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Galactose-1-phosphate-uridyltransferase (E.C. 2.7.7.11): A Simple Routine Method for Detecting Individuals Heterozygous for the Silent Allele Gt^0

G. Siebert, J. Kömpf, and H. Ritter

Institut für Anthropologie und Humangenetik der Universität Tübingen, Wilhelmstraße 27, D-7400 Tübingen, Federal Republic of Germany

Summary. A simple routine method for detecting individuals who are heterozygous for the silent gene Gt^0 is presented. This method consists of a combination of electrophoresis and densitometry. The results confirm the theoretical expectation that these individuals would exhibit about 50% of the enzyme activity found in the corresponding normal phenotype.

Introduction

Deficiency of the enzyme galactose-1-phosphate-uridyltransferase (Gt) is known to be the cause of a severe disease of the newborn, galactosemia. This inborn error, if not treated, will lead to an early death of the affected individual. Consequences of starting the treatment (e.g., strict diet) too late will consist in mental and physical defects of various degrees.

Individuals heterozygous for the silent allele are noted to have no clinical symptoms of the disease. However, for the purpose of genetic counselling clear identification of persons who are heterozygous for the silent gene Gt^0 is extremely important.

This paper presents a simple routine method combining electrophoresis and densitometry for detecting carriers of the silent gene.

Material and Methods

A kindred from the Swabian Alb and healthy persons chosen by chance from the southwestern area of Germany were investigated. Blood samples were either deep-frozen until use or investigated immediately after venepuncture.

To obtain an equal amount of protein in every case, the sample preparation was carried out as follows: 1 ml erythrocytes was washed three times in 0.9% NaCl solution, after which 400 µl erythrocytes were transferred into a glass tube with conical bottom and 200 µl of a solution consisting of 10% Triton X-100 and 40% saccharose was added to each sample. This was followed by sonification. Of the hemolysate 50 µl was applied to the gel.

Electrophoresis was carried out in a continuous Tris-His-HCl buffer system according to Bissbort and Kömpf (1973), with modifications. The bridge buffer used was 0.2 *M* Tris, 0.15 *M* His-HCl, pH 7.8, the gel buffer was a 1:6.6 dilution of the bridge buffer, and the proportion of starch was 18%. Voltage: 10 V/cm for 14 h, with cooling.

Staining mixture: 30 mg Gal-1-P, 10 mg UDPG, 20 mg NADP, 20 mg MTT, a trace of Glu-1.6 P_2, 100 µl G-6-PDH, 100 µl PGM, 50 µl Meldola blau 1% solution (Boehringer) in 5 ml buffer consisting of 0.2 *M* Tris, 0.2 *M* His-HCl, and 2.5 m*M* $MgCl_2$, at pH 7.4.

The gel was covered with a cellulose acetate strip soaked with staining solution and incubated at 40°C for 1 h.

Densitometry was carried out at a wavelength of 546 nm. The height of peaks measured is directly proportional to the amount of formazan produced in the gel per time unit, which means an equivalent for activity of the different gene products *gt*.

Results and Discussion

Fig. 1 shows some different allelic combinations of Gt^{2LA} and Gt^1, for comparison. The rare phenotype 2LA-gt exhibits a markedly reduced enzyme activity. The migration pattern remains unchanged: activity within the bands representing homomeres is strikingly

Offprint requests to: G. Siebert

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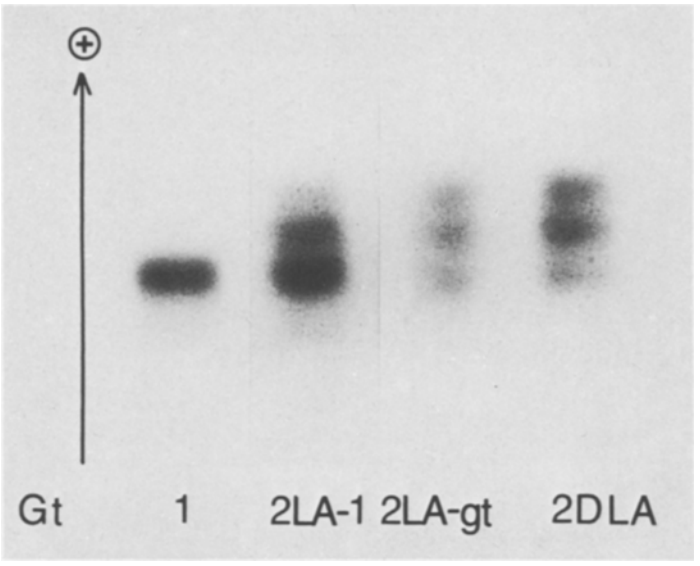

Fig. 1. Some different allelic combinations of Gt^{2LA}, with the phenotype Gt 1 in the first position for comparison. Note the characteristic marked three-banded pattern of the normal phenotype Gt 2LA-1 and the relatively much lowered enzyme activity of the rare phenotype 2LA-gt

