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An improved method for semen α-L-fucosidase typing – distribution in the Wuhan population of China

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Summary. An improved method for the separation of the genetic variants of α-L-fucosidase in human semen is described. The method involves, isoelectric focusing in ultrathin-layer PAG containing a mixture of ampholines, pH 5–7 and pH 3.5–9.5, and separator HEPES. The Fu pattern obtained is simple, easy to interpret, and reproducible. The occurrence of fucosidase phenotypes in 189 semen samples from the population of Wuhan was investigated, and the frequencies of the Fu alleles were calculated.

Key words: α-L-Fucosidase, gene frequencies – Semen, α-L-Fucosidase

Zusammenfassung. Die α-L-Fucosidase (Fu) der menschlichen Spermaproben wurde mittels einer verbesserten Isoelektrofokussierung in einer ultradünnen Schicht von Polyacrylamidgel (PAG) in einem gemischten pH-Bereich von 5–7 und 3.5–9.5 unter Zusatz von HEPES analysiert. Die erzielten Fu-Muster sind einfach und leicht interpretierbar und reproduzierbar. Bei 189 Spermaproben aus der Bevölkerung Wuhans wurde das Vorkommen der Fu-Phänotypen untersucht. Es wurden folgende Genfrequenzen berechnet: $Fu^1 = 0.7857$ und $Fu^2 = 0.2143$.

Schlüsselwörter: α-L-Fucosidase in Spermaproben – Fu-Phänotypen, Genfrequenzen

Introduction

The genetic polymorphism of human α-L-fucosidase (Fu, EC3.2.1.51) was first determined in leucocytes by Turner et al. [1]. Using isoelectric focusing they demonstrated three common phenotypes controlled by two codominant alleles, Fu^1 and Fu^2, at an autosomal locus. In the last few years several studies have revealed that the isozyme can be detected in most human tissues and in semen,

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urine, etc. [2–4]. The results suggest that this polymorphism would provide a useful genetic marker the medico-legal grouping of biological materials.

An improved IEF method for typing human semen α-L-fucosidase has been designed to enhance the sensitivity of Fu typing.

Up to now there have been no reports on Fu gene frequencies for Chinese populations. Therefore, we present here the gene frequencies of Fu in a population of central China (Wuhan region).

Materials and methods

Fresh semen samples were collected at the Fertility Clinic of Tongji, Xiehe and Qiaokou Hospital from 189 unrelated men living in Wuhan. Among these specimens there were 146 with normospermia (sperm counts over 40×10^6/ml) and 43 with oligospermia (sperm counts under 40×10^6/ml). All samples were stored at −30°C before use.

Fu typing was performed by ultrathin-layer isoelectric focusing on a polyacrylamide gel (230 × 110 × 0.3 mm). Each gel was made to a final concentration of acrylamide 5.25% (w/v), *N*,*N*′-methylenebisacrylamide 0.25% (w/v), *N*-2-hydroxyethyl-piperazine-*N*′-ethane-sulphonic acid (HEPES, GIBCO) 3% (w/v), sucrose 15% (w/v), ampholine, pH 5–7, 1.6% (w/v) (LKB), and pH 3.5–9.5, 0.8% (w/v) (LKB), riboflavin 0.002% (w/v).

The electrode paper strips were soaked with 1 *M* phosphoric acid for the anode and with 0.5 *M* sodium hydroxide for the cathode. The distance between the electrode wicks was 8.0 cm.

The gel was prefocused at 300 V for 30 min. Semen samples were applied to a 3 × 5 mm filter paper and placed 1.5 cm from the anode. The papers were removed after 20 min. Focusing was carried out for 30 min at a constant voltage of 1000 V, for 15 min at 1400 V, then for 45 min at 1800 V. Circulating water at 4.5°C was used during the run.

