GENETIC VARIANTS OF THE HUMAN DIAPHORASE 
DIA₃ IN JAPANESE: REPORT OF 
A NEW RARE ALLELE, DIA₃₄

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Summary Sperm-lysates from 264 unrelated Japanese males were tested for the polymorphism of the human diaphorase DIA₃ by isoelectric focusing in thin-layer polyacrylamide gel over pH range 3.5-9.5 gradient. The occurrence of a new rare allele, DIA₃₄, in addition to the previously described three alleles in German and British populations; has been demonstrated in our population. The estimated allele frequencies of the commonly encountered phenotypes, i.e. DIA₃₁-1, 2-1, 3-1, 2-2 and 3-2, were: DIA₃₁=0.837, DIA₃₂=0.143 and DIA₃₃=0.020.

INTRODUCTION

Electrophoretic variants of the human diaphorase (DIA₃) were first demonstrated by Caldwell et al. (1976) using electrophoresis in polyacrylamide gel. In a population sample from USA, they described three electrophoretic patterns as 'sperm specific' which are determined by two common autosomal alleles. Subsequently, using two different techniques, polyacrylamide gel electrofocusing and agarose electrophoresis, for investigating the diaphorase (DIA₃) polymorphism, Kühnl et al. (1977) reported the existence of an additional third common allele in Germans beside the previously observed two in the USA population. Diaphorase (DIA₃) activity was also observed in extracts of the ovary, oviduct and uterus; so the term 'gonadal diaphorase' was proposed instead of 'sperm diaphorase' which was given by Caldwell et al. (1976). Similarly, Edwards et al. (1979) identified the three common diaphorase (DIA₃) genes in a survey of the British population, when a modification of the starch gel electrophoresis method used by Fisher et al. (1977) was employed in their study. On the other hand, Fisher et al. (1977) applied the notation 'diaphorase DIA₃' as the activity was observed not only in the sperm and female
reproductive tract tissues but also in several others such as foetal tissues including placenta and adult brain extracts. Their notation was adopted here.

This study deals with the polymorphism of the human diaphorase (DIA₃) in the Japanese.

MATERIALS AND METHODS

Samples. Semen samples were collected from 264 unrelated Japanese males (16 volunteers and 148 outpatients) from Miyagi and Yamagata prefectures, Japan. Seminal plasma was separated by high speed refrigerated centrifugation at 12,000 rpm, and sperm-lysates were prepared according to the method of Black and Sensabaugh (1978) and analysed fresh or kept frozen at −20°C until tested within few days.

Isoelectric focusing. The run was performed in the LKB Multiphor 2117 electrofocusing apparatus (Bromma, Sweden) in conjunction with the auto-conversion power unit, Model 2000-200 Auto Deluxe KPI (Kanagawa, Japan). Polyacrylamide gels of 0.3 mm thickness were prepared as described elsewhere (Sebetan and Akaishi, 1981), providing an gel concentration (T)=5% and degree of cross linkage (C)=7.5%. A 3.5% mixture of LKB ampholine carrier ampholytes over pH ranges 3.5–9.5 and 5–8 (6:1, v/v). After polymerization of the gel in the presence of riboflavin and UV light was completed, the mould was kept in the refrigerator overnight before use. Paper strips were saturated with 1 M phosphoric acid and 1 M ethanolamine and used at the anode and cathode, respectively. Pieces of filter paper 5×7 mm (Toyo No. 1) were placed on the gel surface at a distance of 2 cm from the anodal electrode strip and 10 μl of sperm-lysates were added. The power unit was adjusted to supply initial voltage of 300 V and maximum of 1,250 V. The total focusing time was about 3 hr. A cooling system with circulating water at 2°C was used during running. Visualization of the isozyme band patterns was accomplished by the following mixture; 5 mg of NADH, 0.1 mg of 2,6-dichlorophenolindophenol sodium and 2.5 mg MTT were dissolved in 2 ml of 0.2 M Tris-NACL buffer (pH 8.4), then added to 1% melted agar in 8 ml of the same buffer. The mixture was poured on the surface of the gel and the isozyme pattern could be seen after the gel was incubated at 37°C for about 20 min. Preservation of the gel was carried out as described previously (Sebetan et al., 1982).

RESULTS AND DISCUSSION

Electrofocusing pattern obtained from the five common diaphorase DIA₃ phenotypes encountered in our population sample is illustrated in Fig. 1. The homozygous phenotypes are represented by major cathodal zone of two close isozymes with corresponding minor anodal pattern. The heterozygotes showed composite pattern consisting of two major and two minor zones, except for the heterozygote phenotype DIA₃ 3-1 with three isozyme zones, since the isoelectric points of the