UTILIZATION OF HAIR BULBS FOR THE DETECTION OF HETEROZYGOTES OF THE ELECTROPHORETIC G6PD VARIANT

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Summary Scalp hair follicles from a subject heterozygous for an electrophoretically fast-moving G6PD variant were examined by using micro-vertical polyacrylamide gel electrophoresis. Twenty-five or 35 hair bulbs and 17 of 35 outer sheaths showed only one G6PD type, B or C. Thus it is desirable to examine only the hair bulb for the detection of heterozygote of the electrophoretic G6PD variant.

INTRODUCTION

Serum, urine, blood cells, and fibroblast skin cultures have been used as sample for the diagnosis of many inherited metabolic disorders. However in some disorders the diagnosis cannot be achieved by serum, urine, or blood cells (Dancis *et al.*, 1968; Nyhan *et al.*, 1970). The cell culture technique needs much time, much labor, and much money. On the contrary the hair follicle is easy to get and can be used as sample directly.

Hair follicles are especially useful for the detection of heterozygotes of the Lesch-Nyhan syndrome. The detection of heterozygotes of the Lesch-Nyhan syndrome cannot be achieved by blood cells because of a selective overgrowth by the normal cells in the bone marrow. Therefore the detection of heterozygotes of the Lesch-Nyhan syndrome has been achieved by cell culture techniques involving either cloning or growth in a selective media. The cloning approach takes at least 6 weeks and growth in selective media requires half this time. But the activity of hypoxanthine guanine phosphoribosyltransferase of single hair follicle can be determined immediately without any cell culture technique. Consequently the rapid detection of heterozygotes of the Lesch-Nyhan syndrome is possible.

Although hair follicles have been used for the detection of heterozygotes of the

Lesch-Nyhan syndrome (Gartler *et al.*, 1971b; Silvers *et al.*, 1972; Franke *et al.*, 1973), at least 20 hair follicles were needed.

We examined hair bulbs and outer sheaths separately from a subject heterozygous for an electrophoretically fast-moving glucose 6-phosphate dehydrogenase (G6PD) variant, and demonstrated that the heterozygote is more easily detected by using only hair bulbs.

MATERIALS AND METHODS

Scalp hair follicles were collected from a Japanese female heterozygous for an electrophoretically fast-moving G6PD variant. The enzymatic properties of this variant have not been characterized as yet. Therefore the band which had faster mobility than normal G6PD B is tentatively named as G6PD C.

Fig. 1. G6PD polyacrylamide gel electrophoresis run of a single hair bulbs and outer sheaths from an electrophoretically fast-moving variant heterozygote (B/C). Bottom band C, Top band B.

Fig. 2. Relative composition of single hair bulbs or outer sheaths in terms of B/C G6PD activity.

Only hairs with visible bulbs and outer sheaths were used. After pulling, they were dissected into bulbs and outer sheaths. Each bulbs and outer sheaths were dipped into 5 μ l of distilled water and were frozen and thawed three times followed by centrifugation at 13,000 g for 5 min at 4°C. Five μ l of 0.5 M Tris-HCl buffer, pH 6.8, containing 2% Triton X-100 and 40% glycerin is added to the clear supernatant and this mixture was used as sample.

Electrophoresis was carried out according to the procedure by Ogita and Yamamura (1976, 1977) for 4 hr at constant current (1.5 mA/cm) by using a micro-vertical electrophoretic apparatus. Running gel buffer was 0.75 M Tris-HCl buffer, pH 8.8. Stacking gel buffer was 62.5 mM Tris-HCl buffer, pH 6.8, and electrode buffer was 50 mM Tris-glycine buffer, pH 8.3.

Following electrophoresis the gel was incubated for 30 min at 37°C in 50 ml of a solution containing 5 ml of 0.5 M Tris-HCl buffer, pH 8.0, 20 mg of glucose 6-phosphate, 10 mg of NADP, 100 mg of $MgCl_2 \cdot 6H_2O$, 25 mg of nitroblue tetrazolium, and 5 mg of phenazine methosulphate. Zones of G6PD activity appeared as purple bands.