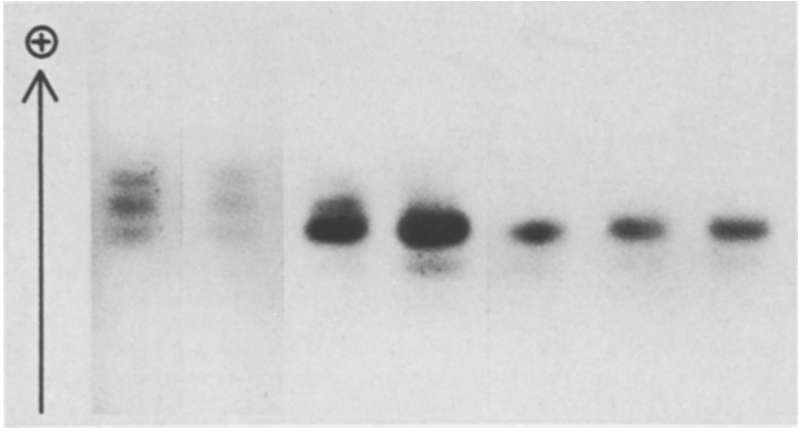

Fig. 2. Phenotypes discovered in a single family (*left* to *right*): Gt 2D, Gt 2D-gt, Gt 2D-1, Gt 1, and three cases of 'Gt 1 weak'

low. The heteromere seems to be stained more strongly. The overall activity of phenotype Gt 2DLA is unmistakably higher, the anodal homomere appears to be stronger. The difference in overall activity and activity distribution from the Gt 2LA-gt phenotype can be clearly seen.

The phenotypes presented in Fig. 2 were discovered in a single family. Differences between the zymograms of Gt 2D, Gt 2D-gt, and Gt 2D-1 are clearly defined: a relatively strong enzyme activity of the phenotype Gt 2D can be seen. The band in the middle position seems to be the most active. The Gt 2D-gt phenotype shows the same pattern of activity distribution as Gt 2D, but the overall activity seems to be weaker. The 'normal' Gt 2D-1 phenotype shows the well-known pattern of three bands, with activity decreasing from the slowest to the fastest.

In comparison with the normal Gt 1 phenotype there seems to be some lack of activity within the zymograms representing Gt 1 weak. The zymograms being studied were measured in a densitometer to obtain numerical support for these findings (Fig. 3).

Individuals who are heterozygous for the silent genes Gt^0, *Gt 1-gt* (*Gt 1* weak), *Gt 2D-gt*, and *Gt 2LA-gt* have substantially lowered enzyme activity.

The difference in the distribution of overall activity is expressed numerically in Table 1. The differences consist in (a) the height of peaks in the densitogram; and (b) the relative overall activity (percentage relative to the homozygous Gt 1).

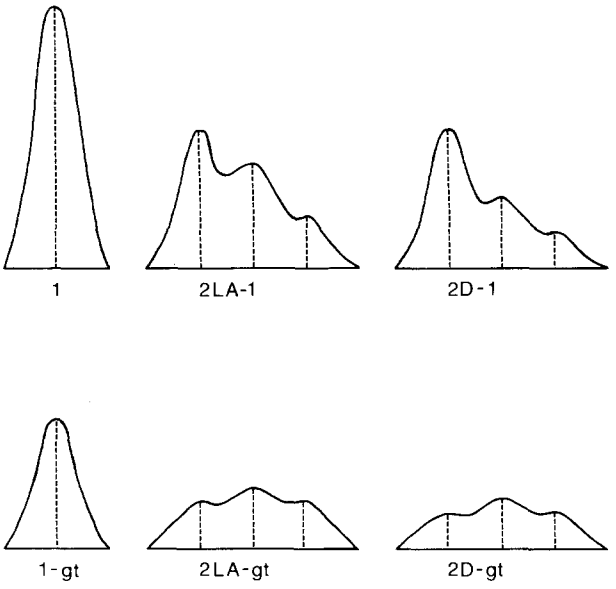

Fig. 3. Densitograms of different Gt phenotypes. *Upper row:* Gt 1, Gt 2LA-1, and Gt 2D-1, *lower row:* Gt 1 "weak", Gt 2LA-gt, and Gt 2D-gt. Enzyme activity is appreciably lower in the densitograms in the lower row. Magnification is the same for all densitograms in both rows

Table 1. Overall enzyme activity in different Gt phenotypes

Phenotype	Relative height of peaks			Relative activity	% (*Gt 1*) activity
	Gt 1	*Gt 2–1*	*Gt 2*		
1	7.5	—	—	7.5	100
2D-1	4.0	2.0	1.0	7.0	93
2LA-1	4.0	3.0	1.5	8.5	113
1-gt	3.8	—	—	3.8	51
2D-gt	1.0	1.5	1.0	3.5	47
2LA-gt	1.4	1.8	1.3	4.5	60

Table 2. Relative activities (%) for different allelic combinations

Gt alleles	*1*	*gt*
1	100	51
2D	100	50
2LA	100	53

The degree of enzyme activity reduction in different allelic combinations with Gt^0 becomes still more obvious when activity comparisons are made between the phenotypes Gt 1 and Gt-1gt; Gt 2D-1 and Gt 2D-gt; and Gt 2LA-1 and Gt 2LA-gt. This is shown in Table 2. Consideration of the corresponding combinations reveals clearly that the activity of phenotypes 1-gt, 2D-gt, and 2LA-gt adds up to approximately 50% of that in the corresponding phenotypes Gt 2, Gt 2D-1, and Gt 2LA-1. Our findings are thus in keeping with expected levels of enzyme activity for these combinations.

Thus, with the aid of this method it is possible to detect the silent gene Gt^0 in every single allelic combination. In genetic counselling concerning inborn errors it is always desirable to be able to detect individuals who are heterozygous for the silent gene, i.e., to tell the genotype from the phenotype. In the case of galactosemia this is now possible.

Reference

Bissbort, S., Kömpf, J.: Galaktose-1-Phosphat-Uridyltransferase (E.C.: 2.7.7.11): Elektrophoretische Darstellung. Z. Rechtsmed. **72**, 119—120 (1973)

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