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Transferrin Subtyping in Dental Pulps

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ABSTRACT: Serum transferrin (TF) subtypes were also found in dental pulps by isoelectric focusing and immunoblotting. The types observed in dental pulps completely agreed with those in serum samples from the same individuals. The allele frequencies in 105 samples were TF*C1 = 0.757 and TF*C2 = 0.243. Reliable subtyping was possible for 4 weeks following extraction of the teeth. The TF system can provide a useful genetic marker for the medicolegal individualization of teeth.

KEYWORDS: pathology and biology, TF subtyping, isoelectric focusing, immunoblotting, individualization, teeth, odontology

Inherited electrophoretic variation of serum transferrin (TF) was first discovered by Smithies [*1*] with a common C type and several rare variant types. The introduction of isoelectric focusing revealed the polymorphic occurrence of TF subtypes that are controlled by TF*C1 and TF*C2 [*2*,*3*].

The TF system has now become an accepted useful genetic marker in medicolegal investigations since using sensitive immunoblotting techniques the subtypes have been demonstrated also in urine, blood stains, and semen [*4*,*5*]. This paper is the first description of the demonstration of TF subtypes in dental pulps present in the teeth.

Materials and Methods

Teeth were extracted from 145 patients who received treatment at the Dental Clinic of Yamanashi Medical University Hospital. 105 samples were examined immediately after extraction and 40 samples after storage at room temperature for various intervals. A piece of tooth was crushed with a hammer, and the dental pulp was picked out from the pulp cavity. The pulp tissue weighing 10 to 20 mg was mashed with a glass rod in 20 μL of 1% Triton X-100. Blood samples were also taken from the same subjects as control.

8 μL of dental pulp lysate or serum was treated with 2 μL of 1 M potassium phosphate buffer (pH 7.0) containing 50 U/mL neuraminidase from *Clostridium perfringens* (type V, Sigma, St. Louis) for 18 h at room temperature. The desialated dental pulp lysate was diluted 1:20 and the desialated serum 1:100 with distilled water.

Isoelectric focusing was performed using a Bio-Phoresis Electrophoresis Cell (Bio-Rad, Richmond, Ca) and a Model 3000 Xi Power Supply (Bio-Rad). Polyacrylamide gel (230 × 110 × 0.5 mm) was composed of 20 mL of stock solution (5.25% acrylamide/0.25%

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N,N'-methylenebisacrylamide) containing 1 mL of Ampholine pH 5 to 7 (Pharmacia, Uppsala, Sweden), 0.3 mL of 0.01% riboflavin and 2.5 g sucrose. The electrode paper strips were soaked with 1 M phosphoric acid for the anode and with 1 M sodium hydroxide for the cathode. After prefocusing at 1000 Vmax and 5 mAmax for 60 min, the desialated and diluted samples were applied to the gel surface 2 cm from the cathode using 5 × 6 mm filter paper strips (Whatmann No. 3, Maidstone, UK). Electrofocusing was conducted at a constant voltage of 1000 V for 180 min. The specimens were removed after 60 min of focusing. During focusing the gel was cooled by circulating water at 4°C.

A sheet of nitrocellulose membrane (Bio-Rad) was placed onto the gel surface and left for 60 min with 1 kg weight. Following blotting the membrane was rinsed in 20 mM tris/500 mM sodium chloride buffer, pH 7.5, (TBS) for 10 min and immersed in TBS containing 3% gelatin for 30 min. After washing in TBS for 15 min, the membrane was incubated for 60 min in rabbit anti-human TF serum (DAKO, Denmark) that was diluted 1000-fold with TBS containing 0.05% Tween 20 (TTBS). Then the membrane was washed three times in TTBS for 30 min and incubated for 60 min in goat anti-rabbit IgG serum conjugated with alkaline phosphatase (Sigma) that was diluted 1000-fold with TTBS. After three washes in TTBS for 30 min, the membrane was incubated in 50 mL of staining solution (1.8 g sodium hydroxide and 3.7 g boric acid dissolved in 1000 mL of distilled water) containing 25 mg β-naphthyl phosphate, 25 mg Fast Blue BB salt and 60 mg magnesium sulfate for a few minutes.

Quantitation of TF was carried out using rocket immunoelectrophoresis by the method of Laurell [*6*] with minor modifications. Glass plates (8 × 8 cm) were covered with 6.4 mL of 1% solution of Agarose A (Pharmacia) containing 38 μL of rabbit anti-human TF serum (DAKO). After gelation, wells were punched out along a line 1.5 cm from the edge of the gel plate. 5 μL of serially diluted TF references (DAKO) and 10-fold diluted dental pulp lysates was applied to each well. Electrophoresis was run at a constant current of 10 mA for 4 h at 4°C. After electrophoresis, the gel was stained with 0.5% Amido Black 10B. The standard curve was obtained by plotting the height of rockets formed against the corresponding TF value. The amount of TF was estimated from the standard curve.

