A Case Study of Paternity Diagnosis Where Phenotypes CA and CB of Erythrocyte Acid Phosphatase were Found in a Putative Father and a Child, Respectively

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A Case Study of Paternity Diagnosis Where Phenotypes CA and CB of Erythrocyte Acid Phosphatase were Found in a Putative Father and a Child, Respectively

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Abstract. We report a case study in which phenotypes CA and CB of erythrocyte acid phosphatase, quite rare in the Japanese population, were found in a putative father and a child, respectively, in a paternity diagnosis. The phenotypes CA and CB in this case consisted of isozymes showing the same positions and intensities in a polyacrylamide gel isolectric focusing as the corresponding phenotypes in the Caucasian population.

Key words : Paternity, Acid phosphatase, Isoelectric focusing

Introduction

A paternity diagnosis was carried out in a case in which a putative father denied his paternity. Examination of the genotypes of the blood was mainly employed because the child in question was only a year old. Table 1 shows the results of tests for 13 systems: ABO; MNSs, Rh, P, Duffy, Kidd and Lewis as red cell groups; Gc, Hp, Tf and Pi as serum protein groups, and EsD and AcP as red cell enzyme groups. The overall probability of positive paternity according to the Essen-Möller method, excluding the acid phosphatase (AcP) phenotype, was 96.28%, and the overall exclusion rate was 89.33%. The AcP phenotype was, CA in the putative father and CB in the child. The total frequency of the three phenotypes, CA, CB and C, is about 8% in the Caucasian population[1], whereas it is less than 0.1% in the Japanese population[2]. Phenotypes CA and CB of AcP found in the process of a paternity diagnosis is reported herein.

Method of phenotyping erythrocyte acid phosphatase

Erythrocyte samples from the mother, the child and the putative father were analyzed by polyacrylamide gel isoelectric focusing under the following conditions, in comparison with erythrocytes showing phenotypes A and BA of AcP. Polyacrylamide gel medium 0.5 mm in thickness, was used. The amphoteric carrier was Amphorine at pH 5-7. The sample (hemolysate) was adhered to a 6 × 2 mm filter paper, which was placed 2 cm from the anode, and subjected to electrophoresis at a constant voltage of 800 V for two hours. Umbelliferyl phosphate was used as the substrate. A cellulose acetate membrane was soaked in a substrate solution and attached to the gel surface, which was then kept at 37°C for 10 minutes to obtain color development[3].

Results and Discussion

Fig. 1 shows the resulting patterns of polyacrylamide gel isoelectric focusing with erythrocyte samples from the three subjects and those of phenotypes A and BA. Corresponding schemata are also illustrated. Each of the fundamental phenotypes A, B and C consisted of four bands, which were referred to by the numbers 1, 2, 3 and 4, from the cathodic side. CA exhibited six bands, i.e., a_1, a_2, $(a_3 + c_1)$, $(a_4 + c_2)$, c_3 and c_4, whereas CB had four bands, $(b_1 + c_1)$, $(b_2 + c_2)$, $(b_3 + c_3)$ and $(b_4 + c_4)$. for reference, BA exhibited six bands, a_1, a_2, $(a_3 + b_1)$, $(a_4 + b_2)$, b_3 and b_4. The electrophoretic patterns in the photograph agreed very well with the schemata. The phenotype of erythrocyte AcP was identified as CA for the putative father, CB for the child, and B for the mother. The electrophoretic pattern of phenotypes A and BA examined for comparison also agreed with the schemata.

The enzymatic activity is considered to be proportional to the degree of color development of umbelliferone. On this basis, the relative intensities