For the isozyme visualization a piece of filter paper was soaked in a solution of 4-methylumbelliferyl-α-L-fucoside (Sigma) (0.6 mg dissolved in 4 ml 0.1 *M* citrate-phosphate buffer, pH 4.8) and applied onto the entire surface of the gel plate. The gel was incubated at 37°C for 20 min and then placed in a chamber containing ammonia gas for 1 min. The Fu isozymes were visualized under ultraviolet light.

Results and discussion

Results obtained for Fu typing are shown in Fig. 1. The pattern demonstrated is very simple and can easily be interpreted compared with that described in earlier publications. The homozygote phenotype Fu1 is represented by two major bands, while Fu2 has three major bands, the Fu1 bands being only slightly more cathodal than the Fu2 bands. The heterozygote phenotype Fu2-1 shows a combined pattern. In the present study no products of the rare Fu allele were observed.

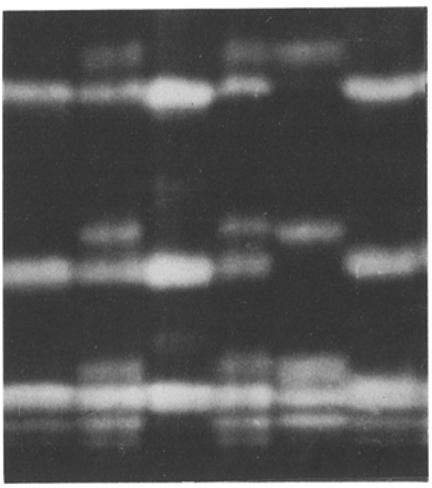

Fig. 1. Isoelectric focusing pattern of α-L-Fu in semen. (*Left* to *right*: 1, 2–1, 1, 2–1, 2, 1; anode at top)

Table 1. Distribution of seminal Fu types in Chinese

Phenotype	No. observed	(%)	No. expected	Gene frequency
1	118	(62.43)	116.67	$Fu^1 = 0.7857$
2–1	61	(32.28)	63.65	$Fu^2 = 0.2143$
2	10	(5.29)	8.68	
Total	189	(100.00)	189.00	

$\chi^2 = 0.3262$; *df*, 1; $P > 0.5$

Besides the major Fu bands, a few anodal minor bands were observed in previous studies [1, 5, 6]. According to the suggestion put forward by Turner et al., these minor bands are due in part to the binding of sialic acid to the primary gene product [5–7]. Because of the existence of the minor bands, the explanation of Fu isozyme pattern seems to be unsatisfactory. In addition, diffusion and a lack of clarity can also cause difficulty with the interpretation. In our experiment some improvements on the method described by Kido et al. [4] have been made in the PAG composition: (1) a carrier ampholine mixture of pH 5–7 and pH 3.5–9.5 was used; (2) the separator HEPES was introduced into PAG; and (3) electrofocusing was carried out using ultrathin-layer gel 0.3 mm thick. This improved technique yielded clearer and narrower Fu isozyme bands. It is of interest to note that the anodal minor bands disappeared and the cathodal minor bands were limited to a narrow region within 1 cm of the cathode and did not interfere with the interpretation of the Fu IEF pattern. The modified technique affords the clearest pattern with high resolution and reliability for Fu typing.

The distribution of Fu phenotypes and gene frequencies in the Chinese population are presented in Table 1. There was good agreement between observed and expected numbers assuming Hardy-Weinberg equilibrium ($P > 0.5$).

Table 2. Comparison of Fu allele frequencies in several populations

Population	No. of cases	Fu^1	Fu^2	References
Poland	271	0.65	0.35	[8]
France	350	0.64	0.36	[9]
German	355	0.74	0.26	[10]
English	109	0.75	0.25	[11]
American (black)	27	0.93	0.07	[1]
Japanese	157	0.739	0.261	[5]
Chinese	189	0.7857	0.2143	This report

Table 2 compares the frequencies of Fu alleles in the Chinese population with selected data from other countries. Differences in the distribution of Fu alleles in the various racial groups are observed. The highest frequency for Fu^1 is found in blacks. The Chinese have a higher frequency for Fu^1 than Europeans and Japanese.

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