Results and Discussion

The isoelectric focusing pattern of TF in dental pulps and serum samples from the same individuals is shown in Fig. 1. The allele products in dental pulps were detected as

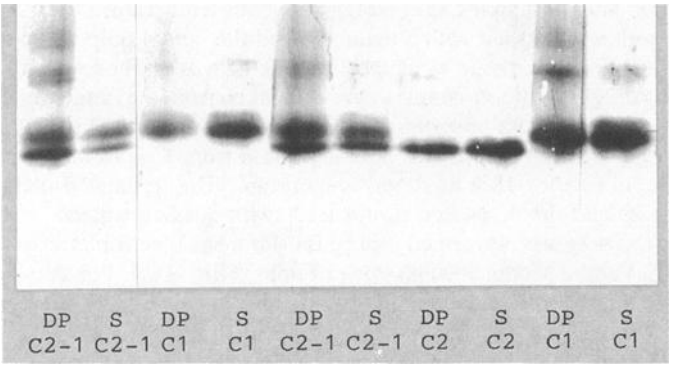

FIG. 1—*Isoelectric focusing pattern of TF subtypes in dental pulps and the corresponding serum samples. DP: dental pulp; S: serum. The anode is at the top.*

clearly and intensely as those in serum samples and the types observed in dental pulps completely agreed with those in the corresponding serum samples. The results for the distribution of TF in sera and dental pulps from 105 individuals are given in Table 1. The observed numbers are in good agreement with the numbers expected according to the Hardy-Weinberg equilibrium.

The amount of TF in 15 samples of dental pulps was determined by rocket immunoelectrophoresis (Fig. 2). The value ranged between 240 and 841 μg/mL and the mean value was 511 ± 42 μg/mL. Since the dental pulp lysates were diluted 4:5 at neuraminidase treatment and 1:20 for isoelectric focusing, the lowest TF value that was subtyped by the present procedure was 10 μg/mL.

Table 2 summarizes the results of TF subtyping obtained from teeth stored at room temperature for various intervals. Reliable subtyping was possible for 4 weeks after extraction of the teeth. The pattern in dental pulps from teeth stored for 2 weeks at room temperature is shown in Fig. 3.

To date, ABO, polymorphic serum proteins (Gm, Km, GC) and polymorphic enzymes (PGM1, PGD, ADA, AK, ESD, FUC, DIA3) have been demonstrated in dental pulps [*7–13*]. The present technique permits reliable and sensitive TF subtyping from a piece of dental pulp for at least 1 month of storage. The TF subtyping in dental pulps can provide a further means for individual identification of teeth. The method is particularly

TABLE 1—*Distribution of TF subtypes in a Japanese population.*

Phenotype	No. observed		(%)	No. expected
	Serum	Dental pulp		
C1	62	62	(59.0)	60.2
C2-1	35	35	(33.3)	38.6
C2	8	8	(7.6)	5.2
Total	105	105	(99.9)	105.0

Allele frequency: TF*C1 = 0.757; TF*C2 = 0.243.
$x^2 = 0.913$; *d.f.* = 1; $0.5 > p > 0.3$.

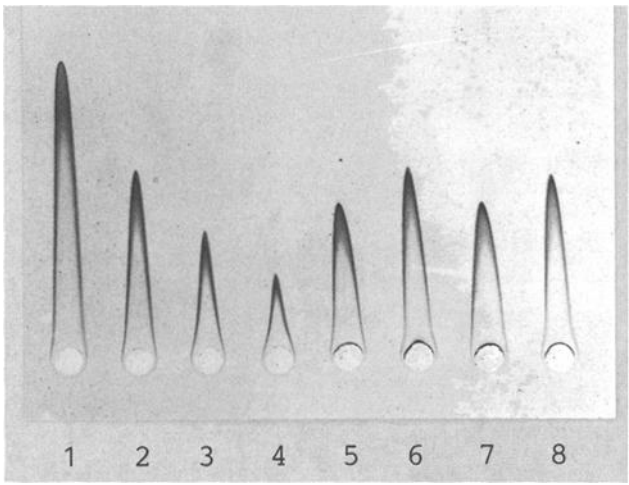

FIG. 2—*Rocket immunoelectrophoresis of TF references and dental pulp lysates. 1–4: serially diluted TF references (1: 134 μg/mL, 2: 67 μg/mL, 3: 33 μg/mL, 4: 17 μg/mL); 5–8: 10-fold diluted dental pulp lysates (5: 45 μg/mL, 6: 62 μg/mL, 7: 45 μg/mL, 8: 59 μg/mL). The anode is at the top.*

TABLE 2—*Positive results of TF subtyping in dental pulps.*

Periods of storage	No. tested	Phenotype		
		C1	C2-1	C2
1 week	10	7	2	1
2 weeks	10	5	3	2
3 weeks	10	5	3	2
4 weeks	10	6	4	

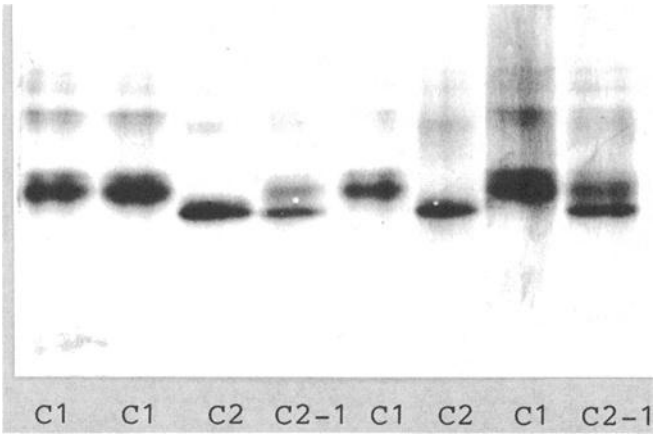

FIG. 3—*Isoelectric focusing pattern of TF subtypes in dental pulps from teeth stored for 2 weeks at room temperature. The anode is at the top.*

useful in the assignment of a broken piece of teeth to the victim when they are separately found, for example, in case of automobile accidents, aircraft crashes or explosions.

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