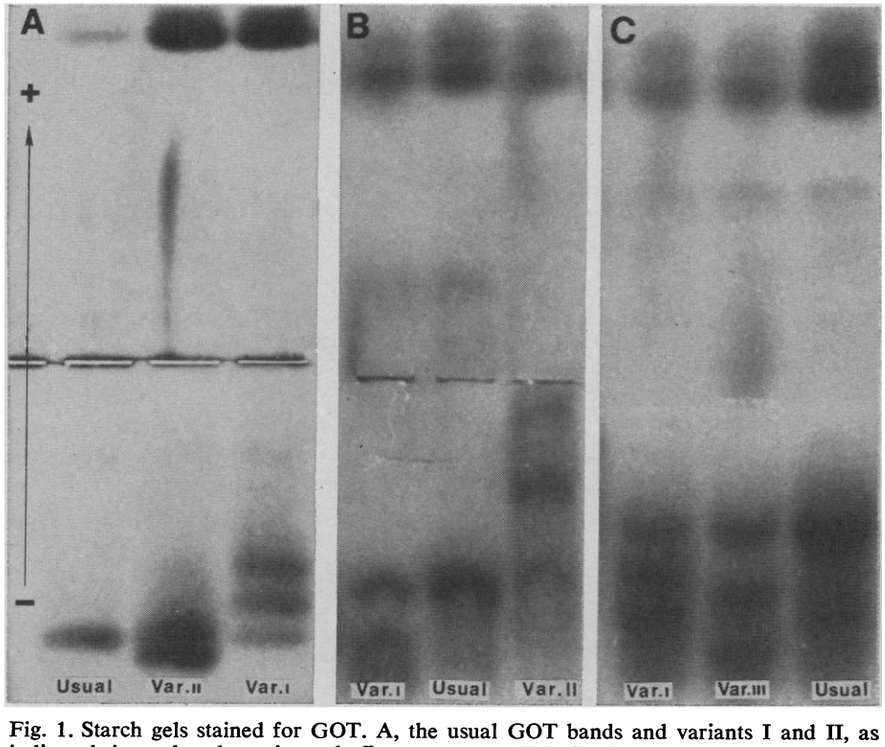

Fig. 1. Starch gels stained for GOT. A, the usual GOT bands and variants I and II, as indicated, in a phosphate-citrate buffer system at *p*H 7. B, the same variants in a barbital buffer system at *p*H 8. C, variant III can be compared with the usual pattern and with variant I, also in the barbital buffer at *p*H 8.

Variant I consists of a band corresponding to the usual major band plus two additional major bands migrating less rapidly toward the cathode. This is in agreement with the dimeric structure of mitochondrial GOT suggested by amino acid analysis and peptide-mapping data (*4*). Neither of the two new bands corresponds in mobility to the minor bands of the usual mitochondrial form of the enzyme.

In this phosphate-citrate system at *p*H 7.0, variant II appeared to form primarily two bands; one corresponding to the usual major band and the second migrating more toward the cathode.

However, when a barbital buffer system at *p*H 8.0 was used (barbital, 0.05*M* in the trays and 0.005*M* in the gel, brought to *p*H 8.0 with concentrated HCl), the electrophoretic patterns were remarkably altered (Fig 1B). The bands of variant I now migrated more rapidly toward the cathode, whereas variant II became resolved into a triplet pattern with bands migrating less rapidly toward the cathode than those of variant I. In each variant one major band corresponded to the major band of the usual form of mitochondrial GOT.

In addition, the barbital buffer system (*p*H 8.0) uncovered a third variant of mitochondrial GOT indistinguishable from variant I in the phosphate-citrate system (*p*H 7.0). The abnormal bands of this third variant (variant III) migrate more rapidly toward the cathode than the corresponding ones of variant I (Fig. 1C).

Prolonged storage, preparation of the samples at different *p*H or in the presence of β-mercaptoethanol, Triton X 100, or diisopropylfluorophosphate (DFP) did not affect the relative mobilities of the three variants. This ex-

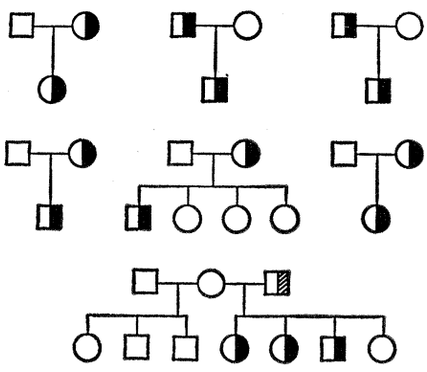

Fig. 2. A diagram of representative pedigrees. Individuals with variant mitochondrial GOT are indicated by half blacked-in symbols. The cross-hatched symbol represents a father who was not examined but who was presumed to be affected.

cludes the possibility that the observed patterns might have been due to oxidation of sulfhydryl groups, incomplete solubilization, or proteolysis of the enzymes. Mixture of the variants obtained by disrupting portions of different placentae with high-frequency sound showed, as expected, a superimposition of the individual variant patterns. Preparation of the samples and electrophoresis in the presence of L-aspartate, α-ketoglutarate, or pyridoxal 5-phosphate did not significantly alter the electrophoretic patterns, which suggests that the bands do not arise from stepwise loss of coenzyme nor from an equilibrium of the two postulated forms of mitochondrial GOT (the pyridoxal and the pyridoxamine forms) (*8*).