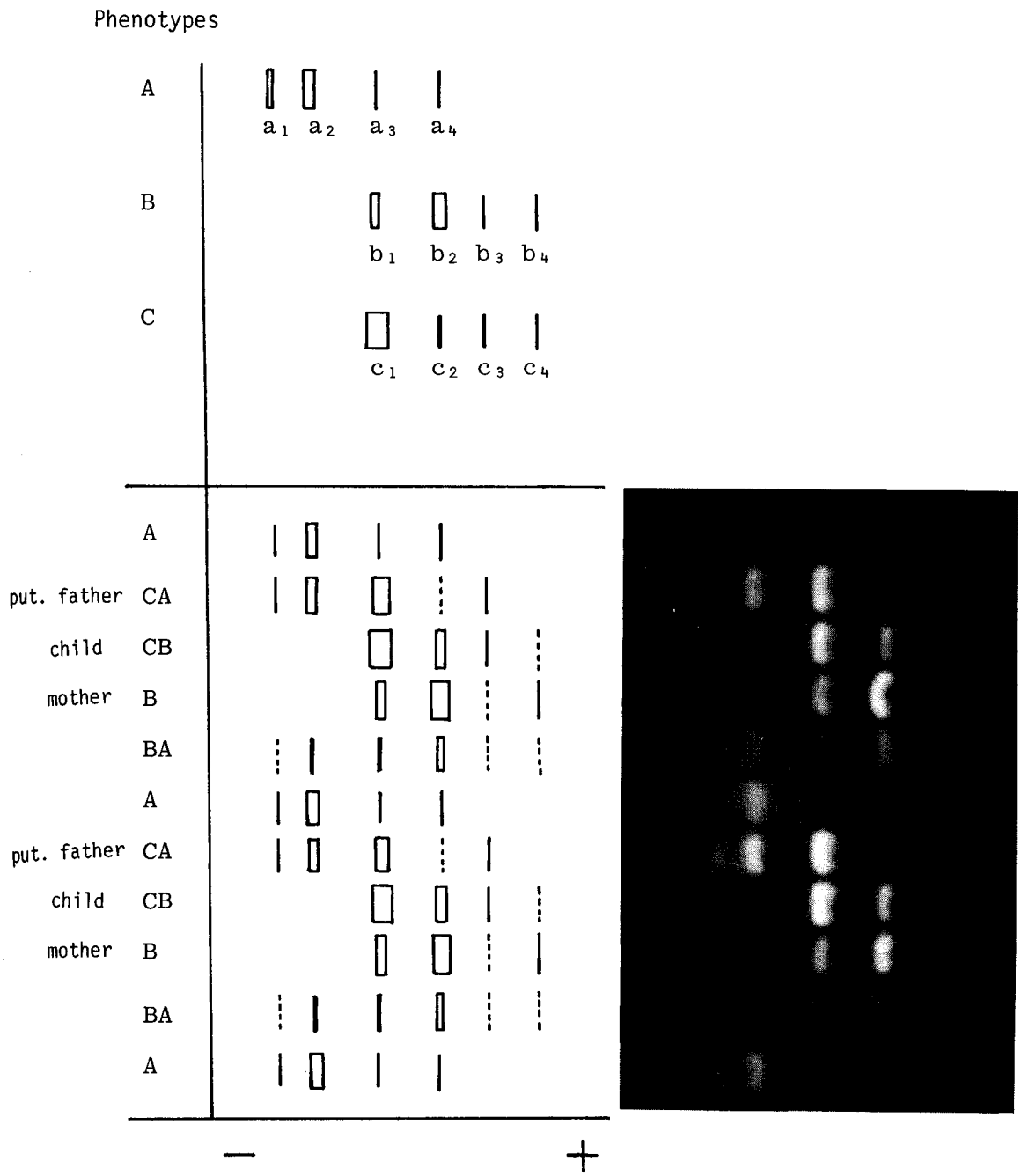

Fig. 1. Zymogram and schematic presentation of erythrocyte acid phosphatase types on polyacrylamide gel isoelectric focusing

of the bands were determined macroscopically, as shown in Table 2. The relative intensities of five phenotypes, A, B, BA, CA and CB, were completely the same as those in the Caucasian population reported by Randall *et al.*[4] and Divall[5].

It has been reported that isozymes in phenotypes CA and CB detected in Hokkaido, Gunma and Okinawa, had very weak activites[5)~7)]. In contrast, the activites of isozymes in phenotypes CA and CB found in the present study were not weak, as compared with those in the phenotype B sampled together and those in the phenotypes A and BA used as

Table 1. Blood group phenotypes of the clients tested

Blood group systems examined		mother	child	put. father
Red cell groups	ABO	O	O	O
	MNSs	Ms	Ms	Ms
	Rh	CCDee	CCDee	CCDee
	P	P^+	P^-	P^-
	Duffy	a^+b^-	a^+b^-	a^+b^-
	Kidd	a^+b^-	a^+b^+	a^+b^+
	Lewis	a^-b^+	a^+b^-	a^-b^+
Serum protein groups	Gc	1S1S	1F1S	1F1S
	Hp	1	2-1	2-1
	Tf	C1C2	C1C2	C1
	Pi	M1	M1M2	M2
Red cell enzyme groups	EsD	2-1	1	1
	AcP	B	BC	AC

Table 2. Relative activity of the isozymes in 5 AcP phenotypes

Phenotypes	Relative activity
A	$a_2>a_1>a_4>a_3$
B	$b_2>b_1>b_4>b_3$
BA	$(b_2+a_4)>(b_1+a_3)=a_2>b_4$
CA	$(c_1+a_3)>a_2>a_1>c_2,\ c_3$
CB	$(c_1+b_1)>(c_2+b_2)>(c_3+b_3)>(c_4+b_4)$

standards. The properties, such as substrate-specificity and thermostability, of these phenotypes rare in the Japanese population are to be studied in the future.

Incidentally, the parents of the putative father are of Japanese nationality, and no signs of racial mixture with Caucasian blood were found in the complexion of the putative father.

Conclusion

In a paternity diagnosis, phenotypes CA and CB, which are very rare in the Japanese population, were found in a putative father and a child, respectively. The phenotypes CA and CB in this case consisted of isozymes showing the same positions and intensities as those for the corresponding phenotypes found in the Caucasian population upon polyacrylamide gel isoelectric focusing.

Refereces

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赤血球酸性フォスファターゼ（AcP）のCA型，CB型が疑父及び子に見出された親子鑑定の1例

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摘要 嫡出子否認事件で，子が満1歳と幼少であるので主として血液の遺伝形質の検査により鑑定を行った．赤血球型：ABO, MNSs, Rh, P, Duffy, Kidd, Lewis, 血清型：Gc, Hp, Tf, Pi, 赤血球酵素型：EsD, AcPの合計13システムを検査した．AcP形質を除いたEssen-Möllerの父権肯定総合確率は96.28%，同じく総合排除率は89.33%であった．AcP形質は疑父がCA型子がCB型と判定された．AcP形質のCA型，CB型，C型の出現頻度の合計は欧米人では約8%であるが[1]，日本人では0.1%以下と報告されている[2]．本親子鑑定にて経験したAcP形質のCA型，CB型について報告した．