RESULTS

Thirty-five hair bulbs and outer sheaths were analysed electrophoretically from

an electrophoretic variant subject (Fig. 1). As shown in Fig. 2 the single hair bulbs or outer sheaths are classified into seven types according to relative composition in terms of B/C G6PD activity: (1) only B, (2) $B \gg C$, (3) $B > C$, (4) $B = C$, (5) $B < C$, (6) $B \ll C$, and (7) only C.

Six hair bulbs showed only B G6PD type and 19 hair bulbs showed only C G6PD type. Namely 25 hair bulbs had only one G6PD type, B or C. On the other hand 5 outer sheaths showed only B G6PD type, and 12 outer sheaths showed only C G6PD type. Thus 17 outer sheaths had only one G6PD type B or C.

The data in Table 1 shows a marked similarity (but not identical) between the G6PD types of the bulb and outer sheath for individual hairs. The bulb and outer sheath G6PD type are alike in 25 cases and different in 10 cases. Of these 10 cases showed different G6PD type between the bulb and outer sheath only one has quite different G6PD type. Namely bulb No. 18 showed only C G6PD type and sheath No. 18 showed $B > C$ G6PD type.

DISCUSSION

Hair follicles have three cellular components, bulbs, outer sheaths, and dermal papillae. The bulb and outer sheath are derived from the epidermis and are differentiated to have their own peptide pattern on SDS-polyacrylamide gel electrophoresis and their own LDH isozyme pattern (Yamamura and Ogita, 1977). The dermal papilla is mesodermal in origin and no G6PD activity was detected (Gartler *et al.*, 1971a).

As the first sign of scalp hair follicle differentiation is observed at about 4 months of fetal life (Pinkus, 1958), and X-chromosome inactivation occurs at about the time of implantation (Lyon, 1968), the epidermis of the scalp has already grown in a coherent clonal fashion at the time of hair follicle initiation. Thus the probability of obtaining a follicle with only one of the X-linked alleles being expressed is great. Actually Gartler *et al.* (1969, 1971a) showed that the probability of hair follicle possessing only one G6PD type was great in heterozygotes of the electrophoretic G6PD variant.

The G6PD types of the bulb are very similar (but not identical) to those of the outer sheath for individual hairs by the data of Gartler *et al.* The data in Table 1 also showed that 25 of 35 cases are alike in G6PD type but 10 of 35 cases are differ-

Table 1. Bulb and outer sheath G6PD type of single hair follicle.

Bulb G6PD type	B	$B \gg C$	$B > C$	$B = C$	$B < C$	$B \ll C$	C
Outer sheath G6PD type	B	$B \gg C$	$B > C$	$B = C$	$B < C$	$B \ll C$	C
No. of observations	5	2	1	1	1	3	12
Bulb G6PD type	B	$B \ll C$	$B > C$	C	C	C	
Outer sheath G6PD type	$B < C$	$B = C$	$B > C$	$B > C$	$B = C$	$B < C$	
No. of observations	1	1	1	1	1	5	

ent. These results suggest that the cellular origin of the bulb is not always identical with that of the outer sheath. Furthermore these results are consistent with embryological and histological fact. Namely hair bulbs are chiefly consisted of the matrix cells which developed from epithelial cells located in restricted region (around the dermal papilla). On the other hand the outer sheaths are basically similar to unspecialized epidermal epithelium and are widely distributed. From this point of view the data that the probability of hair bulbs possessing only one G6PD (25/35) is greater than in case of outer sheaths (17/35) is reasonable.

We demonstrated that the probability to pluck hair bulbs possessing only one G6PD type is greater than in case of outer sheaths. Thus by using only the hair bulb the number of hairs to be examined can be decreased and more accurate detection of heterozygotes of Lesch-Nyhan syndrome or other metabolic disorders which show X-linked recessive inheritance will be possible.

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