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# Genetic Polymorphism of FUC (EC 3.2.1.51.) in Polish Population

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**Summary.** In a sample of the Polish population numbering 271 persons three FUC phenotypes were encountered by cellulose acetate gel isoelectric focusing (CAGIF). The frequencies of $FUC^1$ and $FUC^2$ genes were 0.653 and 0.347, respectively. The FUC system has proven to be of high value for paternity testing.

**Key words:** Alfa-L-fucosidase – Blood groups, FUC polymorphism

**Zusammenfassung.** In einer Anzahl von 271 Personen der polnischen Bevölkerung wurden drei Phänotypen FUC mit der Celluloseacetatfolien-Gelelektrofokussierung nachgewiesen. Die Frequenz der $FUC^1$ und $FUC^2$ beträgt entsprechend 0.653 und 0.347. Das Gruppensystem FUC ist von großer Bedeutung bei der Vaterschaftsbestimmung.

**Schlüsselwörter:** Alpha-L-Fucosidase – Blutgruppen, FUC-Polymorphismus

Alfa-L-fucosidase, FUC, EC 3.2.1.51. is a hydrolase which catalyzes the following staining reaction: 4-methylumbelliferylo-alfa-L-fucoside = L-fucose + 4-methyloumbelliferone. The polymorphism of fucosidase was discovered by Turner et al. [1] in 1974. The authors worked out the method of starch gel electrophoresis in phosphate buffer, pH = 7.0.

In 1975, the same authors [2] described the method of isoelectric focusing in polyacrylamide gel, using pH ranges from 3.5 to 9.5. They observed that the treatment of samples with neuraminidase yields improved results. The isozymes occur in most tissues but are not present in red cells [1, 3].

The present work describes the method of isoelectric focusing on cellulose acetate gel (CAGIF) used for the detection of FUC isoenzymes in leukolysates without neuraminidase and reports the results obtained in the Polish population.

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## Materials and Methods

*Preparation of Samples*

Lymphocytes were obtained from whole fresh blood samples from HLA-tested unrelated adults. Leukolysates were prepared by lysis (20 µl of Triton X 100 for 1 ml packed lymphocytes). The samples were tested immediately or after storage at −20° C.

*Preparation of Cellulose Acetate Gel Strips*

Strips (Cellogel, Chemetron 40 × 170 mm or Celluloseacetatfolien G, Biotest 75 × 150 mm) were washed overnight in methanol and methylated in a shaker in 3.5% boron trifluoride (obtained from 40% $BF_3O/C_2H_5/_2$, Fluka AG) overnight at room temperature. Later on, the methylated strips were washed for 24 h in methanol and for 48 h in distilled water.

*Isoelectric Focusing*

Focusing was performed in an apparatus cooled by running water, in which methylated strips were flooded with hexane [4, 5]. Washed methylated strips were immersed in ampholine solution (6 ml of LKB ampholine pH range 5–7 in 50 ml of 4% sucrose with 5 mg% of thymol). This volume of ampholine solution is sufficient to yield a pH gradient on about fifty 75 × 150 mm strips. The strips were placed in the apparatus, and air bubbles with an excess of ampholine were removed by blotting with filter paper.

*Electrolyte Pads*

The glass filter papers (Whatman GF/D) connecting the methylated strips to anode and cathode were soaked in 0.5% lactic acid in 30% sucrose and in 0.1 *M* NaOH.

Leukolysates were applied (about 2 µl) at 5 cm from the anodic end. Platinum wire electrodes were positioned and pressed firmly into place on the electrolyte pads. The apparatus was continuously flushed with hexane to reduce heating and evaporation of the strips. The typical running conditions included constant 500 V power. The time of electric focusing was about 45 min.

*Staining*

The 5 mg of 4-methylumbelliferyl-alfa-L-fucopyraniside pure (Koch Light) was shaken for 30 min with 1 ml of acetone (this quantity of reagent is sufficient for about 50 staining cycles). Ten milliliters of 0.1 *M* phosphate-citrate puffer, pH = 4.8, was boiled with 0.2 g of agarose and poured onto a glass petri dish. The agarose gel was flooded with substrate in acetone for 15 min, an excess of acetone was decanted, and the strips were placed into the petri dish after focusing. After 1 h incubation at + 37° C the strips were exposed to ammonia vapor and examined under UV light.

## Results and Discussion

Three commonly occurring FUC phenotypes were situated in four zones, the arrangement of which is shown in Fig. 1. The phenotypes of FUC are well distinguishable despite differences in the electrophoretic pattern from that previously described by other authors. The observed phenotypes and their distributions were not significantly different from expectation (Table 1) and were consistent with the hypothesis of hereditary transmission proposed by Turner et al. [1].

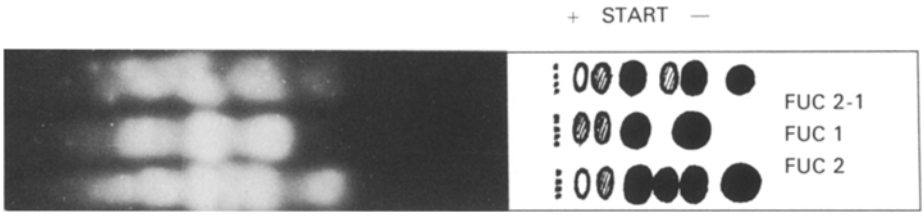


**Fig. 1.** Zymogram of FUC phenotypes

**Table 1.** FUC phenotypes and gene frequencies in the Polish population

| FUC | Phenotypes | | Gene frequencies |
|---|---|---|---|
| | Observed | Expected | |
| 1 | 117 | 115.6 | $FUC^1 = 0.653$ |
| 2-1 | 120 | 122.8 | $FUC^2 = 0.347$ |
| 2 | 34 | 32.6 | |
| Total | 271 | 271.0 | |

$\chi^2 = 0.14$, $df = 1$, $0.50 < P < 0.75$

**Table 2.** Frequency of $FUC^2$ gene in various populations

| Population | Sample | Frequency of $FUC^2$ | Reference |
|---|---|---|---|
| North America: Black people | 27 | 0.07 | [2] |
| North America: Non-Jewish people | 68 | 0.25 | [2] |
| England and Scotland | 109 | 0.25 | [9] |
| North America: Jewish people | 126 | 0.25 | [2] |
| Federal Republic of Germany | 262 | 0.26 | [8] |
| Federal Republic of Germany | 355 | 0.26 | [7] |
| Poland | 271 | 0.35 | This report |
| France | 350 | 0.36 | [6] |

Based on the phenotype frequencies, the frequency of FUC genes in the Polish population was calculated. The frequencies of $FUC^1$ and $FUC^2$ were 0.653 and 0.347, respectively. The frequencies of FUC genes found in the Polish population are similar to those reported in most other populations. Table 2 illustrates the frequency of the $FUC^2$ gene in various investigated populations.

High frequency of $FUC^2$ gene inheritance creates the possibility of utilizing the FUC system in forensic medical practice. As a result of the favorable distribution of FUC genes in the Polish population, this system is of high value for paternity testing.

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