Family studies were performed with white blood cell preparations that were disrupted with high-frequency sound (Fig. 2). All three variants segregate in a simple Mendelian fashion, and the individuals with the variant are heterozygous for an autosomal mutant allele. Sex-linkage is ruled out by transmission from father to son in several families.

Of the 860 placental preparations examined, variant I occurred with a frequency of 1.7 percent among Caucasians (12/705) and 0.67 percent among Negroes (1/148). Variant II was detected almost exclusively among Negroes—of 11 individuals with the variant, 8 were Negroes; 1 was Puerto Rican; 1 was the product of a Puerto Rican father and a Negro mother; and 1 was white. This gives an incidence of 6.1 percent for the Negro sample, including the mixed mating (9/148) and 0.14 percent for the Caucasian sample (1/705). Variant III was found in three Negroes which is a frequency of 2.0 percent (3/148).

Thus, although variants of soluble GOT are extremely rare, there are at least three variants of mitochondrial GOT, all of which are common enough to be considered polymorphisms. The consistent triplet patterns support the hypothesis that both soluble and mitochondrial GOT exist as dimers. The differing patterns of variant I in the two buffer systems and the failure to differentiate between variants I and III in the phosphate-citrate buffer system emphasize the importance of altering electrophoretic conditions in the study of isoenzymes. Segregation of the variants according to simple Mendelian inheritance rather than maternal inheritance, as was the case with a variant of human mitochondrial malate dehydrogenase (*9*), provides a second example of a human mitochondrial enzyme that is under the control of nuclear DNA.

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7. The 0.002*M* pyridoxal 5-PO_4, 0.2*M* L-aspartic acid, and 0.1*M* α-ketoglutaric acid are prepared in 0.1*M* tris, adjusted to *p*H 7.4 with potassium hydroxide pellets and frozen in convenient portions. Fast Blue 2B salt (diazotate-4′-amino-2′,5′-diethoxybenzanilide), 250 mg dissolved in 8.5 ml of water, is added to 1 ml of pyridoxal 5-PO_4, 8.5 ml of α-ketoglutarate and 8.5 ml of L-aspartate, diluted to 50 ml with 0.1*M* tris HCl, *p*H 7.4, poured over the cut surface of the gel and incubated at 37°C. Blue bands identify areas where the diazonium salt couples directly with the oxaloacetate produced by the action of GOT.
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10. We thank A. B. Waters for assistance in the family studies, and A. Molnar and M. Molnar for technical assistance. Supported in part by PHS grants GM15874 and GM09966, contract PH-43-67-1463, the Children's Bureau Project 417, and Dr. H. C. Buswell and Bertha H. Buswell Research Fellowship.

25 March 1970

Visualization of Bacterial Genes in Action

Abstract. *The morphology of active structural and putative ribosomal RNA genes was observed by electron microscopy after lysis of fragile* Escherichia coli *cells. Conclusions drawn are: most of the chromosome is not genetically active at any one instant; translation is completely coupled with transcription; the 16S and 23S ribosomal RNA cistrons occur in tandem, in regions which are widely spaced on the chromosome.*

Techniques developed for the visualization of the structure of active genes in a eukaryotic cell (*1*) were used to observe chromosomes of *Escherichia coli*. A mutant strain was utilized (*2*) which, under certain growth conditions, develops fragile cell walls. Fragile cells in the log phase of growth were osmotically burst by rapid dilution into water, then prepared for electron microscopy (Fig. 1).

At low magnification, the extruded contents of shocked cells appear as masses of thin fibers with attached strings of granules (200 to 250 Å in diameter); these granules are approximately the size of *E. coli* ribosomes (*3*). After osmotic rupture, treatment with deoxyribonuclease destroys the fibers, whereas ribonuclease removes the granular strings from the fibers. From these results, we conclude that the fibers are bacterial chromosomes and the granules are ribosomes which were translating messenger RNA (mRNA) molecules at the time of isolation. The contents are not completely removed from the cells by osmotic shock, although considerable amounts are extruded and spread to varying degrees around the cells. Consequently, we have been able to observe only portions of chromosomes rather than complete genomes.

The diameter of double-helix DNA is ~20 Å (*4*). In our preparations, the *E. coli* chromosome is ~40 Å in diameter. This suggests either that the DNA is combined with some nonhistone proteins in vivo (*5*) or that proteins at-